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Investigating the effect of adding soy, barley and almond milk on the physicochemical and sensory properties of probiotic yogurt

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ABSTRACT

Probiotics are beneficial bacteria that can be added to foods, especially dairy products, and produce their own health effects in the body. In this study, yogurt was prepared with a combination of soy, barley and almonds milk in three concentrations (75:25, 50:50, 25:75 ratio of milk/soy milk, barley and almond) and incubated with 10^8 cfu/ml of two probiotic bacteria *Lactobacillus acidophilus* and *Lactobacillus plantarm* and studied for 21 days. Yogurt samples were tested for probiotic viability, pH, acidity, brix, viscosity, syneresis, and sensory evaluation on 0, 7, 14, and 21 days after production. The results showed that the probiotic bacteria initially increased and then decreased during storage. Yogurt samples containing soy, almond and barley milk had a smaller decrease in the number of probiotic bacteria and at the end of 21 days, they showed an acceptable level of bacterial viability. The trend of decreasing pH and acid production by these bacteria was observed in yogurts over time, and this trend was more in samples containing. Syneresis in all samples increased during time but sample containing soy milk, barley and oats milk had less syneresis. The percentage of soluble solids of all samples showed a decreasing trend during storage, but this trend was slower in the samples of yogurts containing soy, barley and almond milk. The viscosity of the produced products showed an upward trend during the storage time, and the samples containing soy, barley and almond milk had a higher viscosity. Overall acceptability in all our samples was not significantly different from the control sample. Finally, yogurt containing 25% milk and 75% barley milk had better quality than other treatments in terms of physicochemical characteristics.

1-Introduction

Functional foods refer to the foods that improve and enhance the health of the community, while providing essential nutrients to the body. Currently, there is a growing trend towards the consumption of functional foods. Among them, foods and beverages containing probiotics and prebiotic compounds are of paramount importance. Probiotics are live microorganisms that, when consumed in sufficient amounts, help balance the microbial flora of the host [1]. A minimum of 10^7 to 10^6 probiotics per gram is required for health benefits to occur [2].

The majority of probiotic microorganisms are from the category of lactic acid bacteria, such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Streptococcus lactis*. Studies have shown that adding probiotics to food can have many beneficial health effects, including reducing blood cholesterol, improving digestive system function, strengthening the immune system, and reducing the risk of cancer. Lactic acid bacteria are often used commercially in dairy products like yogurt. Adding prebiotic compounds can have a synergistic effect on probiotic products [3].

Yogurt is a widely consumed dairy product made by the fermentation of pasteurized milk by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. Due to its high nutritional value, it has received considerable attention worldwide. Its nutritional value can be further enhanced by adding functional food ingredients, such as probiotics and prebiotics [4]. The addition of nutrients to yogurt and probiotic bacteria has been explored in several studies to improve its nutritional properties. Cui et al. (2021) investigated the effects of adding probiotic bacteria to yogurt made from cow's milk and soymilk. Their research showed that soymilk-based yogurt exhibited less syneresis (whey separation) and greater texture firmness over time [5]. Moreover, the addition of soymilk to probiotic yogurt increased the count of *Lactobacillus acidophilus* LA5, but the taste of the soymilk-based yogurt was less accepted by consumers [6]. Yogurt made from cashew milk had higher probiotic counts than plain yogurt and demonstrated higher antioxidant and phenolic activity [7].

Plant-based dairy products can be used to enhance the nutritional value of various food products, including fermented products like yogurt. These plant-based alternatives contain beneficial fatty acids, such as linoleic acid and linolenic acid, carbohydrates with low glycemic index, fiber, and are rich in B vitamins and vitamin E. They are also a rich source of potassium and contain low levels of sodium [8]. The present study is aimed at producing a probiotic functional yogurt product by adding soymilk, oat milk, and almond milk, and investigating the survival rate of probiotics and sensory characteristics of the produced products.

2- Materials and Methods

2-1. Preparation of Almond Milk, Soymilk, and Oat Milk

Almond, soy, and oat seeds were obtained from the local market in Shiraz and immediately transferred to the laboratory. The seeds were initially prepared by washing and cleaning them, and then soaked in water for 10-16 hours. After the skins were separated, 1 kilogram of each seed was weighed and blended with 3 liters of water in a blender for 10 minutes. The resulting liquid was then strained, boiled (pasteurized at 85°C for 10 minutes), and once cooled to room

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temperature, stored as almond milk, oat milk, and soymilk in the refrigerator (at 4°C) [9].

2-2. Preparation and Cultivation of Microbial Culture

Commercial SVD[1] frozen cultures containing probiotic bacteria *Lactobacillus acidophilus* LA-5 and *Lactobacillus plantarum* PPLP-217 were used. The yogurt starter cultures and probiotic bacteria were provided by the Chr. Hansen Company, Denmark. Starter culture packets were prepared at the Ramak Dairy factory according to the manufacturer's instructions, and the mixture was gently stirred until the starter granules fully dissolved in the milk. The starter culture was added at a concentration of 5% by weight to the milk intended for yogurt preparation.

2-3. Preparation of Probiotic Yogurt Containing Soy, Almond, and Oat Milk

A mixture of soymilk, almond milk, and oat milk (prepared separately in various ratios relative to the milk used – see Table 1) was prepared. The different ratios of soymilk, oat milk, and almond milk were made using skim milk and distilled water. After thermal treatment (85°C for 30 minutes), the samples were cooled to the inoculation temperature (41°C). During inoculation, yogurt starter bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) and two probiotic bacterial strains were added in a 1:5 ratio to the milk. Fermentation was carried out at 42°C until the pH reached 4.6 (acidity of the samples ranged between 0.93 and 0.95). After fermentation, the samples were cooled to 4°C. The control yogurt contained only the starter bacteria without any added non-dairy milk. The samples were stored at this temperature for 21 days, with testing carried out on days 0, 7, 14, and 21.

Table 1 Yogurt treatments

Sample number	Treatment
0	Blank (probiotic yogurt without soy, barley or almond milk)
1	Probiotic yogurt containing 25% soy milk
2	Probiotic yogurt containing 50% soy milk
3	Probiotic yogurt containing 75% soy milk
4	Probiotic yogurt containing 25% barely milk
5	Probiotic yogurt containing 50% barely milk
6	Probiotic yogurt containing 75% barely milk
7	Probiotic yogurt containing 25% almond milk
8	Probiotic yogurt containing 50% almond milk
9	Probiotic yogurt containing 75% almond milk

2-3. Tests

2-3-1. Probiotic Viability

The pour plate method was used to count viable cells. Samples were diluted with sterile saline solution and cultured in MRS Agar[2] medium using pour plate technic and aerobic conditions. They were incubated at 37°C for 48 hours. The colonies were counted using a

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colony counter and reported as log cfu/ml [10].

2-3-2. pH Measurement

To measure the pH, the pH meter was calibrated using standard buffers of pH 4 and 7, and the pH electrode was directly placed in the samples to read the pH [11].

2-3-3. Determination of Brix

In order to examine the changes in soluble solids, including sugars, during the fermentation process of probiotic yogurts, the Brix of the samples was measured. After calibrating the refractometer with distilled water, a few drops of the sample at 20°C were placed on the refractometer's prism. Once the light scattering was removed and two equal bright and dark sections appeared on the display, the concentration of soluble solids in the water was read in Brix. The result was expressed as grams per 100 grams of sample [12].

2-3-4. Viscosity

The viscosity of the samples produced in the present study was measured using a Brookfield viscometer. After initial testing, spindle No. 6 was selected as the appropriate spindle for measuring viscosity (according to the manufacturer's instructions, the suitable spindle for viscosity measurements is the one showing more than 10% torque at the desired speed). The probe's penetration into the samples was set to 30 millimeters. The probe speed before the test was 1 millimeter per second, the test speed was 1 millimeter per second, and the probe speed after the test was set to 10 millimeters per second. All tests were conducted at 5°C with the same conditions. The viscosity of the samples was recorded at a speed of 70 revolutions per minute, 15 seconds after the spindle started rotating. The texture parameters, firmness (grams), and viscosity index (millipascal-seconds) were reported [12].

2-3-5. Syneresis (Whey Separation) Measurement

To measure the syneresis of the yogurt, 25 grams of the sample were weighed on Whatman filter paper No. 41 and placed on a funnel. The amount of whey separated from the funnel after 120 minutes at 4°C was recorded as the syneresis amount. syneresis was expressed as grams per 100 grams of sample [13].

2-3-6. Sensory Evaluation

The sensory characteristics of the yogurt, including flavor, color, texture, and overall acceptance, were assessed using a 5-point hedonic scale by 10 trained evaluators at ambient temperature. A portion of the probiotic dairy product was placed in airtight plastic containers for testing, with a standard size for each sample. The scale was designed so that the maximum score (5) represented excellent quality, and the minimum score (1) represented very poor quality [12].

2-3-7. Statistical Analysis

All experiments were conducted in a completely randomized design with three replications. Means were compared using SPSS 25 and based on Duncan's test at a 5% significance level, with results presented in tables showing English letters and the deviation of data from the mean. The resulting charts were plotted, compared, and analyzed by Excel 2013.

3-Results and Discussion

3-1. Probiotic Bacteria Viability in Yogurt

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According to the statistical results obtained at a 95% confidence level (Table 2), the effect of different concentrations of soymilk, oat milk, and almond milk on the viability of probiotic bacteria in the yogurt samples was significant ($p<0.05$). The viability of the samples initially showed an upward trend, followed by a downward trend over time, so that after 3 weeks of storage, the probiotic yogurt sample containing 75% oat milk and 25% regular milk (T6) and the control sample (T0) exhibited the highest and lowest viability, respectively. The storage time of the probiotic yogurt samples also had a significant effect on the viability of the probiotic bacteria ($p<0.05$). After 21 days of storage, the viability showed a significant increase (Table 2). In general, based on the results, the parameters of time and different percentages of soymilk, oat milk, and almond milk were examined as factors influencing probiotic bacteria viability. The results showed that with an increase in the percentage of soy, oat, and almond milk, the viability of probiotic bacteria significantly increased compared to lower concentrations and control samples.

The results also indicated that the viability of probiotic bacteria initially followed an upward trend and eventually a downward trend during storage. This upward trend until day 14 can be attributed to the richness of the yogurt in nutrients from soy, oat, and almond milks, as well as other components in the yogurt. This caused the probiotic bacteria to grow logarithmically at first, and then decrease as the nutrient levels in the environment decreased. However, the bacterial count never fell below the standard limit of 6log cfu/ml. Shoria et al., (2022) examined the viability and antioxidant activity of probiotic yogurt based on cashew nut milk fermented with Lactobacillus strains. They used strains such as *Lactobacillus casei, rhamnosus, plantarum, delbrueckii*, and *thermophilus*. Their findings showed that probiotic bacteria increased significantly until day 14 of storage, but later, consistent with the current study, they showed a decrease in growth. They attributed this reduction to the decrease in nutrient content and the increase in pH of the environment [6].

In all probiotic products, the number of viable probiotic cells per gram or milliliter of the product at the time of consumption is a key indicator of the product's value. Therefore, this index determines the health-promoting effectiveness of these products [14]. Hence, the number of probiotic bacteria in the product must be high enough so that after consumption, the required number of viable probiotic cells can reach the intestinal environment. The required number of viable bacteria for the product to be effective in promoting health should be at least 6 log cfu/ml at the time of consumption [2]. The survival ability of probiotics in food samples depends on factors such as pH, temperature, storage time (refrigeration), synbiotic compounds like soy, barley, and almond milk, and the presence of competing and inhibiting microorganisms [15].

Lactobacillus bacteria cause an increase in acidity and a decrease in pH over time, as well as the production of compounds such as hydrogen peroxide during storage, which can inhibit growth and viability [16]. In the study conducted by Parks et al., (1967), the viability of Lactobacillus significantly decreased during the last week of a 28-day storage period. The reason for this decrease was attributed to the intensified inhibitory effect of the organic acids produced in the product [17]. According to conducted studies, the inhibitory effect of organic acids depends on factors such as pH, type, and concentration of the acid, bacterial strain, and bacterial growth phase. The results of the study by Lind et al. (2005) showed that propionic acid has a stronger inhibitory effect compared to lactic acid and acetic acid. Moreover, the inhibitory effect of organic acids increases significantly with a decrease in pH [18].

Table 2 The effect of different concentrations of soy, barley and almond milk on changes in the viability (log cfu/ml) of yogurt probiotic bacteria during storage time

Yogurt sample	day			
	0	7	14	21
T0	8.00 ± 0.00 aD	8.34 ± 0.10 aC	7.36 ± 0.22 aB	6.66 ± 0.31 aA
T1	8.00 ± 0.00 aA	8.46 ± 0.05 abC	8.68 ± 0.11 bcC	8.22 ± 0.19 bcB
T2	8.00 ± 0.00 aA	8.87 ± 0.11 cdC	8.91 ± 0.12 bdC	8.60 ± 0.14 cdB
T3	8.00 ± 0.00 aA	8.83 ± 0.22 cdB	9.12 ± 0.10 dC	8.90 ± 0.12 eBC
T4	8.00 ± 0.00 aA	8.81 ± 0.11 cdC	8.66 ± 0.10 bcBC	8.51 ± 0.00 cdB
T5	8.00 ± 0.00 aA	9.04 ± 0.08 deC	9.11 ± 0.13 dC	8.78 ± 0.08 deB
T6	8.00 ± 0.00 aA	9.15 ± 0.01 eB	9.65 ± 0.28 eC	9.36 ± 0.21 fBC
T7	8.00 ± 0.00 aA	8.51 ± 0.33 abB	8.46 ± 0.02 bB	8.12 ± 0.09 bA
T8	8.00 ± 0.00 aA	8.38 ± 0.13 aB	8.72 ± 0.17 bcC	8.19 ± 0.04 bcAB
T9	8.00 ± 0.00 aA	8.72 ± 0.04 bcB	9.02 ± 0.10 dC	8.56 ± 0.27 dB

* Lowercase letters in each column indicate a significant difference of p <0.05 and uppercase letters in each row indicate a significant difference of p <0.05.

* T0 blank sample, T1 Yogurt with 25% soy milk, T2 yogurt with 50% soy milk, T3 yogurt with 75% soy milk, T4 yogurt with 25% barely milk, T5 yogurt with 50% barely milk, T6 yogurt with 75% barely milk, T7 yogurt with 25% almond milk, T8 yogurt with 50% almond milk, T9 yogurt with 75% almond milk

3-2. pH of Yogurt

Based on the statistical results obtained at a 95% confidence level (Table 2-4), the effect of different concentrations of soy, barley, and almond milk on the pH changes of yogurt samples was significant ($p < 0.05$). According to Table 3, the pH of the samples decreased over time, after 3 weeks of storage, the probiotic yogurt sample containing 75% barley milk and 25% soy milk (T6) and the control sample (T0) showed the lowest and highest pH values, respectively. The storage time of the probiotic yogurt samples also had a significant effect on the pH changes ($p < 0.05$), with a noticeable decrease in pH after 21 days (Table 3). In general, the results showed that the parameters of storage time and different concentrations of soy, barley, and almond milk were significant factors affecting the pH changes, with the pH of the samples decreasing significantly with higher concentrations of these milks compared to lower concentrations and the control sample.

Table 3 The effect of different concentrations of soy, barley and almond milk on changes in the pH of yogurt probiotic bacteria during storage time

Yogurt sample	day			
	0	7	14	21
T0	6.76 ± 0.01 cdC	6.45 ± 0.08 fB	5.93 ± 0.05 gA	5.85 ± 0.04 fA
T1	6.74 ± 0.02 bcD	6.42 ± 0.02 efC	5.83 ± 0.09 fB	5.43 ± 0.03 dA
T2	6.77 ± 0.01 dD	6.33 ± 0.08 deC	5.71 ± 0.06 deB	5.32 ± 0.04 cA
T3	6.72 ± 0.01 abD	6.22 ± 0.02 cC	5.60 ± 0.06 cB	5.12 ± 0.05 bA
T4	6.71 ± 0.02 aD	6.26 ± 0.11 cdC	5.67 ± 0.03 cdB	5.43 ± 0.04 dA
T5	6.73 ± 0.01 abD	6.12 ± 0.05 abC	5.45 ± 0.02 bB	5.09 ± 0.07 bA
T6	6.72 ± 0.01 abD	6.07 ± 0.02 aC	5.27 ± 0.04 aB	4.82 ± 0.05 aA
T7	6.71 ± 0.01 aD	6.30 ± 0.06 cdC	5.80 ± 0.02 edB	5.68 ± 0.05 eA

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T8	6.71 ± 0.00 aD	6.21 ± 0.01 bcC	5.71 ± 0.04 edB	5.61 ± 0.08 eA
T9	6.71 ± 0.00 aD	6.21 ± 0.06 bcC	5.64 ± 0.04 cdB	5.26 ± 0.05 cA

* Lowercase letters in each column indicate a significant difference of p <0.05 and uppercase letters in each row indicate a significant difference of p <0.05.

* T0 blank sample, T1 Yogurt with 25% soy milk, T2 yogurt with 50% soy milk, T3 yogurt with 75% soy milk, T4 yogurt with 25% barely milk, T5 yogurt with 50% barely milk, T6 yogurt with 75% barely milk, T7 yogurt with 25% almond milk, T8 yogurt with 50% almond milk, T9 yogurt with 75% almond milk

In this study, the results showed that the pH of all samples decreased significantly over the storage period (Table 3). The pH drop and the increase in acidity during storage could be the result of enzymes produced by the starters during fermentation. This decrease in pH and the increase in acidity have been attributed to acidification during storage due to the beta-galactosidase enzyme, which remains active at temperatures of 0-5°C [19]. The reduction in pH and increase in acidity over the 21 days of storage may also be due to autolysis (self-degradation) of some of the previously killed probiotic cells due to unsuitable environmental conditions or storage (Afzaal, 2022) [20]. Sohrabvandi et al. (2013) reported that this self-degradation of probiotic bacteria leads to cell disruption and the release of amino acids, peptides, and proteins into the medium, which ultimately results in a decrease in pH and an increase in acidity. The variation in the amount of lactic acid produced by lactic acid bacteria depends on their ability to ferment sugars [21].

Soy, oat, and almond milks contain compounds enhancing the activity of microorganisms and their enzymes. It is likely that the enzymes present in the yogurt, as well as some enzymes produced by probiotics, especially during the early storage period, lead to the breakdown of proteins and carbohydrates. As a result, organic acids (particularly lactic acid) are produced [22]. In a similar study, Lollo et al. (2013) examined the effect of probiotics and prebiotic compounds, such as lycopene, on probiotic dairy beverages. The results, consistent with the current study, showed that with the increase in these compounds, the viability of probiotics significantly increased compared to the control sample. Furthermore, as the percentage of prebiotic compounds increased, the pH decreased, and acidity increased during storage [23].

3-3. Total Soluble Solids (Brix) of Yogurt

As shown in Table 4, the Brix value of the samples decreased over time, such that after 3 weeks of storage, the probiotic yogurt sample containing 25% almond milk and 75% regular milk (T6) and the control sample (probiotic yogurt without soy, oat, and almond milk) (T0) showed the highest and lowest Brix values, respectively. The storage time of the probiotic yogurt samples also significantly affected the changes in Brix ($p<0.05$), and after 21 days of storage, a significant decrease in Brix was observed.

Table 4 The effect of different concentrations of soy, barley and almond milk on changes in the brix of probiotic bacteria of yogurt during storage time

Yogurt sample	day			
	0	7	14	21
T0	18.29 ± 0.10 aC	17.11 ± 0.09 aB	16.61 ± 0.32 aA	16.54 ± 0.17 aA
T1	22.31 ± 0.15 cC	22.06 ± 0.06 cC	20.56 ± 0.73 deB	19.05 ± 0.05 fA
T2	24.36 ± 0.32 dC	23.86 ± 0.33 dC	20.43 ± 0.50 cB	18.36 ± 0.10 deA
T3	26.90 ± 0.09 fC	26.62 ± 0.20 fC	22.47 ± 0.10 fB	18.15 ± 0.24 dA

T4	24.71 ± 0.80 [dC]	24.01 ± 0.00 [dC]	20.20 ± 0.10 [cdB]	18.55 ± 0.18 [eA]
T5	27.71 ± 0.22 [gD]	27.19 ± 0.22 [gC]	24.08 ± 0.11 [gB]	17.72 ± 0.24 [cA]
T6	30.08 ± 0.47 [hC]	29.68 ± 0.58 [hC]	25.19 ± 0.20 [hB]	17.02 ± 0.25 [bA]
T7	21.40 ± 0.05 [bB]	21.04 ± 0.05 [bB]	19.37 ± 0.42 [bA]	19.51 ± 0.24 [gA]
T8	22.45 ± 0.01 [cC]	22.17 ± 0.42 [cC]	19.66 ± 0.57 [bcB]	18.98 ± 0.02 [fA]
T9	25.50 ± 0.50 [eC]	6.21 ± 0.06 [eC]	21.16 ± 0.24 [eB]	18.98 ± 0.02 [fA]

* Lowercase letters in each column indicate a significant difference of p <0.05 and uppercase letters in each row indicate a significant difference of p <0.05.

* T0 blank sample, T1 Yogurt with 25% soy milk, T2 yogurt with 50% soy milk, T3 yogurt with 75% soy milk, T4 yogurt with 25% barely milk, T5 yogurt with 50% barely milk, T6 yogurt with 75% barely milk, T7 yogurt with 25% almond milk, T8 yogurt with 50% almond milk, T9 yogurt with 75% almond milk

The addition of bacteria to yogurt samples containing different concentrations of soy, oat, and almond milks resulted in a reduction in Brix across all treatments, with the reduction being greater in yogurt samples with higher concentrations of soy, oat, and almond milks. Since Brix refers to soluble solids in water, and the sugars in yogurt water are part of Brix, fermentation by the bacteria converts sugars into organic acids (lactic acid) and volatile compounds, leading to a decrease in sugar levels and, ultimately, a reduction in Brix. Therefore, the reduction in Brix can largely be attributed to the consumption of sugar by the probiotics that are freely present in the yogurt [14]. Lupien-Meilleura et al. (2016) studied the effect of probiotics on the characteristics of dairy beverages during storage at refrigeration temperatures. The results of this study were consistent with the current study, showing that the level of soluble solids decreased during storage [24]. Shah et al. (2010) reported that the Brix value of various dairy beverage models containing *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Bifidobacterium lactis* decreased from 11.8g to 9.1g per 100g after 6 weeks of storage at 4°C, aligning with the findings of this study [25]. Besides, Shoria et al. (2022) studied the viability of probiotics and antioxidant activity in cashew-milk-based yogurt fermented with Lactobacillus probiotics. Their results indicated that the addition of probiotics to yogurt significantly reduced the percentage of soluble solids compared to the control sample [6].

4-4. Viscosity of Yogurt

According to the statistical results obtained at a 95% confidence level (Table 5), the effect of different concentrations of soy, oat, and almond milk on the viscosity changes of yogurt samples was significant ($p<0.05$). The viscosity of the samples increased over time, such that after 3 weeks of storage, the probiotic yogurt sample containing 75% oat milk and 25% regular milk (T6) showed the highest viscosity. The viscosity in the control sample and the yogurt samples with lower concentrations of soy, oat, and almond milk was significantly lower than the other samples. The storage time of the probiotic yogurt samples also significantly affected the changes in viscosity ($p<0.05$), and after 21 days of storage, the viscosity showed a significant increase.

The viscosity test results indicated that the samples containing soy, oat, and almond milks at various concentrations had higher viscosity than the control sample throughout the storage period. This increase in viscosity could be directly related to the concentration of soy, oat, and almond milks, as these compounds bind free water present in the sample, leading to an increase in viscosity

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[26]. On the other hand, it can be stated that the addition of soy, oat, and almond milk could lead to the formation of dense and compact networks, which prevent movement and involvement of the dispersed phase in the suspension phase. As a result, this increases viscosity and reduces syneresis. Fan et al. (2022) examined the effects of several plant-based additives, such as oat milk, on the quality characteristics of yogurt. Their results showed that the addition of oat milk created a more cohesive network in the yogurt, leading to an increase in yogurt viscosity [27]. In addition, Rashidi et al. (2022) studied the use of soymilk in the production of functional cottage cheese powder. Their results indicated that an increase in soymilk led to higher viscosity and better consistency in the reconstituted cottage cheese [28]. In this context, Cheng et al. (2017) stated that when whey proteins join, the amount of whey proteins at the surface of casein micelles increases, which leads to greater cohesion of the casein micelles, network formation, and improved texture of the yogurt. Moreover, in all treatments studied, the viscosity factor significantly increased over time. This could be influenced by the gel-strengthening phenomenon, which is associated with the storage of samples at low temperatures [29].

Table 5 The effect of different concentrations of soy, barley and almond milk on changes in the viscosity(µpascal/sec) of probiotic bacteria of yogurt during storage time

Yogurt sample	day			
	0	7	14	21
T0	1500.22 ± 0.20 ^{aA}	1501.79 ± 0.48 ^{aB}	1505.12 ± 0.34 ^{aC}	1506.93 ± 0.69 ^{abD}
T1	1503.58 ± 0.52 ^{cdA}	1504.92 ± 0.68 ^{bB}	1506.05 ± 0.07 ^{abC}	1507.68 ± 0.39 ^{bcD}
T2	1503.45 ± 0.56 ^{cdA}	1505.42 ± 0.62 ^{bB}	1507.26 ± 0.16 ^{cdC}	1508.91 ± 0.39 ^{deA}
T3	1504.41 ± 0.69 ^{deA}	1506.43 ± 0.84 ^{bcB}	1508.46 ± 0.47 ^{edC}	1510.96 ± 0.80 ^{gD}
T4	1502.77 ± 0.65 ^{bcA}	1507.19 ± 0.22 ^{cdB}	1509.11 ± 0.16 ^{fC}	1508.32 ± 0.70 ^{cdBC}
T5	1505.23 ± 0.23 ^{efA}	1507.73 ± 0.92 ^{cdB}	1508.33 ± 0.70 ^{defB}	1510.22 ± 0.19 ^{fgC}
T6	1507.01 ± 0.38 ^{gA}	1508.77 ± 0.14 ^{dA}	1509.41 ± 0.12 ^{fB}	1512.82 ± 0.72 ^{hC}
T7	1502.24 ± 0.17 ^{bA}	1506.49 ± 0.87 ^{bcB}	1506.41 ± 0.50 ^{bcB}	1506.11 ± 0.10 ^{aB}
T8	1504.23 ± 0.28 ^{dA}	1506.60 ± 0.39 ^{bcB}	1507.46 ± 0.67 ^{cdeB}	1509.38 ± 0.55 ^{efC}
T9	1505.90 ± 0.97 ^{fA}	1508.15 ± 0.25 ^{cdB}	1509.66 ± 0.90 ^{fB}	1510.30 ± 0.24 ^{fgC}

* Lowercase letters in each column indicate a significant difference of $p < 0.05$ and uppercase letters in each row indicate a significant difference of $p < 0.05$.

* T0 blank sample, T1 Yogurt with 25% soy milk, T2 yogurt with 50% soy milk, T3 yogurt with 75% soy milk, T4 yogurt with 25% barely milk, T5 yogurt with 50% barely milk, T6 yogurt with 75% barely milk, T7 yogurt with 25% almond milk, T8 yogurt with 50% almond milk, T9 yogurt with 75% almond milk

3-5. Syneresis (Whey Separation) of Yogurt

According to Table 6, the syneresis of the samples increased over time, such that after 3 weeks of storage, the probiotic yogurt sample containing 75% oat milk and 25% regular milk (T6) and the probiotic yogurt sample without soy, oat, and almond milk (T0) showed the lowest and highest syneresis, respectively. The storage time of the probiotic yogurt samples also significantly affected the changes in syneresis ($p<0.05$), and after 21 days of storage, there was a significant increase in syneresis.

The addition of soy, oat, and almond milk at different concentrations individually reduced syneresis in the probiotic yogurt treatments compared to the control treatment. This can be explained by the formation of strong connections between the added compounds (soy, oat, and almond milk) and the yogurt gel network [30]. In similar studies, researchers demonstrated that the use of additive compounds, such as the ones studied in this research, increases the water-binding capacity in yogurt curds, which leads to a reduction in syneresis [31]. Furthermore, studies have shown that an increase in the denaturation of whey proteins improves the water-holding capacity, thus reducing syneresis [32]. It has also been shown that the denaturation of beta-lactoglobulin and its interaction with casein micelles significantly affects the gel properties in fermented milk [33].

The syneresis in all of the treatments produced in this study was significantly lower over time compared to the control sample. This is due to the formation of the gel network and the rapid water absorption by the soy, oat, and almond milks, which, through the bonding between the protein molecules in the milk over time, results in reduced syneresis and improved water retention in the yogurt. Moreover, soy, oat, and almond milks help in gel formation, further reducing syneresis [33].

Table 6 The effect of different concentrations of soy, barley and almond milk on changes in the percent of syneresis of probiotic bacteria of yogurt during storage time

Yogurt sample	day			
	0	7	14	21
T0	62.73 ± 0.46 aA	62.04 ± 0.43 cA	69.19 ± 0.91 dB	78.07 ± 0.79 eC
T1	61.38 ± 0.11 aA	61.61 ± 0.20 abcA	66.19 ± 0.05 bcB	74.36 ± 0.37 dB
T2	61.20 ± 0.08 aA	61.49 ± 0.24 abB	65.95 ± 0.10 abcC	72.87 ± 0.70 cD
T3	60.18 ± 0.05 aA	61.68 ± 0.20 abcB	65.06 ± 0.06 abC	71.15 ± 0.06 bD
T4	61.17 ± 0.06 aA	61.61 ± 0.31 abcA	65.96 ± 0.23 abC	73.15 ± 0.25 cC
T5	61.24 ± 0.00 aA	61.68 ± 0.21 abcA	65.66 ± 0.35 abB	71.84 ± 0.72 bC
T6	59.48 ± 0.37 aA	61.30 ± 0.05 aB	64.97 ± 0.85 aC	69.47 ± 0.35 aD
T7	61.60 ± 0.14 aA	61.80 ± 0.05 abC	70.05 ± 0.14 dB	74.61 ± 0.19 dC
T8	61.66 ± 0.23 aA	61.75 ± 0.26 abcA	66.87 ± 0.08 cB	72.83 ± 0.32 cC
T9	61.47 ± 0.24 aA	61.55 ± 0.16 abA	65.81 ± 0.72 abcB	71.53 ± 0.79 bC

* Lowercase letters in each column indicate a significant difference of p <0.05 and uppercase letters in each row indicate a significant difference of p <0.05.

* T0 blank sample, T1 Yogurt with 25% soy milk, T2 yogurt with 50% soy milk, T3 yogurt with 75% soy milk, T4 yogurt with 25% barely milk, T5 yogurt with 50% barely milk, T6 yogurt with 75% barely milk, T7 yogurt with 25% almond milk, T8 yogurt with 50% almond milk, T9 yogurt with 75% almond milk

3-6. Sensory Properties of Yogurt

The effect of different concentrations of soy, oat, and almond milk on the sensory attributes (aroma, taste, color, and overall acceptance) of the yogurt samples was not significant (p>0.05). In this study, the probiotic yogurt samples containing different concentrations of soy, oat, and almond milk were comparable to, or even scored higher than, the control yogurt sample in terms of color, taste, and aroma (Table 7). The yogurt sample (probiotic yogurt containing 75% soymilk and 25% regular milk) scored the

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lowest for aroma, sample T8 (probiotic yogurt containing 50% almond milk and 50% regular milk) scored the lowest for color, and sample T4 (probiotic yogurt containing 25% oat milk and 75% regular milk) scored the lowest for taste. Finally, there was no significant difference in overall acceptance between the yogurt samples with different concentrations of soy, oat, and almond milk and the control yogurt sample.

Sensory evaluation of probiotic products has always been one of the most important tests. In this study, the probiotic yogurt produced was tested by judges for aroma, color, taste, and overall acceptance. The results indicated that the probiotic yogurt samples made with soy, oat, and almond milk were accepted and deemed acceptable by the judges in terms of the sensory characteristics evaluated. In a study by Shoria et al. (2022), the survival rate of probiotics and antioxidant activity of cashew milk-based yogurt fermented with Lactobacillus probiotic strains were investigated. They reported that the produced samples showed no significant difference compared to the control sample [6].

Table 7 The effect of different concentrations of soy, barley and almond milk on changes in the percent of syneresis of probiotic bacteria of yogurt during storage time

Yogurt sample	color	taste	smell	Overall acceptance
T0	4.40 ± 0.51 e	4.40 ± 0.47 bc	4.00 ± 0.47 bc	4.20 ± 0.42 a
T1	4.00 ± 0.47 bcde	4.90 ± 0.31 e	4.00 ± 0.47 bc	4.40 ± 0.51 a
T2	3.44 ± 0.69 ab	4.66 ± 0.48 de	4.00 ± 0.81 bc	4.22 ± 0.42 a
T3	3.60 ± 0.84 abc	4.60 ± 0.51 de	3.70 ± 0.67 a	4.10 ± 0.31 a
T4	4.30 ± 0.48 de	3.30 ± 0.82 a	4.60 ± 0.51 c	4.00 ± 0.66 a
T5	4.10 ± 0.56 cde	4.50 ± 0.70 de	4.40 ± 0.51 c	4.20 ± 0.42 a
T6	4.10 ± 0.56 cde	3.60 ± 0.69 bc	4.20 ± 0.63 bc	4.50 ± 0.70 a
T7	3.80 ± 0.63 abcd	4.30 ± 0.48 de	4.10 ± 0.31 bc	4.50 ± 0.52 a
T8	3.30 ± 0.48 a	4.10 ± 0.56 cd	4.20 ± 0.32 bc	4.00 ± 0.47 a
T9	3.80 ± 0.63 abcd	3.90 ± 0.31 cd	4.60 ± 0.16 c	4.30 ± 0.48 a

* Lowercase letters in each column indicate a significant difference of $p < 0.05$

* T0 blank sample, T1 Yogurt with 25% soy milk, T2 yogurt with 50% soy milk, T3 yogurt with 75% soy milk, T4 yogurt with 25% barely milk, T5 yogurt with 50% barely milk, T6 yogurt with 75% barely milk, T7 yogurt with 25% almond milk, T8 yogurt with 50% almond milk, T9 yogurt with 75% almond milk

4- Conclusion

Since dairy products contain beneficial substances like minerals, antioxidants, dietary fibers, and vitamins, they can provide a suitable environment for the production of probiotic dairy products. However, the survival capability of probiotics in dairy-based foods is influenced by factors such as the presence of antimicrobial compounds and essential nutrients required for their growth, creating an optimal environment for them. In this study, compounds such as soy, oat, and almond milk were added to functional yogurt, improving its physicochemical and sensory properties.

Fortification of probiotic yogurt with soy, oat, and almond milk at different concentrations, as well as storage time, influenced the survival of probiotics. The results showed that increasing the

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concentration of soy, oat, and almond milk led to an increase in the survival rate of probiotics, and this trend continued over time. As the concentration of soy, oat, and almond milk increased, a significant decrease in pH and an increase in acidity were observed. The Brix index in yogurt samples decreased with increased storage time, but this decreasing trend was less pronounced in the samples containing higher concentrations of soy, oat, and almond milk. Furthermore, the storage time had a significant effect on the viscosity index of the yogurt. Adding different concentrations of soy, oat, and almond milk resulted in higher viscosity. On the other hand, the syneresis in the samples enriched with soy, oat, and almond milk showed a significant reduction, although syneresis was observed in all yogurt samples over the storage period. Sensory acceptance results showed no significant difference among all the samples. Overall, the results of this study demonstrated that adding compounds such as soy, oat, and almond milk could improve the physicochemical and sensory properties of probiotic yogurt. In this study, the sample containing oat milk (T6) (probiotic yogurt with 75% oat milk and 25% regular milk) exhibited higher quality in all parameters.

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بررسی تاثیر افزودن شیر سویا، جو و بادام بر ویژگی‌های فیزیکوشیمیایی و حسی ماست پروبیوتیکی

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چکیده

پروبیوتیک‌ها باکتری‌های مفیدی هستند که می‌توانند به مواد غذایی به خصوص فرآورده‌های لبنی اضافه شوند و اثرات سلامت بخشی خود را در بدن ایجاد کنند. در این مطالعه از دو باکتری پروبیوتیکی لاکتوباسیلوس اسیدوفیلوس و لاکتوباسیلوس پلانتارم به میزان ۱۰۸ cfu/ml در ماست همراه با شیر سویا، جو و بادام در سه غلظت (۷۵:۲۵، ۵۰:۵۰، ۲۵:۷۵ نسبت شیر/شیر سویا، جو و بادام) استفاده شد و به مدت ۲۱ روز مورد بررسی قرار گرفت. در روزهای ۰، ۷، ۱۴ و ۲۱ نمونه‌های ماست تحت آزمون‌های تعیین قابلیت زنده‌مانی، pH، اسیدیته، بریکس، ویسکوزیته، آب‌اندازی و آزمون‌های حسی قرار گرفتند. نتایج نشان داد که باکتری‌های پروبیوتیک در طی نگهداری در ابتدا روندی صعودی و سپس نزولی را طی نموده‌اند. نمونه ماست های حاوی شیر سویا، بادام و جو مقدار کاهش کمتری در میزان باکتری پروبیوتیک داشتند و در پایان ۲۱ روز حد قابل قبولی از زنده مانی باکتری را نشان دادند. روند کاهش pH و تولید اسید توسط این باکتری ها در ماست ها طی زمان مشاهده شد و این روند در نمونه های حاوی شیر سویا، جو و بادام بیشتر بود. آب اندازی در تمامی نمونه ها روند افزایشی در طول زمان نشان داد ولی ماست های حاوی شیر سویا، بادام و جو آب اندازی کمتری داشتند. درصد مواد جامد محلول تمامی نمونه‌ها در طی نگهداری روندی کاهش را نشان داد ولی در نمونه ماست های حاوی شیر سویا، جو و بادام این روند کندتر صورت گرفت. ویسکوزیته محصولات تولید شده در طی زمان نگهداری روندی صعودی را نشان دادند و نمونه های حاوی شیر سویا، جو و بادام، ویسکوزیته بالاتری داشتند. مقبولیت کلی در تمام نمونه های ماست با نمونه شاهد تفاوت معنی داری نداشت. در نهایت ماست حاوی ۲۵ درصد شیر و ۷۵ درصد شیر جو از نظر ویژگی‌های فیزیکوشیمیایی نسبت به سایر تیمارها کیفیت بهتری برخوردار بود.