Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

DETERMINATION OF TEXTURE PROFILE ANALYSIS AND MICROBIOLOGICAL EVALUATION OF YOGURT PRODUCED BY COMMERCIAL AND HEIRLOOM CULTURE

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ARTICLE INFO

Article History:

Received: 2024/7/8
Accepted:2024/7/31

Keywords:

Homemade,

Industrial,

Yogurt,

Texture,

fermentation culture

DOI: **10.22034/FSCT.21.155.137.**

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ABSTRACT

Yogurt, a widely consumed fermented milk product, is known for its health benefits attributed to the presence of probiotic bacteria. This study explores the impact of starter cultures on the texture and microbiological quality of yogurt. The production involved two types of starter cultures: a commercial culture and an heirloom culture. Various parameters, including chemical analysis of raw milk, yogurt production steps, texture parameter analysis, syneresis, water holding capacity, and microbial analysis, were examined. Statistical analysis was performed to indicate the effect of the type of culture on the production of yogurt. Results indicated that the physicochemical analysis of raw cow milk revealed parameters within recommended quality standards. Significant differences in hardness, gumminess, chewiness, resilience, and syneresis between commercial and heirloom yogurts were found. Microbiological analysis demonstrated higher Lactic acid bacteria counts in commercial yogurt compared to heirloom yogurt. The study provides insights into the influence of starter cultures on textural and microbiological characteristics, emphasizing the importance of standardized production methods for consistent yogurt quality.

1. Introduction

Milk products are the most consumed fermented foods in the world. Yogurt is defined as a semisolid fermented milk product with a special taste made from the activity of the symbiotic mixture of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*, which convert milk sugar (lactose) into lactic acid and acetaldehyde which increases the acidity of the yogurt, that way forming a gel and causing it to have a sour taste [1] [2] [3]. This process is anaerobic, meaning that it is processed in the absence of oxygen. Furthermore, acidity formation inhibits the growth of spoilage and pathogenic bacteria, and it contributes to the texture, flavor, and composition of yogurt [3]. Yogurt promotes a healthy lifestyle by lowering the risk of colon cancer, enhancing digestion, and offering a host of other advantages attributed to the presence of antimicrobial compounds including lactic acid and bacteriocins, low levels of cholesterol and lactose. In addition, yogurt is also a good source of protein, carbohydrates, vitamins, and minerals (including calcium) [4]. However, yogurt is also readily perishable due to its great susceptibility to bacterial contamination [5]. Yogurt's structural alterations are mostly caused by the proteolytic activity of bacteria and the denaturation of whey proteins as are β-lactoglobulins and α-lactalbumins, during heat treatment of milk before fermentation. Early detection of insufficient processing, packaging, and refrigeration via microbiological techniques reduces the risk to public health. Thus, microbiological assessments of yogurt are required by monitoring the microbiological quality of raw milk, bulk milk, and the final milk products after production and during storage. On the other hand, rheology is the study of the flow and deformation of matter. In the context of yogurt, it involves understanding how the yogurt flows and deforms under different conditions. Knowledge of yogurt's rheological properties is essential in the processing and handling stages of production. This includes the steps from milk processing to fermentation and post-fermentation processing. Yogurt exhibits non-Newtonian flow properties with strong time dependence, specifically thixotropic (changes in viscosity over time) and viscoelastic (combination of viscous and elastic behaviors) types [6]. Understanding these properties is critical for designing effective processing methods. Physical attributes such as lack of syneresis (separation of whey), perceived viscosity, acidity, aroma perceptions, and textural properties are important for consumer acceptance. Monitoring and controlling rheological properties during production help ensure consistency and quality in the final product.

Therefore, the aim of this study was the production of yogurt by using two types of starter cultures including commercial starter culture and heirloom culture, and the examination and comparison of the texture profile analysis (TPA) and microbiological analysis for two products.

2- MATERIALS AND METHODS

2.1. Chemical analysis of raw milk

The samples of milk were mixed and analyzed in ECOMILK for fat, density, proteins, dry matter, and freezing point of milk in triplicate. The pH values were measured using a digital laboratory pH meter.

2.2. Yogurt production

Fresh Cow's milk purchased from the local market (milk fat %, protein %, TS %, and acidity SH) was used for yogurt production. Yogurt starter cultures TREDIMIX K 5U (*L. bulgaricus and S. thermophilus*) were purchased from the BIOKOM company in Bulgaria, whereas heirloom culture was purchased from a nearby farm that has been preserved and used for generations.

The samples of yogurt were produced according to the article by Kose et al. 2018 [7] with few modifications in triplicate. Here 1L of raw milk was heated to 92°C for 2-3 min for pasteurization and then allowed to cool to 47 °C before inoculation with starter culture (8 g/100 L yogurt bacteria mixture *Lactobacillus bulgaricus* and *Streptococcus thermophiles*, (based on the guidelines of the producer) for commercial yogurt and yogurt (1-2 % yogurt, 48 hours after production) for hairloom yogurt

[DOI: 10.22034/FSCT.21.155.137]

[8] [9]. Then, yogurts were incubated at 42.5 °C until a pH reached between 4.3-4.5. The yogurt samples were cooled at 4 °C in a refrigerator before they were analyzed. The flowchart of the yogurt production is shown below (Figure 1):

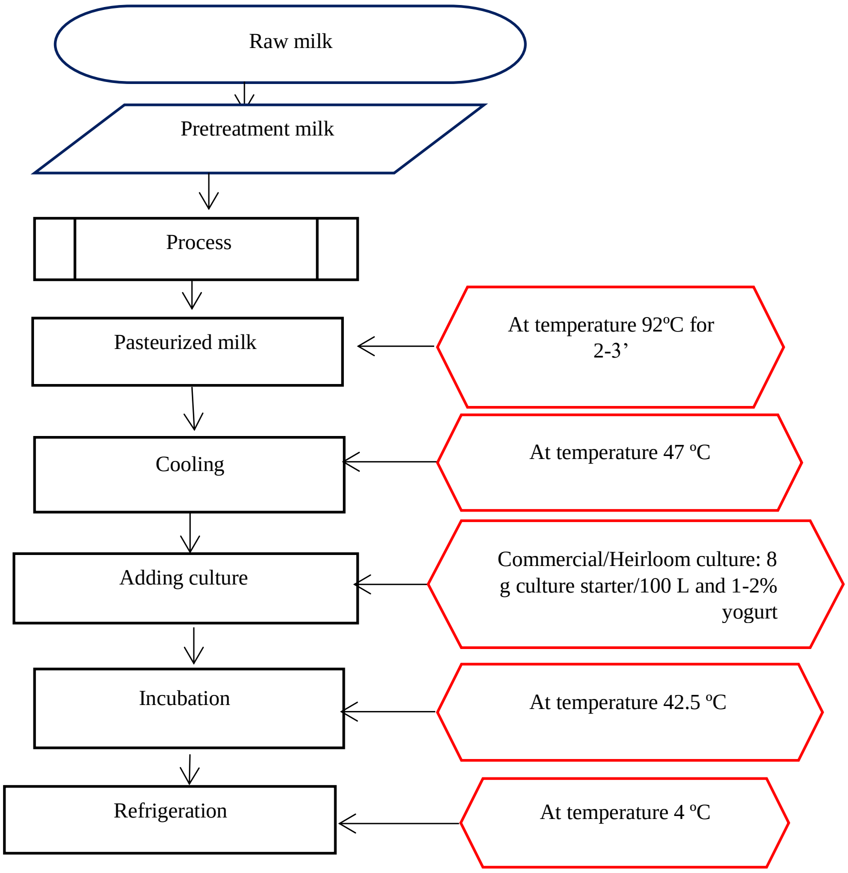

Figure 1. The flowchart of commercial and heirloom yogurt production

2.3. Texture profile analysis of yogurt (TPA)

Yogurts were analyzed for texture parameters. Texture Analyzer (TexturePro CT V1.8 Build 31) was used to measure texture parameters like hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness, and resilience. This method was performed with a slight modification of the method used previously [10] [11]. Textural properties were analyzed by performing two sequential compression tests with a cylindric-shaped probe with a diameter of 25 mm that moves to a depth of 7.5 mm. Samples were compressed up to 70% of their original length. Pre-test speed, test speed, and return test speed during textural analysis were 2, 1, and 1 millimeter per second, respectively. The analyzer was connected to a computer that documented data via a software program called test software testXpert® II. Samples were analyzed over time on days 1, 2, 3, and 6.

2.4. Syneresis

Syneresis was performed in triplicate. Yogurt samples were placed on a filter paper resting on the top of a funnel. After drainage, the quantity of whey collected in a 200 ml graduated cylinder was used as an index of syneresis [12][13]. Syneresis was calculated as follows [14]

Syneresis (%) = supernatant/yogurt *100%

2.5. Water Holding Capacity (WHC)

WHC was performed in triplicate. It was determined using the centrifugation method. WHC is a measure of the water capacity to retain in the yogurt and it could be considered as the opposite of syneresis [15]. WHC was calculated by using the following equation: [16] WHC (%) = (Weight of the native yogurt

[DOI: 10.22034/FSCT.21.155.137]

– Weight of the discarded whey)/Weight of the native yogurt *100

2.6. Microbial Analysis

Testing for Lactic acid bacteria (LAB), Yeast and Mold, and aerobic bacteria was performed for two types of yogurt using De Man–Rogosa–Sharpe agar (MRS) [2] [17] , Potato Dextrose agar (PDA) [5], and Plate Count Agar (PCA) agar [5], respectively. Enumeration of microorganisms was carried out by aseptically mixing a yogurt sample (1 ml) with 9 ml of buffered peptone water. The sample was thoroughly mixed and serial dilutions were performed using peptone water as the diluents. 25 ml melted (45 °C) MRS agar, PDA, and PCA were added in Petri dishes followed by inoculation with 200µl of diluted yogurt (10^3, 10^4, and 10^5) in triplicate for each dilution. The Petri dishes were covered and incubated anaerobically at 37 °C for 24-48 hours for LAB and aerobic bacteria whereas Petri dishes for yeast and mold were covered and incubated at 28 °C for 96 hours. The viable microbial count was calculated as follows:

Cfu/ml=cfu/plate x dilution factor

2.7. Statistical analysis

All tests were carried out in triplicates, and each value represents the mean of three readings; while the error bars represent the standard deviations. The results were analyzed statistically to determine the significant differences using a t-test. The null hypothesis, H0, is postulated as *“there is no significant difference between yogurt produced by commercial culture and heirloom culture”*, while the alternative hypothesis, H1, is postulated as *“there is a significant difference between yogurt produced by commercial culture and heirloom culture”*. Type 3 (different groups with different variance) t-tests were performed with a significance level α of 0,05. A changed variable is a type of starter culture used for yogurt production.

3- RESULTS

3.1. Physico-chemical analysis of raw milk

The physicochemical parameters of raw cow milk samples collected from the local market were performed in triplicate and are shown in Figure 2.

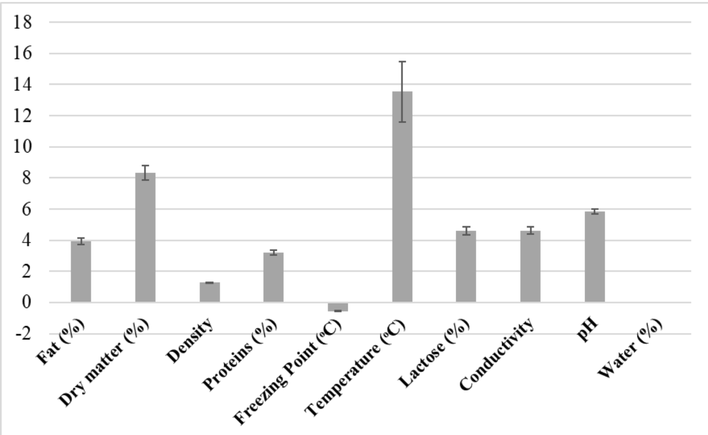

Figure 2. Physical-chemical parameters of raw cow milk

3.2. Texture profile analysis of yogurt

TPA parameters (hardness, springiness, adhesiveness cohesiveness, chewiness, gumminess, and resilience) of the yogurt samples were analyzed during the storage of two yogurts in triplicate on days 1, 2, 3, and 6 and are given in Tables 1 and 2. Utilizing the data presented in Table 3, a type 1 t-test was conducted to compare the syneresis, yield, and WHC capacity levels between commercial and heirloom yogurt.

Table 1. TPA parameters of commercial yogurt samples (±SD).

Texture parameters	**Commercial Yogurt**			
	Day 1	Day 2	Day 3	Day 6

[DOI: 10.22034/FSCT.21.155.137]

Hardness 1 (g)	107±9.5	117.67±8.0	118±10.39	114.67±5.5
Hardness2 (g)	93.3±4.5	100.67±8.62	105.33±9.81	99±7
Adhesiveness (mJ)	3.2±2.45	2.97±1.7	2.567±2.23	0.833±1.18
Springiness (mm)	42.9±6.68	50.17±9.77	47.747±9.96	44.44±9.06
Gumminess (g)	53.3±3.21	58±4	56±6.55	59.67±10.01
Chewiness (mJ)	22.27±2.32	28.4±3.67	26.03±5.05	27.57±5.67
Cohesiveness	0.5±0.017	0.43±0.015	0.48±0.02	0.52±0.09
Resilience	0.05±0.01	0.0367±0.01	0.043±0.015	0.053±0.0152
DaH (%)	50.26±4.21	58.28±7.68	58.92±7.74	59.04±6.82

Table 2. TPA parameters of Heirloom yogurt samples (±SD).

Texture parameters	**Heirloom Yogurt**			
	Day 1	Day 2	Day 3	Day 6
Hardness 1 (g)	90.3±12.7	99±9.89	95±6.55	91±7.81
Hardness2 (g)	70±8.7	79.5±4.94	75.3±3.5	77.67±8.62
Adhesiveness (mJ)	3.03±2.9	3.5±1.56	3.27±0.92	2.46±0.9
Springiness (mm)	38.82±1.64	45.195±10.14	47.49±7.35	44.98±7.18
Gumminess (g)	43.7±12.5	46±2.82	44.33±3.21	48.3±3.05
Chewiness (mJ)	16.83±5.57	20.1±3.25	20.57±1.74	21.13±2.49
Cohesiveness	0.47±0.066	0.46±0.014	0.467±0.0057	0.53±0.034
Resilience	0.047±0.0057	0.035±0.007	0.03±0.0057	0.04±0
DaH (%)	44.65±2.84	55.97±12.36	57.63±8.83	57.14±8.5

Table 3. Yield, syneresis, and WHC analysis of commercial and heirloom yogurt

	Commercial				**Heirloom**			
	Yogurt (g)	WHC (%)	Syneresis (%)	Yield (%)	Yogurt (g)	WHC %	Syneresis (%)	Yield (%)

[DOI: 10.22034/FSCT.21.155.137]

Average	514.486667	51.4487	43.3833333	66.4533	516.59	51.659	35.61	63.3294
Standard deviation	54.0205075	5.40205	3.65505586	9.29384	40.9320767	4.09321	2.23242021	5.80301

3.3. Microbial analysis

Figure 3 shows the results obtained after microbial examination of the commercial and heirloom samples. There was no evidence of aerobic bacteria and mold and yeasts growing in none of the yogurts showing there was no contamination in yogurt manufacture. On the other hand, LAB was present in yogurt samples (Figure 3) which were examined in a microscope also (Figure 4).

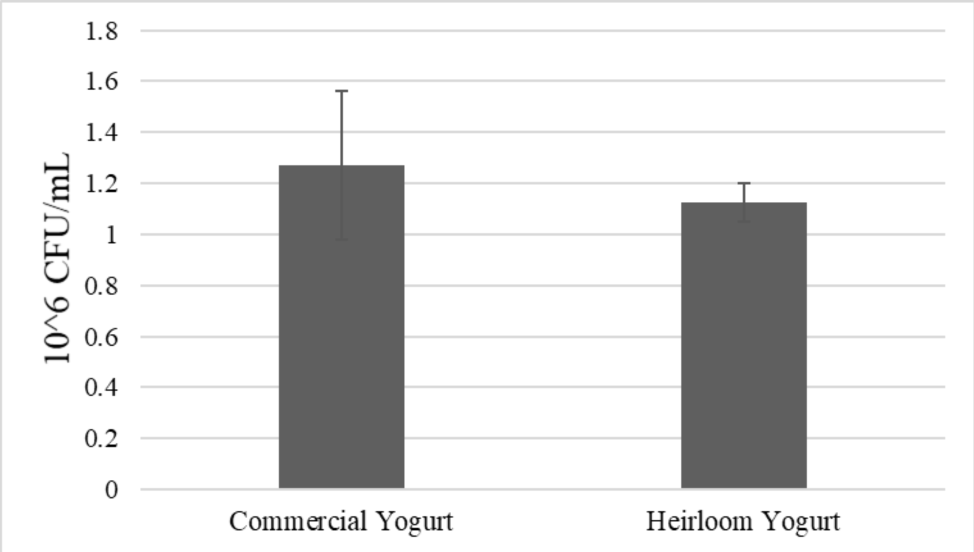

Figure 3. Viable count of the total number of LAB in commercial and heirloom yogurt

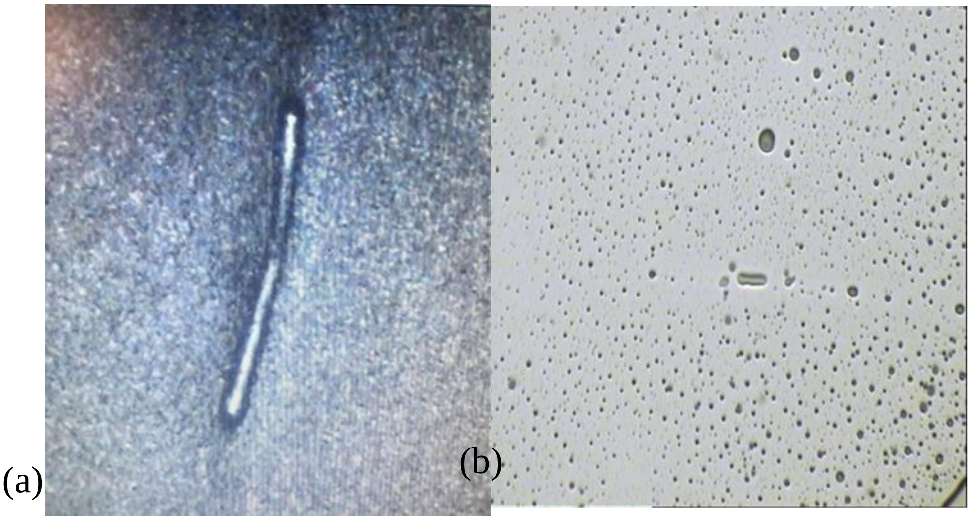

Figure 4. LAB found in yogurt sample (a) *Lactobacillus spp.*, (b) *Streptococcus thermophiles*

4-Discussion

Physico-chemical analysis is an important parameter to monitor the quality of milk and its product [18] [19]. As it shows the fat (%) was found in the range of 3.93±0.22, dry mater (%) was found at 8.33±0.46, density at 1.27g/cm^3±0.01, proteins (%) at 3.2±0.15, the freezing point at (-0.548°C±0.029), temperature (13.53°C ± 1.95), lactose (%)

[DOI: 10.22034/FSCT.21.155.137]

(4.6±0.24), conductivity (mS/cm) (4.62±0.23), and pH (5.84±0.13). Based on World Health Organization (WHO) standards and other scientific works, quality milk contains 1.02-1.03 g/cm^3 density, and our results show that all milk samples are within the required range of 1.02-1.03 g/cm^3. The pH values for all milk samples are below the permissible limit of WHO (pH=6.6) and they are slightly acidic. According to our results, the protein contents (3.37%) and lactose contents (4.86%) are slightly higher as compared with the permissible standards of WHO and other scientific works, which are 2.6% and 2.8%, respectively. The study showed that the average fat content for milk samples collected is 3.93 % which is good enough and above the minimum standard of WHO which is 3.5%. In general, all milk samples met the World Health Organization (WHO) and other national and international standards. However, there were exceptions in the lactose and protein content levels.

Texture profile analysis is crucial in understanding and controlling the quality of food products, including yogurt. The texture is influenced by factors such as milk composition, starter culture, homogenization conditions, incubation temperature, stabilizers, and storage conditions [5]. The mentioned texture parameters have been chosen to interpret meaningful results related to the type of culture used for yogurt production. Statistical analysis was performed for each texture parameter to check for any significant differences between the two types of yogurts. In general, yogurt obtained from heirloom culture is characterized by lower texture values than the yogurt induced from commercial culture.

The first texture parameter to be analyzed for statistical significance was hardness. Hardness (g) is defined as the peak force during the first compression cycle or the maximal force required for a given deformation. Decreased protein content in yogurt mix can decrease casein interactions and result in decreased hardness [10] [20]. Hardness at cycles 1 and 2 refers to the measurement taken at different stages inside the mouth during the consumption of the product [16]. The hardness of commercial yogurt increased systematically in two cycles reaching the value of 118±10.39 g and 105.33±9.81 g in cycles 1 and 2 respectively, whereas the hardness of Heirloom yogurt was found to be lower in two cycles, reaching the value of 95±6.55 g and 75.3±3.5 g at day 3. This parameter was analyzed in two cycles, as expected for both cycles null hypothesis was rejected indicating that in both cycles hardness has a significant difference between the two types of yogurts with a p-value of 0.000597 for cycle one and 0.000704 for cycle two. When the hardness values are compared with the literature, our results were similar to the values determined by Wen et al. 2014 [21] and higher to the values determined by Helal et al. 2018 [22].

Adhesiveness (mJ) is the force of attraction between the food and a solid in contact with it. It is important to show the stickiness of the food and the force required to remove material adhering to the mouth during eating. Adhesiveness reflects the association with the probe surface [10] [20] [22]. The adhesiveness value is reported in Tables 2 and 3 and the effect of type of culture in yogurt production was very limited and the differences were not significant between commercial yogurt and heirloom yogurt (2.567±2.23 mJ at day 3 and 3.27±0.92 mJ at day 3, respectively). The p-value calculated with the results of adhesiveness was 0.083 which is higher than α-0.05 meaning that we cannot reject the null hypothesis and that there is no significant difference in adhesiveness. Vieira et al. 2019 reported a value of adhesiveness (mJ) between 4.0 and 8.0 that are slightly higher than the adhesiveness values of yogurt samples reached in this study [14].

Springiness (mm) represents the recovery ability of the sample against the first deformation. It is important for product quality during transportation and storage [10] [20]. Springiness values of the commercial yogurt samples were found to be 47.747±9.96 at day 3 whereas for heirloom yogurt samples were found to be 47.49±7.35 at day 3. T-test performed in springiness data resulted in a p-value of 0.1038, failing to reject the null hypothesis signifying no differences between the two types of yogurts. Springiness values for two types of yogurt were found to be higher compared with the values of springiness found by Ragab et al. 2019 [23] and Helal et al. 2018 [22].

Gumminess (g) is defined as the product of hardness and cohesiveness [10] [20]. High hardness in yogurt results in high gumminess [23] which is in line with the results shown in Table 1. Gumminess values of commercial yogurt samples ranged between 53.3±3.21 g - 59.67±10.01 g whereas gumminess values of heirloom yogurt ranged between 43.7±12.5 g - 48.3±3.05 g. Gumminess is considered in the literature to correlate with hardness [23] proven also by our results (R=0.92 and p-value 0.001013). Unsurprisingly, the p-value of statistically operated data gained a p-value of 0.000121, indicating that there is a significant difference between the gumminess of yogurt produced with commercial culture and the one produced with heirloom culture.

Cohesiveness indicates structural integrity and bond strength. It reflects the force of internal bonds stabilizing the yogurt gel. Greater cohesiveness and springiness relate to stronger gel structures [10] [20]. The cohesiveness value of the commercial yogurt samples was found between 0.43-0.58 whereas for heirloom yogurt samples was found between 0.46-0.53. The results show that commercial yogurt has a firmer structure in samples with flaxseed compared with heirloom yogurt due to the high value of cohesiveness. On the other hand, The p-value calculated with the results of cohesiveness was 0.195 which is higher than α-0.05 meaning that we cannot reject the null hypothesis and that there is no significant difference in cohesiveness between two products. The values of cohesiveness were reported in the range of 0.33-0.48 by Helal et al. 2018, which is in range with our values of cohesiveness [22].

Chewiness (mJ) is measured in terms of the energy required to masticate solid food. It is calculated as the product of hardness, springiness, and cohesiveness [10] [20]. The chewiness value of the commercial yogurt samples was found to be 26.03±5.05 at day 3 whereas for heirloom yogurt samples was found to be 20.57 mJ±1.74 at day 3. Chewiness is correlated with hardness [7] [20] (R=0.9213 p-value 0.00114). The resulting p-value of 0.00122, by which we can reject the null hypothesis meaning that there is a significant difference between the two types of yogurts. Kose et al. 2018 reported that the chewiness value of the traditional and industrial yogurt samples was in the range of 11.87 to 112.61, that are comparable with the values of chewiness reached in our study [7]. A significant difference was found for the data of resilience and deformation at hardness, with p-values of 0.044 and 0.0319, respectively. Resilience shows the ability of the yogurt to regain its original form after force is applied [7]. These findings indicated that the textural parameters of yogurt might be affected by the starter culture used.

Understanding and controlling these texture parameters are essential for ensuring the desired quality and consumer acceptance of yogurt products. The information provided emphasizes the importance of various factors in influencing yogurt texture and the need for standardized production methods.

Utilizing the data presented in Table 3, a type 1 t-test was conducted to compare the syneresis levels between commercial yogurt (M=43.38, SD=3.65, n=3) and heirloom yogurt (M=35, SD=2.23, n=3). The null hypothesis posited that there would be no statistically significant difference in syneresis between the two yogurt types. The obtained t-test result yielded a p-value of 0.01619, by which we can reject the null hypothesis. Concluding that there is a significant difference in syneresis between two types of yogurt.

Conversely, a parallel t-test was executed to assess the yield of commercial yogurt (M=66.46, SD=9.29, n=3) against that of heirloom yogurt (M=63.32, SD=5.8, n=3). The null hypothesis asserted that there would be no significant difference in yield between the two yogurt variants. The outcome of this t-test supported the null hypothesis with a p-value of 0.3654, indicating that there is no significant difference between the yield of the two types of yogurt. The same statistical conclusion was reached for a water-holding capacity test with a

[DOI: 10.22034/FSCT.21.155.137]

p-value of 0.4839. The evaluation of set yogurt relies significantly on WHC, a crucial determinant. The presence of denatured whey proteins plays a key role in improving WHC, leading to a decrease in whey syneresis. This reduction is essential as whey syneresis negatively affects the overall quality of the yogurt [24].

Yogurt consumption is beneficial to human health primarily due to the bacteria it contains. As Figure 3 shows, in commercial yogurt, the number of LAB was found in higher amounts (cfu/ml) than in heirloom yogurt. For commercial yogurt, the viable number of LAB was found at values of 1.27 $^\wedge 10^6$ cfu/ml, whereas for heirloom the viable number of LAB was found at values of $1.12^\wedge 10^6$ cfu/ml. For yogurt to be considered effective, it is generally accepted that it should contain at least 10^7 colony-forming units (cfu) of viable bacteria per milliliter. Various organizations have established standards to ensure yogurt contains sufficient viable bacteria: The Fermented Milks and Lactic Beverages Association requires $\geq 10^7$ viable bacteria/mL in dairy products whereas the Swiss Food Regulation and the International Standard of FIL/IFD (1991) mandate $\geq 10^6$ cfu/g. The Spanish Yogurt Quality Standard requires $\geq 10^7$ cfu/mL [25]. On the other hand, Harmann and Marth 1984 recommend yogurt contain at least 1 million viable organisms per gram at the time of sale after extensive study [26].

5- CONCLUSIONS

This study investigated the impact of two different starter cultures, commercial and heirloom, on the texture and microbiological quality of yogurt. The production process involved various parameters, including raw milk analysis, yogurt production steps, texture profile analysis (TPA), syneresis, water holding capacity (WHC), and microbial analysis. The key findings and conclusions drawn from the study are summarized below:

The raw cow milk used in the study met the recommended quality standards, with parameters such as fat content, dry matter, density, proteins, freezing point, temperature, lactose, conductivity, and pH within acceptable ranges.

Yogurt was produced using both commercial and heirloom starter cultures, with slight modifications in the production process.

The flowchart of yogurt production highlighted the key steps involved, including pasteurization, inoculation with starter cultures, incubation, and cooling.

Texture parameters (hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness, resilience) were analyzed using TPA for both commercial and heirloom yogurts.

Significant differences were observed in hardness, gumminess, chewiness, resilience, and syneresis between commercial and heirloom yogurts.

Commercial yogurt exhibited higher hardness and gumminess compared to heirloom yogurt, while heirloom yogurt showed lower syneresis.

Syneresis, representing the separation of whey, was significantly higher in commercial yogurt compared to heirloom yogurt.

WHC showed no significant difference between the two types of yogurts, indicating similar water retention capabilities.

Microbial analysis revealed the absence of aerobic bacteria and mold and yeast contamination in both commercial and heirloom yogurts.

LAB were present in both types of yogurt, with higher counts in commercial yogurt compared to heirloom yogurt.

Statistical analysis, including t-tests, supported significant differences in various texture parameters and microbial counts between commercial and heirloom yogurts.

The results emphasized the influence of the starter culture on the textural and microbiological characteristics of yogurt. The study provided valuable insights into the influence of starter cultures on the texture and microbiological properties of yogurt. Standardized production methods are crucial for ensuring consistent yogurt quality.

The findings contribute to a better understanding of factors affecting yogurt texture and highlight the importance of selecting appropriate starter cultures for desired product characteristics. This research enhances our knowledge of yogurt production and quality control, providing a foundation for further studies and potential improvements in yogurt manufacturing processes.

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