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RESEARCH ARTICLE

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Technological and Sensory Characterization of Yogurt Produced with Lactic Acid Bacteria Isolated from Traditional Yogurt of Eastern Afghanistan

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ABSTRACT

Traditional fermented yogurts represent an important source of indigenous lactic acid bacteria (LAB) with desirable technological, sensory, and health promising properties. However, the technological potential of LAB from Afghan traditional yoghurt remains largely unexplored. This study aimed to assess the technological and sensory attributes of LAB isolated from Afghan traditional yogurts. In this sense, four safe LAB strains, which were isolated from Afghan traditional yogurt, were used in the production of yogurts. The final product then were subjected to technological and sensory examinations through strain and product-based tests, and compared with commercial control yogurt. The results showed that all LAB isolates were positive for amylolytic activity and showed different responses to exopolysaccharide (EPS) production. All strains, particularly Pediococcus acidilactici (NSG2), showed a better response in terms of acidifying activities, reduced pH, titratable acidity, and reduced syneresis compared to the control ($p < 0.05$). The texture parameters of all four strains were significantly different, except the stiffness of yogurt compared to the control group ($p < 0.05$). Sensory evaluation by both expert and consumer panels indicated that yogurt produced with indigenous LAB isolated from Afghan yogurt samples showed superior aroma, texture, flavor, and overall acceptability. In conclusion, all isolates from Afghan traditional yogurt, particularly NSG2, showed outstanding technological and sensory attributes, reflecting their potential for industrial use.

Keywords

Lactic Acid Bacteria, Technological Properties, Sensory Attributes, Traditional Yogurt, Afghanistan

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Abbreviations

ANOVA: Analysis of Variance
CFU: Colony Forming Unit
EPS: Exopolysaccharide
LAB: Lactic Acid Bacteria

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Introduction

Yogurt is an extensively enjoyed fermented dairy product that is popular around the globe [1]. Its consumption is linked to various health benefits, including better bone health, improved gut health, reduced cardiovascular disease risk, enhanced diabetes management, boosted immunity, and alleviation of allergy symptoms [2]. According to the Codex definition, yogurt is a type of fermented milk created through the symbiotic action of Streptococcus thermophilus and Lactobacillus delbrueckii subspecies bulgaricus [3]. Moreover, yogurts may contain additional cultures such as Lactococcus lactis, Lactobacillus casei, or various species of Bifidobacterium. In such instances, the product is classified as yogurt with added bacteria. The primary microorganisms found in yogurt belong to Lactic Acid Bacteria (LAB), which ferment lactose, the natural sugar in milk, into lactic acid [4]. Numerous studies have been carried out that have identified different LAB from naturally fermented milk products, with Lactococcus lactis subsp. cremoris and Lactococcus lactis subsp. lactis being the predominant microbiota, along with other mesophilic lactobacilli (Lactobacillus casei/Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus fermentum, or Lactobacillus acidophilus), Enterococcus faecium, Leuconostoc species, and Pedococcus [5].

The quality of yogurt is influenced by different factors, including the type and composition of the milk used, the fermentation kinetics, acidification rates, the starter cultures used, and the post-production handling and storage practices. These variables work together to define the final physicochemical, rheological, and sensory characteristics of the yogurt [6]. Even when using the commercial starter cultures Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, the modification or inclusion of different LAB strains can alter the characteristics of yogurt, as these strains differ in their acidification processes and their capacity to generate structure-forming metabolites, especially exopolysaccharides (EPS) [7]. Experimental evidence highlights that the choice of cultures significantly impacts the final product. Yogurt cultures can improve structural stability and minimize syneresis. At the same time, adjunct/probiotic LAB (such as Lactiplantibacillus plantarum 299v or Lactobacillus acidophilus La-5) can enhance gel firmness and/or increase acidification depending on the strain and matrix. Consequently, mixed-culture systems are frequently employed to achieve an optimal balance between texture, stability, and sensory acceptance [8].

Traditional yogurt, harbor a wide variety and dense number of indigenous microbiotas, primarily consisting of LAB, which contribute unique sensory characteristics to the food. These microorganisms play a significant and well-documented role in the nutritional and dietary qualities of the product, influenced by types of mammalian milk used (e.g., cow, sheep, or goat), diverse microbial patterns, and various production techniques employed [9]. Furthermore, the acidity levels, flavor development, and texture of yogurt are directly affected by the starter culture's characteristics. While various commercial starter cultures, are available globally, they may not always accurately replicate the physicochemical and sensory attributes of traditional starter cultures in the final product. Moreover, one major risk associated with using a single starter culture for continuous yogurt production is bacteriophage contamination. Therefore, it's crucial to identify new and wild yogurt starters that possess different technological characteristics from local traditional yogurts to broaden the availability of yogurt starter cultures and improve the technological features of yogurt while maintaining traditional organoleptic qualities [10]. In this context, traditional Afghan yogurt represents a valuable opportunity for discovering new and wild cultures, particularly LAB. However, no published research currently details and assesses the various properties of LAB isolated from Afghan yogurt, particularly produced in the Eastern region of Afghanistan. Consequently, this study represents the first investigation into the technological and sensory characteristics of different LAB isolated from Afghan traditional yogurt.

Results

The results of this study are presented by distinguishing between the strain-based functional characteristics of LAB and the technological and sensory attributes of the yogurts produced from these strains.

Strain-based characteristics

Amylolytic activity and exopolysaccharide production

The amylolytic activity and EPS production of the selected LAB strains are presented in Table 1. All strains showed positive amylolytic activity. Regarding EPS production, strain-dependent differences were observed depending on the carbohydrate source. KSK1 exhibited positive EPS production in the pres-

Abbreviations-Cont'd

MCT: Multiple Comparison Test
MRS: de Man-Rogasa-Sharpe
PC: Principal Component
PCA: Principal Component Analysis
TA:Titratable Acidity

Table 1.
Amylolytic activity and EPS production of the selected isolates.

Strain	Variable				
	Amylolytic activity	EPS production			
		Lactose	Fructose	Glucose	Sucrose
KSK1	Positive	Positive	Positive	Positive	Positive
LAG2	Positive	Positive	Positive	Negative	Negative
NW2	Positive	Negative	Negative	Negative	Negative
NSG2	Positive	Negative	Negative	Negative	Negative

ence of lactose, fructose, glucose, and sucrose, while LAG2 produced EPS when grown on lactose and fructose but not on glucose or sucrose. In contrast, NW2 and NSG2 did not show EPS production with any of the tested carbohydrates.

Acidification activity

The acidification activity of the selected LAB strains was evaluated over a 24-hour incubation period, as shown in Figure 1. At the initial time point (0 h), no significant differences were observed among the strains, with initial pH values ranging from 6.51 to 6.53 ($p > 0.05$). After 6 h, all strains showed a significant decrease in pH, and differences among them became statistically significant ($p < 0.05$). Additional reductions in pH were observed at 12 and 24 h. Among the tested isolates, NSG2 consistently exhibited the lowest pH values at all fermentation times after 0 h.

Product-based characteristics

Survival of Isolates

The survival of the isolates in yogurt during 28 days of refrigerated storage, are presented in Table 2. After day one, significant differences in the viability of all isolates were observed ($p < 0.05$). Over the 28-day storage period, major difference in the survival of storage was recorded for KSK1 (0.95 Log CFU/g) followed by control (0.81), NW2 (0.48), and LAG2 (0.30). In contrast, NSG2 survival rate was increased during the storage where the Log CFU/g of the isolate was increased by approximately 0.23 Log CFU/gr.

pH and titratable acidity

The results of pH values of yogurt samples produced with different LAB strains during 28 days of refrigerated storage are presented in Figure 2. On day 1, significant differences were observed among all samples ($p < 0.05$), with pH ranging from 3.93 to 4.11. These highly significant differences persisted on days 7, 14, 21, and 28 of storage ($p < 0.01$). Although all samples showed a decreasing trend throughout storage, the extent of decrease varied among strains. By day 28, pH values ranged from 3.61 to 3.84 in LAB-fermented samples, while the control sample had higher pH at 3.84.

Changes in titratable acidity (TA) of yogurt sam-

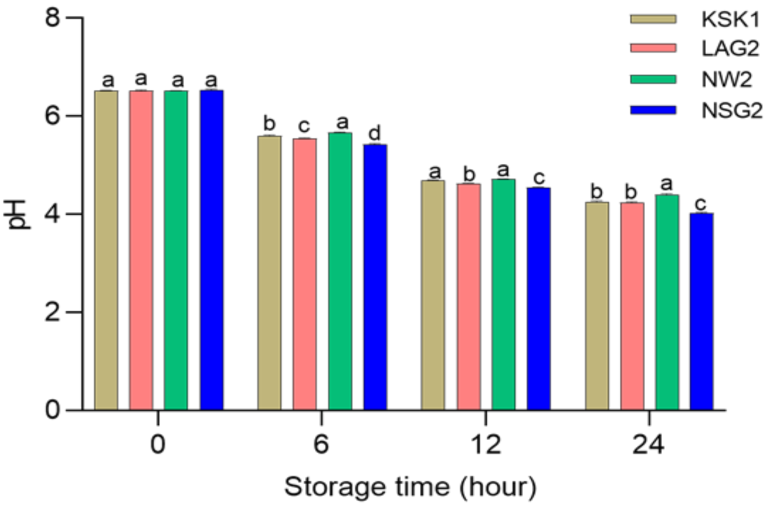

Figure 1.
Acidification activity of four selected isolates during 24 hours of storage at 37 °C.
The bars show the mean of duplicate values, and error bars represent the standard error. The data analysis showed no significant difference (p < 0.05), while in the subsequent storage times, a significant reduction in pH values was observed (p < 0.05).

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Table 2.
Survival of the isolates during 28 days of refrigerated storage.

Strain	Survival (Mean±SD) *				
	Day one	7th day	14th day	21st day	28th day
KSK1	7.97 ± 0.67[b]	8.53 ± 0.04[c]	8.47 ± 0.03[c]	8.08 ± 0.05[c]	7.52 ± 0.03[d]
LAG2	8.82 ± 0.02[a]	9.22 ± 0.01[a]	9.13 ± 0.03[a]	8.85 ± 0.01[b]	8.52 ± 0.02[b]
NW2	8.41 ± 0.02[ab]	8.93 ± 0.02[b]	8.53 ± 0.03[bc]	8.11 ± 0.02[c]	7.93 ± 0.03[c]
NSG2	8.62 ± 0.01[ab]	9.22 ± 0.02[a]	9.10 ± 0.01[a]	8.94 ± 0.01[a]	8.85 ± 0.05[a]
Control	8.34 ± 0.01[ab]	8.53 ± 0.03[c]	8.57 ± 0.03[b]	8.09 ± 0.02[c]	7.53 ± 0.02[d]

* Number in each cell represents the mean ± SD of duplicate samples.
a-d Means with different small scale alphabet superscripts in the same column are significantly different.

ples produced with different LAB strains over 28 days of refrigerated storage are presented in Figure 2 Figure 3. On day 1, titratable acidity values ranged from 0.61 to 0.69% lactic acid, and no significant differences were observed among samples ($p > 0.05$). During storage, TA increased progressively in all samples, and the difference was statistically significant in all storage days ($p < 0.05$). On day 7, 14, 21, and 28, TA values ranged from 0.66 to 0.78%, 0.73–0.85%, 0.76–0.92%, and 0.81-0.94% lactic acid, respectively.

Dry matter and syneresis

The dry matter content and syneresis of yogurt samples produced with different LAB strains during 28 days of refrigerated storage are presented in Table 3. Dry matter content differed significantly among samples ($p < 0.05$), ranging from 9.55% to 11.00%. The control sample had the highest dry matter content, while the LAB-fermented samples showed comparable values, with slight variations among strains.

Syneresis values on day 1 ranged from 13.87% to 24.05%. The control sample exhibited significantly higher syneresis compared to all LAB-fermented yogurts ($p < 0.05$). Syneresis progressively increased in all samples throughout storage, with significant differences observed among all LAB-fermented yogurts on all storage days. On day 7, syneresis values ranged from 15.27% to 27.97%. By day 14 and day 21, these values further increased to 17.87–32.42% and 19.35–39.20%, respectively. By day 28, syneresis values in LAB-fermented samples ranged from 20.47% to 26.72%, while the control sample exhibited the highest syneresis at 40.32%.

Coagulation activity

The results of the coagulation activity of yogurt samples produced with different LAB strains are presented in Table 4. Differences in coagulation time were observed among the strains. LAG2 and NSG2 exhibited rapid coagulation, occurring within 4 - 6 hours of incubation at 42 °C. KSK1 and the control sample showed intermediate coagulation activity, with coag-

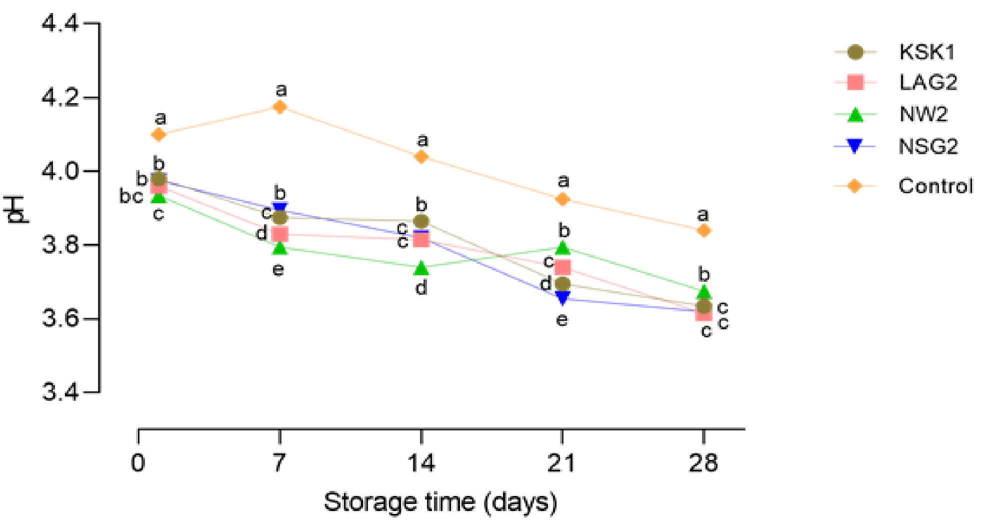

Figure 2.
pH values of different yogurt samples during 28 days of refrigerated storage.
Each symbol shows the mean of pH value calculated from duplicate samples, while the error bars show the SD. Different small alphabets in the same storage day show significant differences. The results showed significant differences among different types of yogurt in all storage days ($p < 0.05$).]

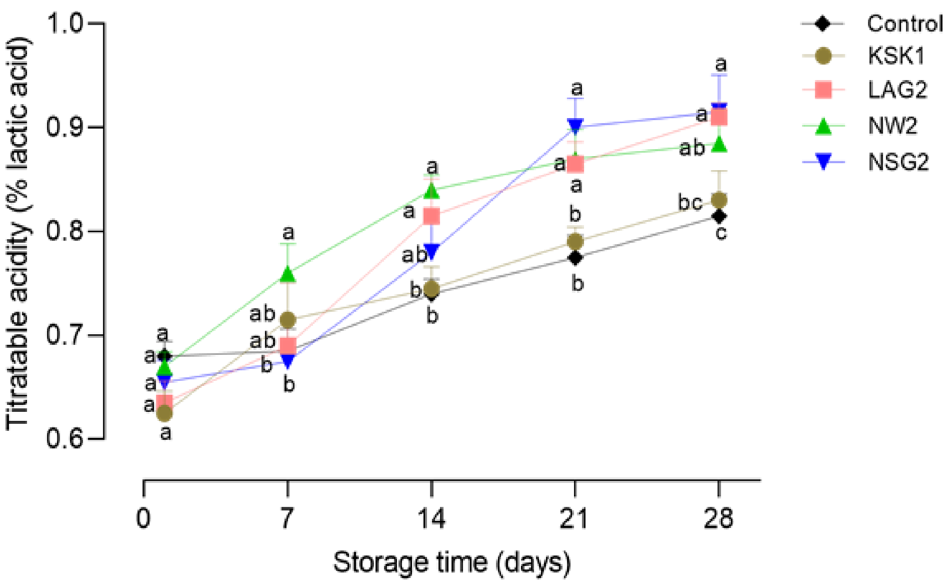

Figure 3.
Titratable acidity of different yogurt samples during 28 days of refrigerated storage.
Each symbol shows the mean of the titratable acidity value calculated from duplicate samples, while the error bars show the SD. Different small alphabets in the same storage day show significant differences. The results showed significant differences among different types of yogurts in all storage days except on day one ($p < 0.05$).]

Table 3.
Dry matter and syneresis of the produced yoghurt samples.

Strain	Dry matter	Survival (Mean±SD) *				
		Day one	7th day	14th day	21st day	28th day
KSK1	10.30 ± 0.13^{b}	14.32 ± 0.32^{c}	15.27 ± 0.25^{e}	17.87 ± 0.18^{c}	20.82 ± 0.32^{b}	22.05 ± 0.42^{d}
LAG2	9.55 ± 0.21^{c}	16.65 ± 0.28^{b}	17.57 ± 0.25^{c}	19.42 ± 0.18^{b}	19.35 ± 0.14^{c}	20.47 ± 0.32^{e}
NW2	10.50 ± 0.14^{b}	14.42 ± 0.32^{c}	18.17 ± 0.18^{b}	19.00 ± 0.28^{b}	20.55 ± 0.28^{b}	26.72 ± 0.25^{b}
NSG2	10.25 ± 0.21^{b}	13.87 ± 0.04^{c}	16.65 ± 0.14^{d}	17.95 ± 0.07^{c}	19.50 ± 0.07^{c}	23.62 ± 0.18^{c}
Control	11.00 ± 0.10^{a}	24.05 ± 0.07^{a}	27.97 ± 0.04^{a}	32.42 ± 0.25^{a}	39.20 ± 0.21^{a}	40.32 ± 0.32^{a}

* Number in each cell represents the mean ± SD of duplicate samples.
a-e Means with different small scale alphabet superscripts in the same column are significantly different.

Table 4.
Coagulation activity of LAB strains.

Strain	Coagulation*
KSK1	Intermediate
LAG2	Rapid
NW2	Slow
NSG2	Rapid
Control	Intermediate

* Rapid: 4-6 hours, Intermediate: 6-12 hours, and Slow: more than 12 hours.

ulation observed between 6 and 12 hours. In contrast, NW2 displayed slow coagulation, requiring more than 12 hours to coagulate under the same conditions.

Texture profile analysis

The findings of texture profile characteristics of yogurt samples fermented with different LAB strains are presented in Figure 4. With the exception of stiffness ($p > 0.05$), all other evaluated parameters showed significant differences among the strains ($p < 0.05$). Hardness ranged from 0.67 to 0.98 N, with higher values observed in yogurts produced with LAG2. Cohesiveness values ranged from 0.39 to 0.69, with relatively higher cohesiveness observed in the control sample (0.69) compared to most LAB-fermented samples. Springiness values were generally similar across samples, ranging from 11.01 to 14.57 mm, though yogurts fermented with LAG2 displayed higher springiness (14.57 mm) compared to the other strains. Gumminess values ranged between 0.62 and 0.72 N. Chewiness (expressed as energy) ranged from 4.88 to 5.63 mJ across all samples. Adhesiveness values ranged from 1.56 to 2.96 mJ, with higher adhesiveness observed in yogurts produced with NW2. Stiffness values, expressed as N/mm, ranged from 0.061 to 0.071.

Sensory evaluation

The sensory evaluation of the produced yogurts by the expert panel on day 7 is depicted in Figure 5.

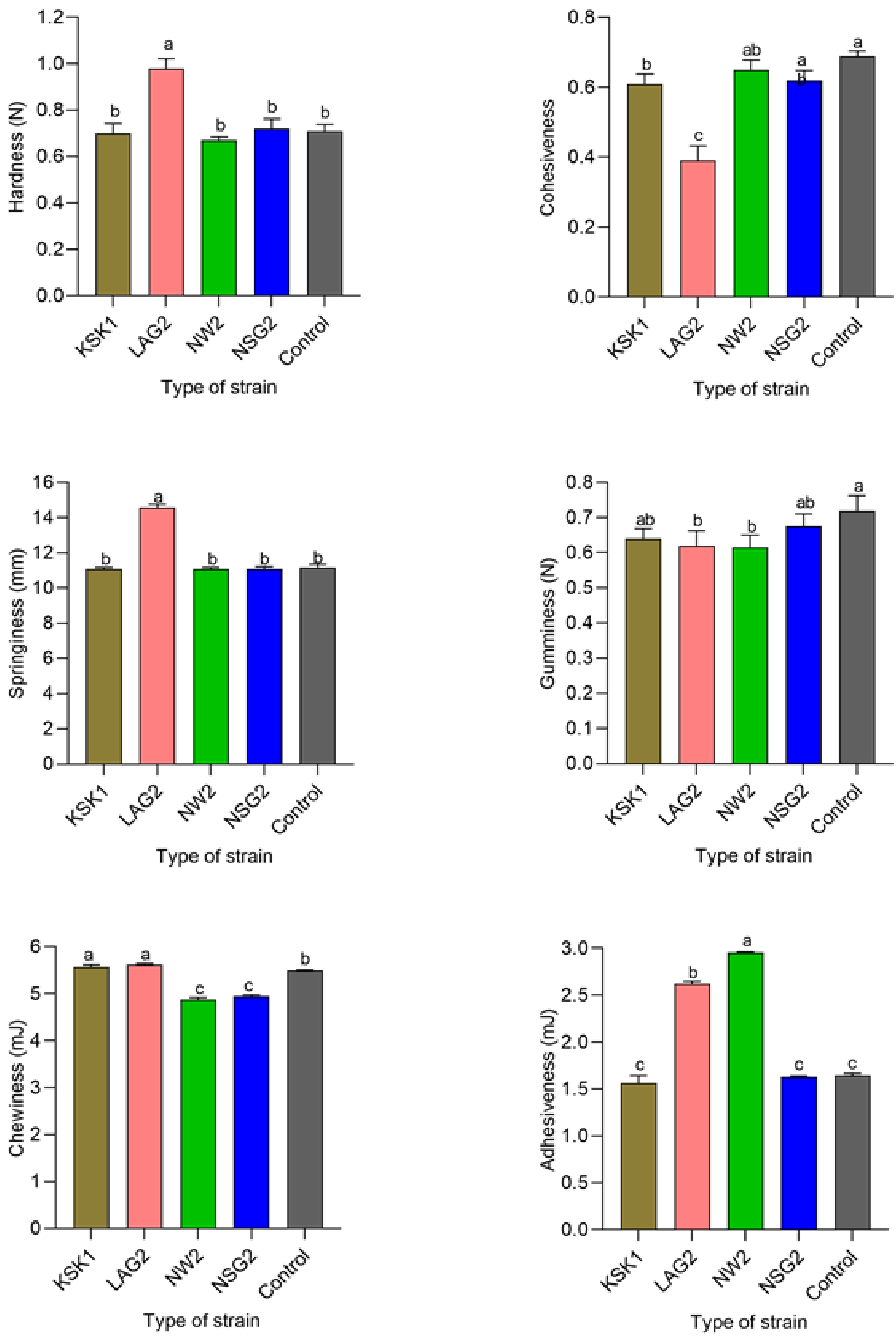

Figure 4.
Texture profile of yogurt samples produced with the isolates.
Figure 4 shows the Bars show the mean of two samples, while error bars depict the SD. Bars with different small alphabets are significantly different from each other. The results showed that all the parameters of texture profile of the yogurt samples were significantly different with each other ($p < 0.05$) except stiffness of the yogurt samples ($p > 0.05$)]

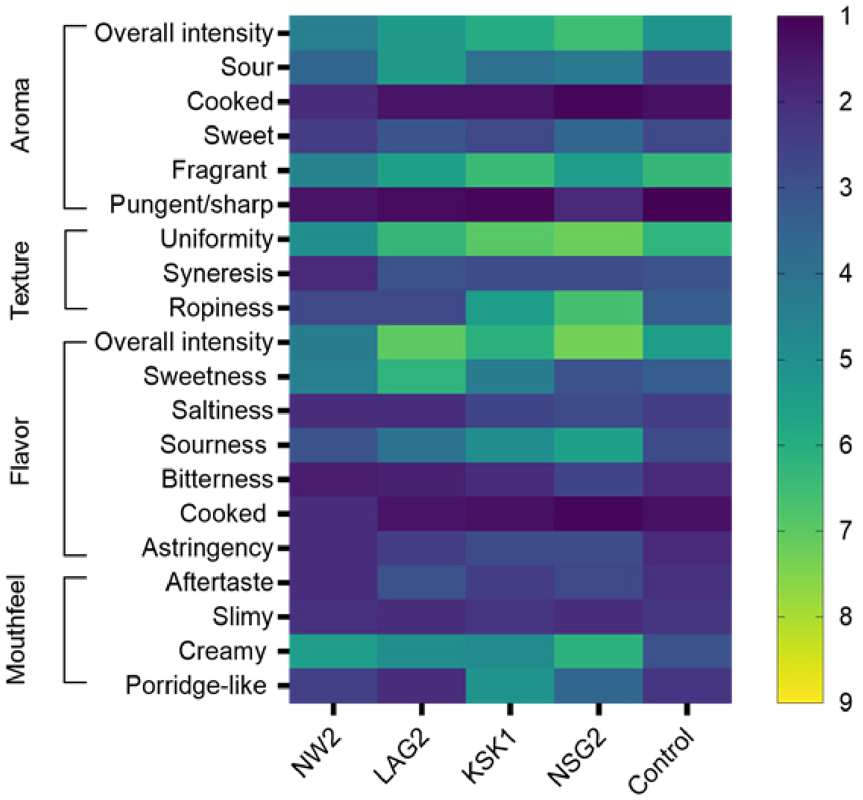

Figure 5.
Heatmap of the sensory evaluation scores by expert panel of the produced yogurt samples. Score for each attribute is the mean of 10 expert panel.

Sensory score for different attributes of the samples ranged from 1 to 7.3. NSG2 had the highest score for overall aroma intensity (6.5). For other Aroma attributes, scores were below the medium score (5) except for the fragrant attribute, where KSK1 scored highest (6.4) and NW2 lowest (4.5). Regarding texture characteristics, the high score for uniformity was reported for NSG2 (7.2), while NW2 scored lowest (4.9). Syneresis was reported with low scores for Control and LAG2 samples at 3. Ropiness was higher for NSG2 (6.6) and lower for NW2 and LAG2 (2.7). NSG2 also scored highest for overall flavor intensity (7.3), followed by LAG2 (7), KSK1 (6.1), control (5.4), and NW2 (4.3). The majority of other flavor related attributes were below the medium score (5). In terms of mouthfeel, the creamy attribute received the highest scores, with NSG2 scoring highest (6.1) and control sample lowest (3).

Principal Component Analysis (PCA) of the sensory attributes is illustrated in Figure 6. High variability (75.87%) is reported by both principal components (PC). The projection of attributes onto the factor plane indicated that, cooked and sweet flavors, cooked aroma, and slimy mouthfeel negatively influenced PC 1. Conversely, other aroma, texture, taste, and mouthfeel descriptors positively effected PC 1. On the other hand, PC 2 was positively influenced by sour, sweet, pungent, and cooked aroma, cream mouthfeel, and sweet, cooked, aftertaste, astringency, and overall intensity flavor descriptors are reported. Conversely, PC 2 was negatively influenced by mouthfeel (slimy, porridge like), aroma (fragrant, and overall intensity), flavor (bitterness and saltiness), and texture descriptors (ropiness, uniformity, and syneresis).

The PCA correlation analysis revealed a clear separation between control and treatment groups, although the distinction between LAG2 and NSG2 was not evident. LAG2 and NSG2 were characterized by sour, pungent, and sweet aroma, creamy mouthfeel, sourness, astringency, aftertaste, and overall intensity flavor descriptors. On the other hand, KSK1 was associated with texture attributes (ropiness, uniformity, and syneresis), aroma (fragrant and overall intensity), flavor (saltiness and bitterness), and mouthfeel (porridge-like). NW2 was characterized by high intensities of cooked aroma and sweet and cooked flavor. The control sample was solely intensified in slimy mouthfeel.

Consumer panel sensory evaluation scores obtained from 30-members are presented in Figure 7. NSG2 received the highest mean scores for flavor (7.35), texture (7.20), and overall acceptance of 7.15. KSK1 followed with the second-highest ratings, particularly for aroma (6.70) and texture (6.90), achieving an overall acceptance of 6.55. The control sample exhibited intermediate values with an overall acceptance of 6.40, while NW2 and LAG2 recorded the lowest scores across all attributes, both achieving an overall acceptance of 6.10.

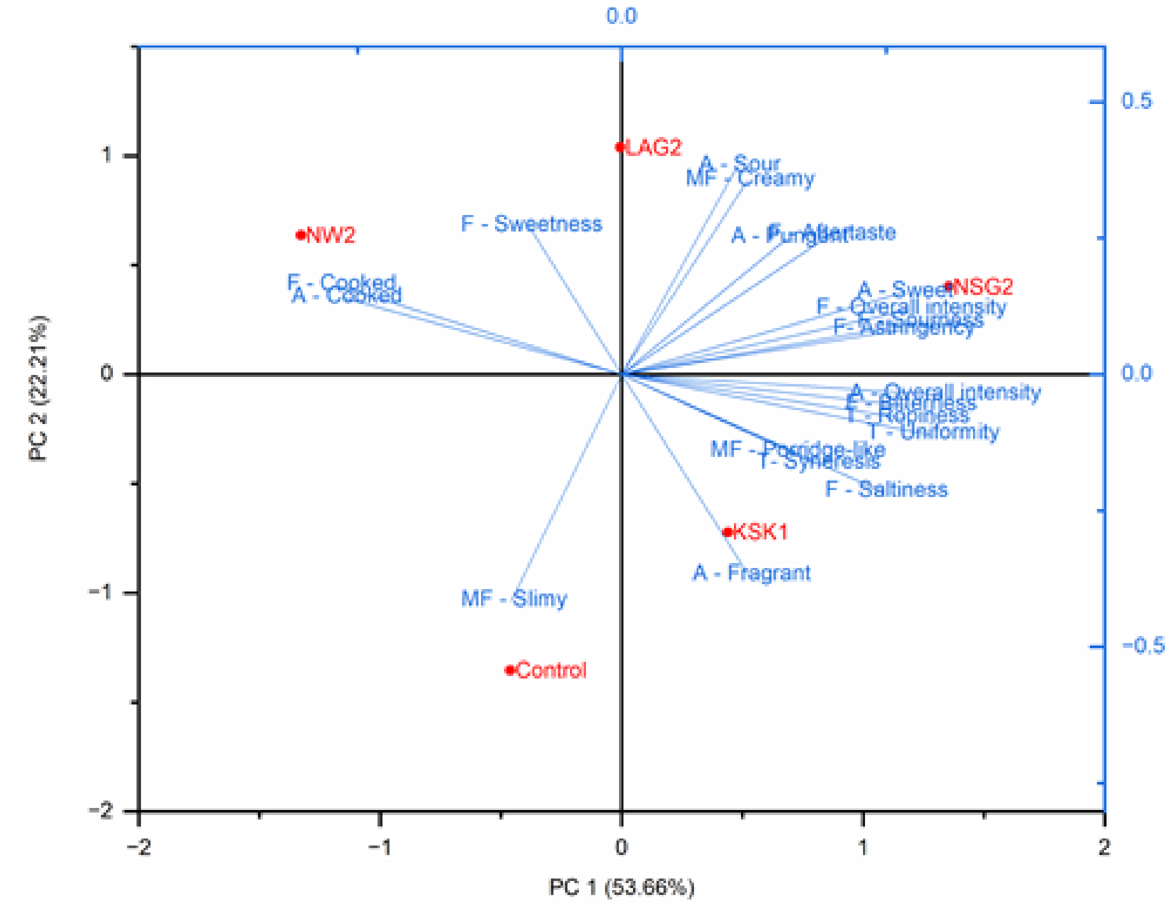

Figure 6.
PCA biplot for the distribution of different yogurt samples and its correlation with the sensory attributes.

Discussion

The present study aimed to assess the technological and sensory attributes of yogurt produced using indigenous LAB strains isolated from traditional Afghan yogurt. Notably, the study highlights the novelty of Afghan traditional yogurt as an underexplored source of indigenous LAB, demonstrating strain-specific performance in both technological and sensory aspects. The results clearly indicate that the selection of starter culture significantly impacts fermentation performance, physical stability, texture, and sensory quality. All tested strains exhibited positive amylolytic activity. However, EPS production varied influenced by the specific strain and the carbohydrate source uti-

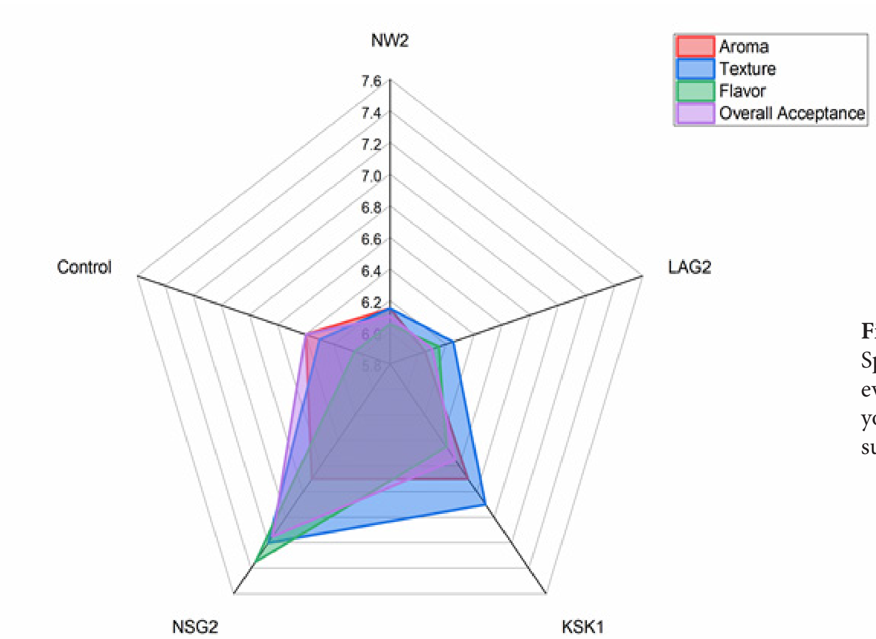

Figure 7.
Spider plot of sensory evaluation of the produced yogurt samples by consumers.

lized. Regarding fermentation dynamics, the NSG2 exhibited the most rapid acidification and maintained lower pH values during refrigerated storage, whereas titratable acidity showed a progressive increase across all samples. In terms of physical stability, yogurt produced with indigenous strains generally exhibited lower syneresis compared to the control. Coagulation times varied significantly, with LAG2 and NSG2 displaying faster activity than NW2. Texture profile analysis revealed significant variations in most parameters, with the exception of stiffness. Ultimately, sensory evaluation conducted by the expert and consumer panels revealed that yogurt produced with indigenous LAB strains offered superior quality attributes compared to the control sample.

The production of organic acids, predominantly lactic acid, by starter cultures is the primary outcome product of yogurt fermentation. This process acidifies the milk matrix, initiating milk coagulation and the formation of the yogurt gel [11-13]. Consequently, these changes are typically observed as an increase in titratable acidity and a decrease in pH over time. These acidification processes are strongly strain-dependent and influence coagulation kinetics and product quality [6, 8]. The results of the present study align with this established phenomenon, demonstrating that the indigenous LAB isolated from Afghan yogurts possess distinct acidification capacities. Notably, NSG2 showed the highest acidification activity compared with the control and the other isolates, which corresponded to shorter coagulation times. Conversely, the NW2 strain showed slower fermentation kinetics. Ziarno et al. [8] similarly reported that the acid production is strain-dependent. In their study, samples supplemented with L. plantarum 299v and L. acidophilus La-5 reached the the lowest pH levels. Furthermore, Li et al. [14] observed a strain-dependent pH decline in soy yogurt samples, where L. acidophilus N4 rapidly reached the terminal fermentation point (pH 4.6) within 240 minutes, while other strains required between 260 and 280 minutes.

Syneresis, commonly known as whey separation, is a key quality defect that negatively affects consumer acceptance. This defect is linked to the gel's water-holding capacity and the structural integrity of the protein network [15, 16]. This defect is highly strain-dependent, and studies reported the different intensities for different yogurt types produced with different LAB [17, 18]. In the present study, syneresis progressively increased in all yogurt samples during the 28-day storage period. However, yogurts produced with the indigenous LAB strains exhibited significantly lower syneresis compared to the control. Notably, the control sample, despite having the most pronounced dry matter content, showed the greatest syneresis by the end of the storage period. In contrast, yogurts fermented with indigenous strains demonstrated improved physical stability. This enhanced stability may be attributed to factors such as EPS production (e.g., KSK1) and the formation of a more compact gel structure (e.g., NSG2). These findings align with previous research indicating that EPS production by LAB contributes to reduced syneresis and enhanced textural stability in yogurt systems [19]. Additionally, strain-dependent differences in gel structure and whey separation during storage are well documented. For instance, one study reported lower syneresis in yogurt containing Lactobacillus rhamnosus and Lactobacillus paracasei compared to the control group [20].

Texture profile analysis provides an objective characterization of yogurt's structural integrity through the measurement of mechanical properties associated with gel formation [7]. In the present study, most texture parameters differed significantly among yogurt samples, with the exception of stiffness, which remained comparable. Yogurts fermented with different strains showed distinct textural profiles, with LAG2 producing a firmer/more elastic gel and NW2 showing higher adhesiveness. These findings confirm the strain-dependent nature of structure formation. Comparable strain-related variations in yogurt texture have been reported previously, where differences in texture profile were linked to the LAB strains. Ili´c et al. [7] reported that the addition of selected probiotic strains alongside commercial starters increased firmness and related texture indices compared to other formulations. In addition, EPS production also affects the rheological, sensory, and texture properties of foods, including yogurt [20, 21]. In the present study, LAG2 strain, which produced EPS from lactose, demonstrated good hardness. This observation is supported by research from Ozturkoglu-Budak et al. [22] and Tiwari et al. [23] who reported that Bifidobacterium spp. can produce EPS, thereby enhancing yogurt firmness. Tabrizi et al. [19] also reported that EPS producing LAB significantly increased the yogurt firmness throughout the storage compared to the control group using non-EPS producing LAB. Similarly, Wu et al. [18] also reported that Leuconostoc mesenteroides RSG7, an EPS producer, improved the textural properties, including hardness, cohesiveness, gumminess, chewiness, springiness, and adhesiveness compared to the control group. From an industrial standpoint, strains that enhance firmness and reduce whey separation may help decrease reliance on stabilizers and support cleaner-label yogurt formulations.

Sensory properties are among the required qualities of yogurt, serving as a reflection of the interplay between fermentation processes, matrix structure, and metabolite formation as perceived by consumers [24, 25]. In the present study, sensory evaluation conducted by a trained and consumer panel showed distinct strain-dependent differences in aroma, texture, flavor, and mouthfeel attributes. Notably, yogurt

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produced with NSG2 consistently exhibited higher intensities for key positive attributes, including overall aroma intensity, uniformity, ropiness, creamy mouthfeel, and overall flavor intensity. In contrast, NW2 was associated with lower sensory scores and was characterized by undesirable cooked-related aroma and flavor notes. The control sample was mainly characterized by slimy mouthfeel and lower overall sensory intensity. PCA supported these findings, effectively separating the control from LAB-fermented yogurts and indicating distinct sensory drivers associated with each strains. These findings are consistent with previous research demonstrating that LAB strains differ significantly in their capacity to modulate yogurt sensory characteristics through strain-specific metabolic activity and structural interactions within the gel matrix [26]. In addition, Lestari et al. [27] also reported that the incorporating of probiotic LAB not only improved microbial quality but also maintained sensory acceptability comparable to control yogurt. These sensory outcomes indicate that indeginous LAB, particularly NSG2, represent promising candidate culture for producing consumer-acceptable yogurt with desirable aroma and mouthfeel profiles.

In conclusion, all LAB strains showed good performance in terms of technological and sensory properties. All strains exhibited positive amylolytic activity, produced EPS from all or some carbohydrates, maintained viability during 28 days of refrigerated storage, displayed favorable acidification activities, and sensory properties compared to the control group. Among the indigenous LAB, NSG2 (Pediococcus acidilactici) was the superior candidate. It exhibited excellent acidifying activity, coagulation, and textural profile. These characteristics collectively improved the sensory attributes of the yogurt. These findings support the practical potential of these Afghan indigenous strains as starter or adjunct cultures for industrial yogurt manufacture.

Materials & Methods

Declaration of AI use

During writing this manuscript the author(s) used AI tools to check spelling, and grammar of the content where needed. After using these tools, the author(s) reviewed and take full responsibility for the content.

Study design

This study evaluated the technological and sensory characteristics of four LAB strains isolated from traditional yogurt produced in Eastern Afghanistan (Table 5). The isolates were stored at −18 °C and reactivated prior to analysis. Assessments were conducted at two levels: (i) direct strain-based tests performed on the LAB isolates, and (ii) product-based tests involving the manufacture of yogurt using each isolate as a single-strain starter culture.

Technological and sensory attributes

The evaluation of technological properties includes two types of tests namely strain-based test which included amylolytic activity, qualitative test of exopolysaccharide production [28], acidification capacity [29], and product-based tests which included pH, titratable acidity, syneresis, dry matter [29], coagulation activity, texture profile analysis (hardness, springiness, consistency, and gumminess index) [30], and sensory analysis of the isolates. In addition, the survival of selected isolates was monitored over during 28-day refrigerated storage period [29]. All the tests were conducted in duplicate fashion. The protocols for each test are briefly described as follows:

Strain-based examinations

Amylolytic activity

The overnight culture of selected isolates of LAB in MRS medium was surface-cultured onto plates containing modified MRS agar (MRS medium without glucose but with 0.25% starch). The plates were incubated at 37°C for 48 to 72 hours. Then, a warm iodine solution was p added to the plates as a detection agent. The presence of clear halo zones surrounding the colonies indicated positive α-amylase activity.
Qualitative detection of exopolysaccharide production
For the detection of EPS, the overnight culture of the isolated LAB strains was streaked onto MRS agar supplemented with 20 g/L sugar (glucose, fructose, sucrose, or lactose). Plates then were incubated at 37°C for 36 to 48 hours. The formation of slimy colonies by the LAB isolates on the sugar-containing media was indicative of EPS production. The "Ropy" character of the colonies was also assessed by pulling the colonies with an inoculating loop.

Acidification capacity

The acidification capacity of the selected LAB strains was assessed by inoculating 1% (v/v) of the overnight culture in MRS broth of each LAB into 10 mL of reconstituted skim milk at 37°C. The pH values were measured at the beginning of incubation (0 hours), and subsequently at 6, 12, and 24 hours with a digital pH meter.
Product (yogurt)-based examinations

Yogurt production

Yogurt was prepared using commercial pasteurized whole cow's milk

Table 5.
Isolated LAB from the traditional yogurt of Eastern Afghanistan.

#	LAB strain	Accession #	Province of yoghurt sample	District of yoghurt sample	Code of sample
1	Lactiplantibacillus Plantarum H12	OQ096590.1	Kunar	Sarkani	KSK1
2	Lactobacillus brevis KLDS 1.0727	EU626012.1	Laghman	Alingar	LAG2
3	Pediococcus acidilactici HBUAS56306	MZ959430.1	Nangarhar	Spinghar	NSG2
4	Lactiplantibacillus plantarum NWAFU1559	MG551236.1	Nuristan	Wama	NW2

(Pegah, Iran). The milk was homogenized, heat-treated at 95 °C for 15 min, and then cooled to 42 °C. Selected LAB isolates were activated according to the procedure described by Faraki et al. [31]. Briefly, overnight cultures grown in MRS broth were harvested, washed twice with sterile physiological saline, and resuspended in 2 mL saline. The cell suspension was adjusted to a final concentration of 10^9 CFU/mL and inoculated into the milk at a rate of 2% (v/v). Inoculated samples were incubated at 42 °C until coagulation (indicated by a pH of 4.6). Post coagulation, the samples were stored at 4 °C for 28 days.

Survival of the isolates

The viability of the inoculated LAB during storage was monitored from day zero to day 28. To determine viability, one gram of yogurt accurately weighed and diluted in 9 mL of 0.1% (w/v) peptone water. Then, serial dilutions were performed as necessary. Subsequently, 0.1 mL of the dilutions were plated on MRS agar. The plates were incubated at 37 °C for 48-72 hours under anaerobic conditions. The number of LAB was reported as CFU/g.

pH and titratable Acidity

The pH of the prepared yogurt samples was measured using a digital pH meter. TA was measured using the titration method with 0.1 N sodium hydroxide and phenolphthalein as an indicator. TA was reported as a percentage of lactic acid, which was calculated through the following formulae:

Titratable Acidity (% Lactic acid)=(Volume (mL) NaOH*Normality of NaOH*lacit acid equivalent)/(Volume of sample used (mL))

Syneresis

To determine syneresis, 5 g of yogurt was centrifuged at 5000 rpm for 20 minutes at 4°C and the separated whey was weighed. The following equation was used to calculate the syneresis:

Syneresis (%)=(Weight of whey (g))/(Weight of yogurt (g))*100

Dry matter

The dry matter of yogurt samples was determined using the gravimetric method, and the results were reported as a percentage (w/v).

Coagulation activity

Coagulation activity was assessed based on the time required for milk to coagulate at 42°C after incubation. Strains were categorized as: (i). Rapid. Coagulation within 4-6 hours, (ii). Intermediate. Coagulation between 6-12 hours, (iii). Slow. Coagulation taking longer than 12 hours.

Texture profile analysis

Texture analysis of the prepared yogurt samples was performed using a texture analyzer (Brookfield CT3, USA) according to the manufacturer's standard protocols. Hardness (N), cohesiveness, springiness (mm), gumminess (N), chewiness (mJ), adhesiveness (mJ), and stiffness (N/mm)., were measured for texture analysis. The texture characteristics of the samples were determined on day 7 at 4 ± 2°C without removing the samples from their containers, weighing 150 g. In this test, a cylindrical probe (with a smooth cross-section of 25.5 mm in diameter, a speed of 1 mm/s, and a penetration depth of 15 mm) was used in the yogurt sample immediately after the samples were removed from 4°C [32, 33].

Sensory evaluation

Sensory evaluation of the yogurt samples was conducted on day 7 of refrigerated storage. The evaluation undertaken in a separated room under room temperature and adequate lighting. An expert panel (n = 10), comprising PhD students and lecturers experienced in yogurt sensory assessment from the food hygiene and aquaculture department, performed quantitative descriptive sensory analysis. This panel received two days of training on the definitions of the descriptors and score scale. The expert panel used a 9-point structured intensity scale (1 = very weak/low intensity; 9 = very strong/high intensity) to evaluate predefined sensory attributes encompassing aroma, texture, flavor, and mouthfeel descriptors (Table 6). In parallel, a consumer panel (n = 30) evaluated yogurt acceptability on day 7 using the 9-point hedonic scale (1 = dislike extremely; 9 = like extremely) for the aroma, texture, flavor, and overall acceptability of the yogurt samples. Scores from both panels were recorded on evaluation forms and compiled for statistical analysis.

Statistical analysis

The collected data was analyzed using SPSS statistical software (Version 23, IBM Corp, Armonk, NY, USA). One-way Analysis of Variance (ANOVA) was used to compare the means among different groups. Duncan's Multiple Comparison Test (MCT) was used for post-hoc analysis to identify specific differences between group means. All the numerical variables were reported as mean ± Standard Deviation (SD). To visualize the data for some variables, Graphpad-Prism 9.2 (Version 9.2, GraphPad Software, San Diego, CA, USA) and OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA) software was used. A p-value less than 0.05 ($p < 0.05$) was considered as statistically significant.

Authors' Contributions

F.H. and A.A. conceived and planned the experiments. F.H. and A.A. carried out the experiments. F.H. and A.A. contributed to sample preparation. A.A. contributed to the interpretation of the results. A.A. took the lead in writing the manuscript. Both authors contributed to the first draft, revising.

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Conflict of interest

The authors declare that there is no conflict of interest.

Table 6.
Definitions of the sensory attributes used by the expert panel.

Category of sensory attribute	Descriptor	Operational definition for scoring
Aroma	Overall intensity	Overall strength of the yoghurt smell
	Sour	Intensity of acidic/fermented sour smell.
	Cooked	Intensity of cooked/heat-treated milk odor note.
	Sweet	Intensity of sweet-smelling note (aroma-associated sweetness).
	Fragrant	Intensity of pleasant aromatic/fragrant smell.
	Pungent	Intensity of sharp/pungent odor.
Texture	Uniformity	Degree of structural evenness (smooth, consistent gel; low graininess/lumps).
	Syneresis	Degree of visible whey release/separation.
	Stretchiness	Degree of stringy/ropy behavior when handled or stirred.
Flavor	Overall intensity	Overall strength of flavor perception.
	Sweetness	Intensity of sweet taste.
	Saltiness	Intensity of salty taste.
	Sourness	Intensity of sour/acid taste.
	Bitterness	Intensity of bitter taste.
	Cooked	Intensity of cooked/heat-treated flavour note in the mouth.
	Astringency	Intensity of drying/puckering sensation in the mouth.
	Aftertaste	Intensity/persistence of taste after swallowing.
Mouthfeel	Slimy	Intensity of slippery/mucilaginous mouth-coating sensation.
	Creamy	Intensity of creamy/rich smooth mouthfeel.
	Porridge-like	Intensity of thick/pasty “porridge-like” mouthfeel impression.

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