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Qualitative and Sensory Evaluation of Synbiotic Greek Yogurt Containing *Lactobacillus Rhamnosus* GG and *Bifidobacterium Longum* HH-BL18 during Shelf Life

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ABSTRACT: The Greek yogurt is a suitable alternative for carrying probiotic microorganisms in sufficient counts and has health-promoting effects. The present research aimed to investigate adding inulin on quality characteristics in probiotic Greek yogurt containing *Lactobacillus rhamnosus* GG (GYL) and *Bifidobacterium longum* HH-BL18 (GYB) with 2 % inulin individually (GYLI and GYBI). Microbial evaluation, physicochemical attributes, physical function, sensory features, and rheological characteristics on GYL, GYB, GYLI and also GYBI samples investigated during 1, 7, 14 and 21 days of the shelf life at 4 °C. The results illustrated that probiotic viability did not fall below a recommended limit of 10^6 CFU.g^{-1} during the shelf life. Inulin addition slowed down probiotic viability reduction, and the highest (7.08 CFU.g^{-1}) obtained in GYL on the 21st day. Microbial characteristics demonstrated a growth reduction for spoilage microorganisms in GYLI and GYBI compared to the control. The minimum and maximum pH (4.12) and acidity (1.47 % lactic acid) levels in GYLI found on the 21st day, respectively. The inulin presence indicated no significant effect on protein and fat; however, total solid contents improved. Inulin declined the water retention, but elevated WHC, sensory investigation, and rheological attributes. The overall results portrayed that synbiotic Greek yogurt of GYLI produced with desirable sensory acceptability.

Keywords: *Bifidobacterium longum* HH-BL18, Greek yogurt, *Lactobacillus rhamnosus* GG, Probiotic, Rheology, Synbiotic, Viability.

Introduction

Yogurt is a plentiful source of essential minerals such as phosphorus, calcium, potassium, vitamins (A, B_2, and B_{12}), more-quality biological proteins, and fatty acids that can be an outstanding carrier of probiotics (Arabshahi-Delouee *et al.*, 2023; Zhang *et al.*, 2025). The probiotic survival influenced by several factors during the treating and shelf life; therefore, production of edible products containing an adequate population (at least 10^6 CFU.g^{-1} in consumption time) remains a challenge (Brishti *et al.*, 2025; Nouri, 2023). The probiotic viability is commercially low; however, Greek yogurt can be a suitable carrier due to higher total solids, fat, and buffering capacity that contribute to enhanced survival and stability, potentially offering greater health benefits (Santos *et al.*, 2025). The most commercial probiotics in dairy products belong to strains from the genera Lactobacillus and Bifidobacterium (Ziar *et al.*, 2025). *Lactobacillus rhamnosus* GG

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(*L. rhamnosus* GG) is indeed a gram-positive, facultatively anaerobic and non-spore-forming bacterium that illustrates health benefits, mitigates gut microbiota dysbiosis, and maintains colonic barrier integrity in inflammatory bowel disease and diarrhea disorders (Wang *et al*., 2025). The *Bifidobacterium longum* HH-BL18 (*B. longum* HH-BL18) is a gram-positive and catalase-negative bacterium in an intestinal tract, which widely applied for probiotic products owing to anti-inflammatory and immunomodulatory potentials (Kim *et al*., 2025). *L. rhamnosus* GG encapsulated using an innovative electrospraying method and conventional freeze-drying in a prebiotic yogurt based inulin and whey protein isolate matrices to enhance stability, bioavailability, and controlled release under gastrointestinal conditions (Zepeda-Hernández *et al*., 2025). *B. longum* HH-BL18 as a probiotic culture supplemented with yogurt to improve nutritional value and health-promoting properties (Son *et al*., 2023). The previous studies have demonstrated that prebiotic addition, such as inulin, polydextrose, and modified starch into Greek yogurt can enhance viability of *Lactobacillus casei* probiotics, maintaining counts above 10^7 CFU.mL^{-1} (Tarrah *et al*., 2019; Tavakoli *et al*., 2025). A comparison of *Lactobacillus helveticus* as a probiotic bacterium in Greek yogurt produced via ultrafiltration and centrifugation revealed that viability was 3 to 7 times higher than that of a conventional stirred sample (Costa *et al*., 2022). The addition of three specific prebiotics (inulin, Hi-maize, and β-glucan) into yogurt significantly influenced probiotic survival to improve as compared to the control, and 2.5, 1.5, and 2.5 % β-glucan had the maximum mean viability 8.95 Log CFU.mL^{-1} (Jaman *et al*., 2022). The objective of the present research was to evaluate the probiotic bacteria viability in Greek yogurt containing *L. rhamnosus* GG (GYL) and *B. longum* HH-BL18 (GYB), and individually with inulin as a prebiotic (GYLI and GYBI), and assess their effects on physicochemical and sensory attributes during the shelf life.

Materials and Methods

- *Materials*

Pasteurized whole milk of cow and cream (3 and 40 % fat) with protein concentrate powder procured from Mihan Factory (Tehran, Iran). The probiotic bacterial strains (*L. rhamnosus* GG and *B. longum* HH-BL18) and inulin purchased from Christian Hansen (Hørsholm, Denmark) and Orfati (Oreye, Belgium) companies, respectively.

- *Preparation method of Greek yogurt samples*

In the present research, four probiotic formulations prepared GYL, GYB, GYLI, and GYBI. The pasteurized whole milk homogenously blended with cream and protein concentrate powder to achieve a final formulation containing 4.2 % fat and 7.5 % protein. GYL and GYB mixed individually with inulin as a prebiotic dietary fiber at 2 % (w/w) concentration (GYLI and GYBI). The resulted sample homogenized at 55 °C under 150 bar pressure, followed by pasteurization in a 90 °C water bath for 5 min; subsequently, the mixture was cooled (44 °C prior) to further processing. The conventional yogurt starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. delbrueckii* subsp. *bulgaricus*) added at the concentration recommended by the manufacturer. In addition, a probiotic culture comprising GYL and GYB inoculated at an initial 10^6 CFU.mL^{-1} concentration to ensure adequate viability in the final product. The mixture incubated at 44 °C until pH reached approximately 4.3, after which samples transferred to refrigeration. During subsequent cold storage, pH of samples gradually reduced, reaching values between 3.31 and 3.34. The prepared yogurt treatments maintained at 4 °C and sampled for chemical, microbiological, and sensory analyses on days 1, 7, 14, and 21 post-productions. The sampling procedures conducted following section 11 of the Iranian National Standard No. 695 (ISIRI, 2022).

- ***Microbiological analyses***

Viable probiotic counts were determined using selective media such as De Man, Rogosa, and Sharpe agar and supplemented with cysteine, respectively; then, plates incubated anaerobically at 37 °C for 48 to 72 h (Pascariu *et al*., 2025). The enumeration of molds and yeasts conducted on Potato Dextrose agar acidified with tartaric acid (pH approximately 3.5) to inhibit bacterial growth; afterwards, samples were plated and incubated aerobically at 25 °C for 5 to 7 days (Kalkan *et al*., 2025). The coliform bacteria distinguished and enumerated using Violet Red Bile agar under aerobic incubation at 37 °C for 24 h, following the standard protocols (Albay *et al*., 2025).

- ***Physicochemical assays***

Physicochemical attributes of yogurt evaluated during shelf life to assess product quality and composition. The pH determined by a calibrated digital pH meter with ± 0.01 accuracy, and titratable acidity measured using titration with 0.1 N NaOH and expressed as lactic acid (%). This measurement performed by drying a known sample weight in an oven at 105 ± 1 °C until a constant level achieved. The protein content assessed using the Kjeldahl method, where nitrogen converted using 6.38 conversion factor. The fat quantified using the Gerber method, a widely acid digestion technique that efficiently separated from the dairy matrix, enabling precise percentage (AOAC, 2020).

- ***Syneresis***

Syneresis, defined as whey from yogurt, quantified gravimetrically and expressed as an initial sample weight percentage. For this purpose, a standardized portion of yogurt carefully placed onto qualitative filter paper positioned within a glass funnel, using a rounded scoop to ensure uniform sample size and shape. The setup left undisturbed at 25 ± 1 °C for 4 h, and the whey volume that drained through the filter weighed, and syneresis calculated using Equation 1 (Turgut and Diler, 2025):

$$\text{Syneresis (\%)} = \frac{\text{Expelled whey weight}}{\text{Initial yogurt weight}} \times 100$$

- ***Water-holding capacity (WHC) assay***

WHC, an essential indicator of yogurt structural integrity and ability to retain moisture under applied force, assessed via centrifugation. A 10.00 g aliquot of probiotic yogurt placed in a tube and subjected to centrifugation at 1400 rpm for 12 min at 20 ± 1 °C. The supernatant whey yogurt carefully decanted and weighed; afterwards, WHC calculated according to the following Equation 2 (Costa *et al*., 2022):

$$\text{WHC (\%)} = \frac{(\text{Initial yogurt weight} - \text{whey yogurt wight})}{\text{Initial yogurt weight}} \times 100$$

All measurements performed in triplicate, and results reported as mean values ± standard deviation.

- ***Sensory evaluation and acceptance assay***

Sensory attributes of yogurt evaluated by established protocols for dairy product assessment. The sensory assessment conducted by a five-point hedonic scale to determine consumer acceptance, where scores ranged from 1 to 5, corresponding to "dislike extremely", "dislike", "neither like nor dislike", "like", and "like extremely", respectively. A panel of 30-trained assessors (15 men and 15 women) participated in the evaluation. Each yogurt sample coded with a randomized three-digit number to ensure unbiased investigation. The panelists evaluated the samples under controlled conditions, focusing on key sensory attributes including appearance, texture, flavor, and overall acceptability. The hedonic scores statistically analyzed to quantify consumer preference degree and acceptance for each formulation (Wang *et al*., 2024).

- ***Dynamic rheological characterization***

The viscoelastic behavior is investigated using a rotational rheometer (MCR-302, Anton Paar, and Austria) equipped with parallel plate geometry. The stress sweep assay performed at a constant frequency to measure the linear viscoelastic region. Within this range, the storage (G') and loss (G'') moduli were calculated to assess gel structure and consistency. Measurements conducted during the shelf life to monitor changes in rheological behavior over time (Le Ba *et al*., 2025).

- ***Scanning electron microscopy (SEM) analysis***

Greek yogurt samples (GYL, GYB, GYLI and GYBI) collected on days 1 and 21 of refrigerated storage (4 °C) for microstructural analysis. Central portions (~1 cm^3) frozen at -40 °C, lyophilized, mounted on aluminum stubs and sputter-coated with ~10 nm gold-palladium. Images were captured using a SEM (Hitachi S-4800, Hitachi High-Technologies Corporation, Tokyo, Japan) at a magnification corresponding to a 5 µm scale bar (Dias *et al*., 2021).

- ***Statistical analysis***

A full factorial completely randomized design and employed to evaluate the effects of three independent variables: probiotic bacterial strain, inulin concentration, and shelf life. All formulations and analytical measurement performed in triplicate and duplicate to ensure reproducibility, respectively. Data were investigated through variance analysis using Minitab version 19 (Minitab LLC, USA), means evaluated by Least Significant Difference at $p < 0.05$.

Results and Discussion

- ***Probiotics survivability and quantitative assessment of microbial contamination***

As illustrated in Figure 1, probiotic viability declines significantly over time ($p < 0.05$) that is observed consistently across both strains and all formulation types. Nonetheless, probiotic populations remained within functionally acceptable limits throughout the shelf life across all samples, indicating that formulations maintained satisfactory stability. The samples supplemented with inulin outlined significantly greater probiotic viability during days 7 and 21 of shelf life than those lacking inulin. Additionally, GYL exhibited the highest viability among all evaluated treatments. A significant difference ($p < 0.05$) detected in probiotic viability among yogurt formulations containing varying inulin levels and distinct strains at equivalent time points. The results portrayed a significant impact of inulin concentration and probiotic strain on viability utilized in the present study. Across all treatments, probiotic populations consistently maintained above the recommended viability threshold throughout the shelf life, indicating satisfactory survival. No growth of molds, yeasts, and coliform bacteria detected in any treatment or shelf life interval under optimal hygienic conditions.

The successful development of the probiotic dairy products requires that selected microbial strains retain their viability and functional efficacy throughout the entire shelf life (Dias *et al*., 2021). The production and stability of probiotic products influenced by several factors, including pH, specific strains, hydrogen peroxide, and dissolved oxygen, metabolic concentrations (lactic and acetic acids), matrix buffering capacity, temperature, and additive nature in formulation (Sarwar *et al*., 2022). A marked reduction in probiotic viability observed over the yogurt shelf life, which attributed to harsh processing conditions, reduced pH levels, and elevated acidity inherent to the product matrix (Kumar and Kumar, 2016). The elevated acidity adversely affects probiotic growth and under excessive concentration of free hydrogen ions disrupts mass transfer across cell membrane and alters cytoplasmic pH, thereby damaging and reducing viability, which provides a plausible explanation for decline in survival during shelf life (Olson and Aryana, 2022; Sarwar *et al*., 2022). The previous report represented that supplementation of low-fat yogurt with inulin significantly enhanced *Lactobacillus acidophilus* (*L. acidophilus*) and *L. delbrueckii subsp. bulgaricus* viability, which corresponded factors such as strain type, product formulation, shelf life, and others (Dias *et al*., 2021). A modest enhancement in starter culture viability employed in low-fat yogurt production distinguished with incorporation of 0.5 % inulin (Albay *et al*., 2025; Li *et al*., 2019). The edible products marketed with probiotic claims must contain a minimum survival rate at the consumption point to guarantee the viability threshold necessary for conferring health benefits (Kumar and Kumar, 2016). The results demonstrated that probiotic yogurt containing inulin indicated the absence of mold and yeast (Kamel *et al*., 2021). Inulin appears to suppress yeast proliferation, consequently extending the shelf life of probiotic yogurt; moreover, this preservative effect relates to elevated lactic acid (Kamarinou *et al*., 2022; Pinto *et al*., 2017).

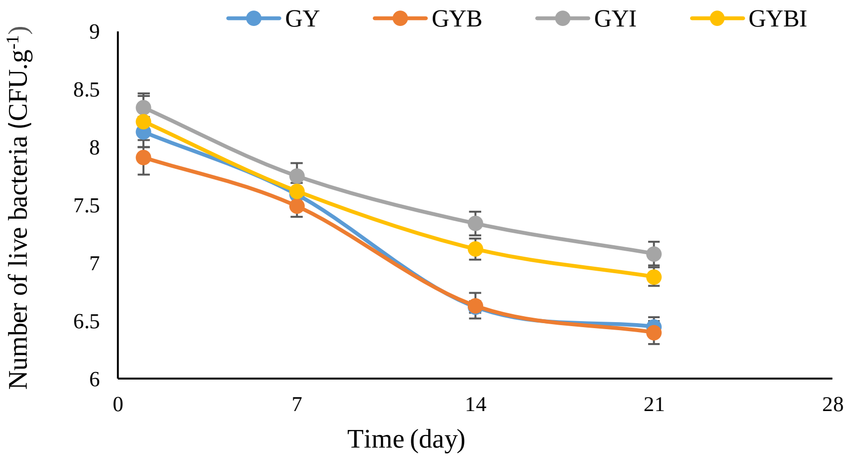


Fig. 1. *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* HH-BL18 viability (Log CFU.g^{-1}) in Greek yogurt samples (affected by inulin) during 21 days of shelf life Probiotic Greek yogurt containing *Lactobacillus rhamnosus* GG (GYL) and *Bifidobacterium longum* HH-BL18 (GYB) and individually with inulin as a prebiotic (GYLI and GYBI)

- ***Comprehensive physicochemical traits: pH, acidity values, fat, protein, and total solids***

Titratable acidity and pH are key quality indicators in yogurt; as portray in Table 1. The initial and final pH samples ranged from 4.31 to 4.34 and 4.12 to 4.27, respectively. The pH of probiotic and synbiotic Greek yogurts significantly declined during refrigerated shelf life (p<0.05). Furthermore, pH values not significantly influenced by specific probiotic strain employed (p> 0.05); however, GYBI and GYLI exhibited a substantially lower level than the control. GYBI and GYLI demonstrated a more pronounced reduction in pH to the control. The titratable acidity measured on the 1st day post-production ranged from 1.24 to 1.27 g lactic acid per 100 g, which enhanced between 1.33 and 1.47 by the end of shelf life across various treatments (Table 1).

The improvement in titratable acidity was statistically significant (p < 0.05) over the shelf life. While probiotic strain type had no significant impact on titratable acidity (p>0.05), GYBI and GYLI exhibited considerably lower levels to the control.

The total solids significantly enhanced with 2 % inulin addition (Table 1). The highest total solids detected in GYBI and GYLI, ranging from 17.33 to 17.66 %. Additionally, no significant changes distinguished in total solids of samples throughout the shelf life, and a probiotic strain had no considerable effect.

Table 1. pH, acidity values (% lactic acid), fat, protein and total solids of Greek yogurt samples (affected by inulin) during shelf life (mean ± standard deviation)

Shelf life (Day)	**1**	**7**	**14**	**21**
Samples				
		pH		
GYL	4.33±0.04^{aA}	4.27±0.01^{aAB}	4.23±0.03^{aB}	4.24±0.04^{aB}
GYB	4.34±0.01^{aA}	4.26±0.03^{aB}	4.27±0.04^{aB}	4.27±0.04^{aB}
GYLI	4.31±0.03^{aA}	4.25±0.04^{aAB}	4.22±0.04^{aB}	4.12±0.05^{aC}
GYBI	4.32±0.03^{aA}	4.28±0.03^{aAB}	4.21±0.03^{aB}	4.14±0.07^{Ac}
		Acidity (% lactic acid)		
GYL	1.25±0.05^{aB}	1.31±0.03^{aAB}	1.33±0.02^{aAB}	1.35±0.04^{aA}
GYB	1.24±0.03^{aB}	1.30±0.02^{aAB}	1.34±0.03^{aA}	1.33±0.03^{aA}
GYLI	1.26±0.05^{aC}	1.33±0.03^{aC}	1.39±0.02^{B}	1.47±0.04^{aA}
GYBI	1.27±0.04^{aC}	1.31±0.02^{aC}	1.37±0.02^{aB}	1.44±0.03^{aA}
		Fat (%)		
GYL	4.30±0.05^{aA}	4.28±0.12^{aA}	4.26±0.07^{aA}	4.25±0.05^{aA}
GYB	4.21±0.03^{aA}	4.22±0.05^{aA}	4.23±0.04^{aA}	4.24±0.04^{aA}
GYLI	4.16±0.06^{aA}	4.18±0.05^{aA}	4.19±0.04^{aA}	4.21±0.05^{aA}
GYBI	4.18±0.07^{aA}	4.20±0.10^{aA}	4.22±0.12^{aA}	4.24±0.07^{aA}
		Protein (%)		
GYL	7.60±0.03^{aA}	7.59±0.04^{aA}	7.57±0.09^{aA}	7.56±0.03^{aA}
GYB	7.61±0.03^{aA}	7.59±0.05^{aA}	7.57±0.05^{aA}	7.56±0.03^{aA}
GYLI	7.57±0.03^{aA}	7.55±0.06^{aA}	7.54±0.06^{aA}	7.53±0.03^{aA}
GYBI	7.65±0.03^{aA}	7.57±0.07^{aA}	7.52±0.04^{aA}	7.49±0.03^{aA}
		Total solids (%)		
GYL	15.04±0.03^{bA}	15.03±0.04^{bA}	15.02±0.05^{bA}	15.01±0.05^{bA}
GYB	15.03±0.05^{bA}	15.02±0.02^{bA}	15.01±0.03^{bA}	15.00±0.03^{bA}
GYLI	16.00±0.02^{aA}	16.01±0.05^{aA}	16.03±0.02^{aA}	16.04±0.03^{aA}
GYBI	17.05±0.03^{aA}	17.04±0.04^{aA}	17.03±0.03^{aA}	17.02±0.04^{aA}

[*] Distinct capital and lowercase letters indicate significant differences between each time (column) and treatments (rows), respectively.

The fat results revealed no significant differences ($p > 0.05$) across distinct samples and shelf life (Table 1). No remarkable differences found in a protein of GYB and GYL; additionally, varying inulin concentrations did not exert a significant influence ($p > 0.05$). Furthermore, protein changes were not statistically significant in samples during the 21-day refrigerated shelf life.

The reduction in pH during the shelf life of fermented dairy products commonly attributed to organic production by lactic acid bacteria, primarily due to synthesis, particularly *L. delbrueckii subsp. bulgaricus* (Dias *et al*., 2021). A similar decline in pH observed during the shelf life in probiotic yogurts containing *L. acidophilus* and bifidobacteria, where initial values ranged from 4.33 to 4.44 on day 0, decreasing 4.16 to 4.22 after 35 days (Jaman *et al*., 2022). A comparable study demonstrated that higher inulin in frozen yogurt led to a significant rise in titratable acidity (Rezaei *et al*., 2014). Another report showed that inulin addition at concentrations ranging from 0.2 to 0.6 % improved titratable acidity and diminished pH in probiotic yogurt over 16 days (Kamel *et al*., 2021). The adding inulin into probiotic Greek yogurt caused reduction and elevation in pH alongside and titratable acidity throughout 28 days, which physicochemical alterations negatively affected sensory acceptability (Dias *et al*., 2021). In yogurt containing *L. rhamnosus* GG and *B. longum* HH-BL18, a gradual decline in pH and acidity that is more titratable observed during the shelf life, indicating ongoing metabolic activity of probiotic strains (Okur *et al*., 2025). The previous studies have shown that inulin and oligofructose supplements improved total solids in fermented prebiotic milk (Li *et al*., 2019; Pinto *et al*., 2017). These findings were consistent with a research on yogurt containing *L. rhamnosus* GG and *B. longum* HH-BL18 to physicochemical composition, such as total solids, protein, and fat, which illustrated no significant differences compared to the control (Kamel *et al*., 2021).

- *Syneresis assessment*

As depicted in Figure 2a, a significant reduction in syneresis index found for yogurt containing prebiotics compared to the control ($p<0.05$). GYB and GYL demonstrated a higher syneresis throughout the shelf life. Furthermore, extending the time remarkably elevated syneresis; however, the probiotic strain did not have a significant influence ($p< 0.05$). Comparable results reported regarding lower syneresis in yogurt with inulin incorporation and other additives such as casein, whey protein, and similar ingredients (Costa *et al*., 2022; Turgut and Diler, 2025). Syneresis occurs as hydrogen ions increases, leading to lower repulsive forces, resulting in aggregation within casein micelles that the present phenomenon pronounced by prolonged shelf life (Turgut and Diler, 2025).

- *WHC analysis*

GYBI and GYLI exhibited higher WHC than those without inulin during shelf life (Figure 2b). A significant reduction ($p < 0.05$) in water loss indicated with inulin addition, alongside more total solids. The WHC did not exhibit substantial variation, suggesting that probiotic strain did not influence on this characteristic. The WHC declined for all yogurt samples, particularly those without inulin, as the shelf life progressed. The elevated WHC in yogurts containing inulin might be attributed to relatively more compact matrix (acting as a texture modifier), absorption and susceptible to water loss (Costa *et al*., 2022). The lower

WHC could result from weaker bonds between H_2O molecules and the yogurt gel network, which influenced by pH reduction and associated biochemical events occurring during shelf life (Aslam *et al*., 2015). The syneresis absence and enhanced WHC that contributed to firmness and stability throughout shelf life, are crucial structural characteristics of yogurt (Kamel *et al*., 2021).

- ***Sensory attributes***

The sensory evaluation results indicate no statistically significant differences among the distinct samples in color and appearance, as illustrated in Figure 3 ($p < 0.05$). The shelf life did not have a significant impact on the visual characteristics of the yogurt samples under study. Although GYBI and GYLI exhibited a glossier and smoother surface, receiving relatively higher scores for visual acceptability compared to yogurt without inulin, the mentioned difference was not statistically significant ($p > 0.05$). The inulin utilized in the present research was inherently devoid of any odor characteristic. According to the results obtained, did not exert a measurable impact on metabolic pathways for either traditional starter cultures or probiotic bacteria that would lead to distinctive aroma or flavor production. The inulin addition up to 2 % concentration probiotic in Greek yogurts significantly improved ($p < 0.05$) flavor acceptability with the maximum scores. The shelf life did not exert a statistically significant effect on flavor profile, which remained acceptable ($p > 0.05$). Throughout the shelf life, texture scores of GYBI and GYLI were significantly more favorable than those of the control ($p < 0.05$). GYBI and GYLI exhibited a smoother and delicate texture compared to their non-counterparts. According to panelist evaluations, neither the probiotic strain type nor the 21-day shelf life at 4 °C had a statistically significant influence on overall acceptability.

The metabolic activity for probiotic bacteria can occasionally lead to component production that adversely affects the aroma and flavor of the final product (Pourjavid *et al*., 2023; Wang *et al*., 2024). The results indicated that probiotic strain type did not have a statistically significant impact on flavor and textural attributes; however, inulin addition to yogurt samples resulted in improved mentioned functions as compared to plain formulations (Crispín-Isidro *et al*., 2015; Sarwar *et al*., 2022). Inulin applied as a fat replacer to increase the creaminess perception in ice cream (Akbari *et al*., 2016). The sensory attributes for low-fat stirred yogurt enhanced as compared to full sample because of inulin and fructan incorporation, primarily gel formation in conjunction with casein micelles, which positively influenced texture and overall

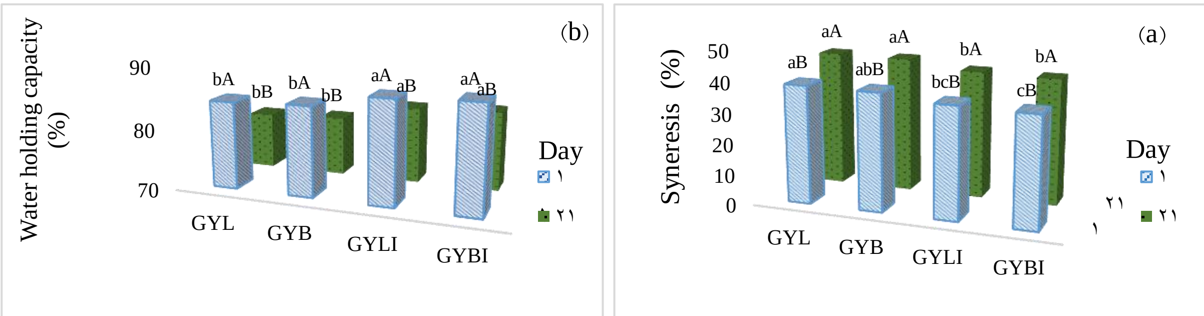


Fig. 2. (a) Syneresis (%) and (b) water holding capacity (%) for Greek yogurts containing probiotics and inulin during 21 days of shelf life at 4 °C Probiotic Greek yogurt containing *Lactobacillus rhamnosus* GG (GYL) and *Bifidobacterium longum* HH-BL18 (GYB) and individually with inulin as a prebiotic (GYLI and GYBI)

mouthfeel (Crispín-Isidro *et al*., 2015). The previous findings depicted that inulin addition improved overall acceptability and flavor of probiotic and low-fat yogurt (Jaman *et al*., 2022; Sarwar *et al*., 2022).

- *Rheological behavior*

The rheological results for yogurt samples throughout the shelf life is illustrated in Figure 4. The progressive increase in G' values reflected a gradual reinforcement of the gel network during the shelf life in GYLI and GYBI. This enhancement attributed to inulin, which promoted gel strength by forming hydrogen bond networks and improving the total solids content. Consequently, a more stable and elastic behavior of gel structure rheology develops over time. G′ modulus either declined or remained relatively stable during shelf life, potentially due to progressive weakening of the protein network, which influenced by enzymatic activity or pH-induced structural changes in GYL and GYB. A gradual enhancement in G'' observed, albeit with a gentler slope for GYLI and GYBI. This suggested a modest improvement of viscous (liquid-like) matrix; however, an elastic structure remained predominant, as evidenced by $G' > G''$. The G'' reduction or stabilization in GYL and GYB attributed to gradual breakdown of matrix components and a consequent decreased gel flowability. The inulin addition enhanced gel framework and G', but reduced structural degradation during shelf life. Inulin combination with probiotics, particularly GYL, may exert a positive synergistic effect on texture and functional properties.

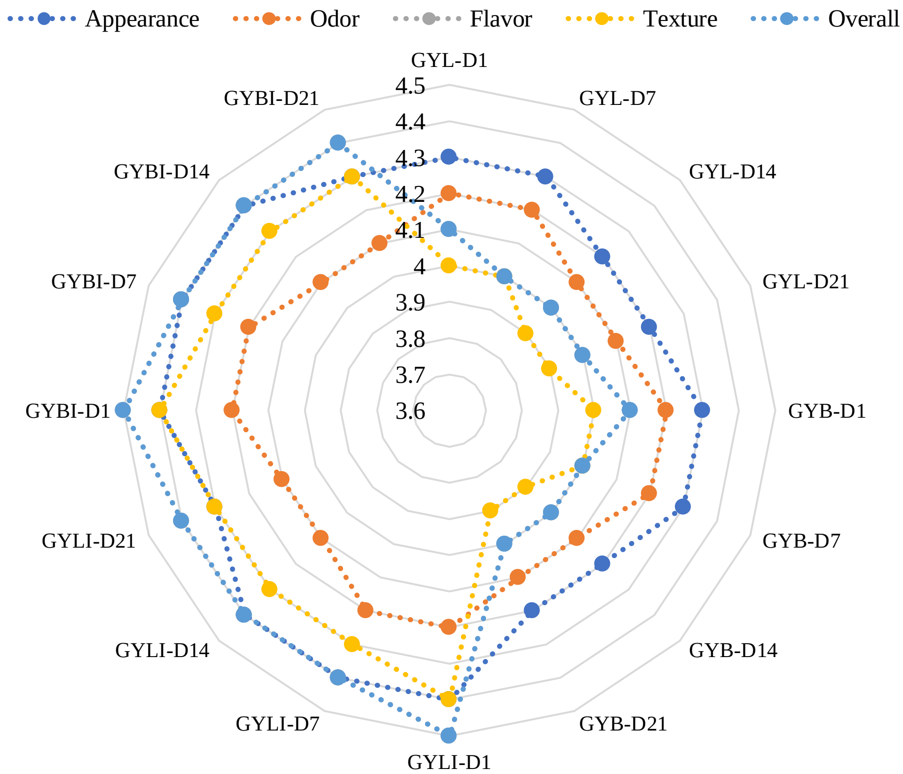
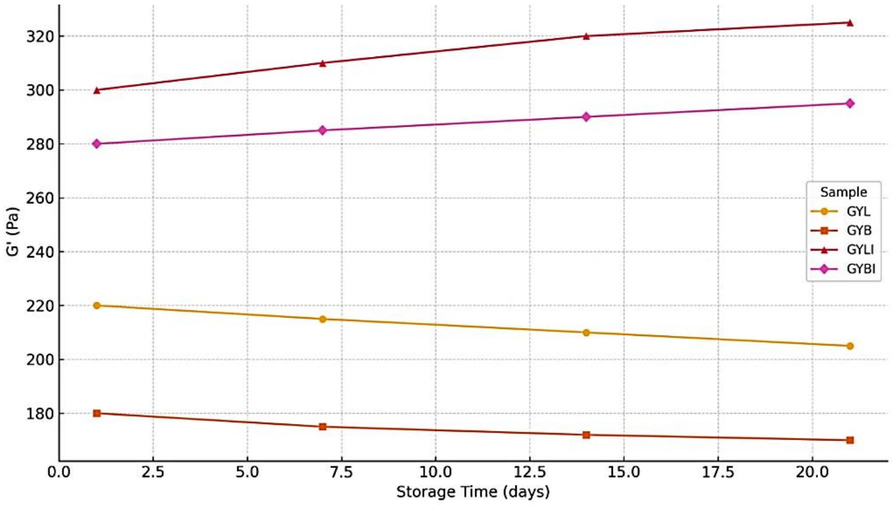


Fig. 3. Sensory attributes for Greek yogurt samples (affected by inulin) during shelf life Probiotic Greek yogurt containing *Lactobacillus rhamnosus* GG (GYL) and *Bifidobacterium longum* HH-BL18 (GYB) and individually with inulin as a prebiotic (GYLI and GYBI)



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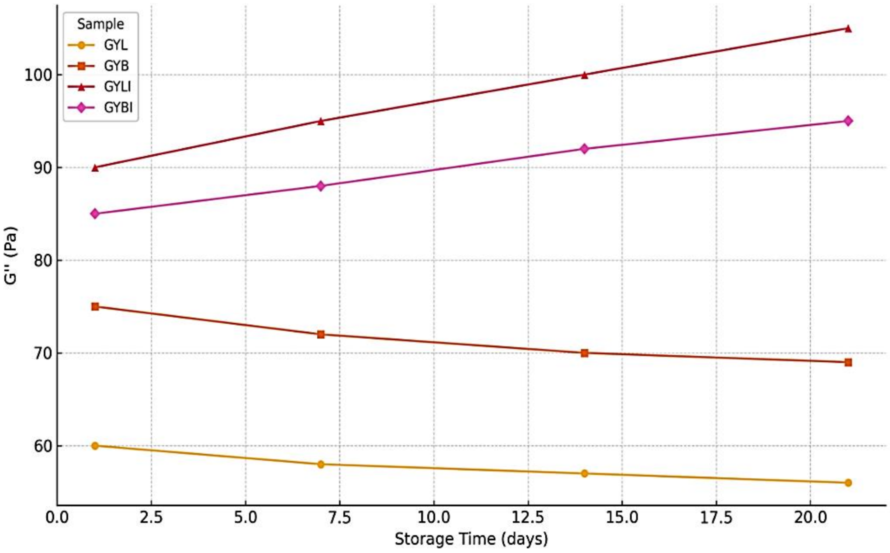

b

Fig. 4. (a) G′ (storage) and (b) G″ (loss) modulus of Greek yogurt samples (affected by inulin) during shelf life Probiotic Greek yogurt containing *Lactobacillus rhamnosus* GG (GYL) and *Bifidobacterium longum* HH-BL18 (GYB) and individually with inulin as a prebiotic (GYLI and GYBI)

A previous study demonstrated that inulin elevated G′, and a beneficial role as a prebiotic in enhancing yogurt structure and had been well documented (Le Ba *et al*., 2025). Furthermore, dietary fibers such as inulin improved rheological characteristics and consistency of dairy products (El-Sayed and El-Sayed, 2021). The previous researchers reported that synergistic incorporation of probiotics and dietary fibers indicated enhanced rheological stability (Le Ba *et al*., 2025; Tian *et al*., 2020).

- ***Structure assessment of samples***

The SEM images revealed microstructural differences among GYL, GYLI, GYB, and GYBI on days 1 and 21 of shelf life that correspond with the known physicochemical trends from this study (Figure 5). On 1st day, GYL appeared as aggregated clusters with some roughness and voids between particles, indicating a less compact protein gel matrix. In contrast, GYLI exhibited a more densely packed and smoother microstructure with fewer visible gaps, suggesting that inulin enhanced the gel network formation early in shelf life. GYB showed larger, more separated particles and smoother surfaces, reflecting a weaker protein network and a more open matrix. Meanwhile, GYBI displayed tighter aggregation and a smaller particle size distribution compared to GYB, indicating improved gel cohesion due to inulin.

By 21st day, GYL became more compact, but still exhibited noticeable pores and cracks, suggesting ongoing protein rearrangement and syneresis. GYLI maintained a cohesive, dense structure with minimal pore development, highlighting inulin role in preserving WHC and structural integrity over time. GYB matrix appeared denser than on the 1st day and showed more visible cracks and irregular voids compared to GYBI, indicating continued moisture loss and protein contraction.

GYBI retained a relatively smooth and interconnected microstructure, reflecting slower deterioration and better WHC than GYB. The GYLI and GYBI consistently displayed finer, more compact microstructures with fewer voids over 21 days, aligning higher WHC and lower syneresis observed in your study. The GYL and GYLI tended to form a more clustered, granular structure, whereas GYB and GYBI exhibited a smoother, larger-particle nature. Structural tightening over shelf life corresponded to gel network contraction due to ongoing acidification with inulin mitigating excessive collapse.

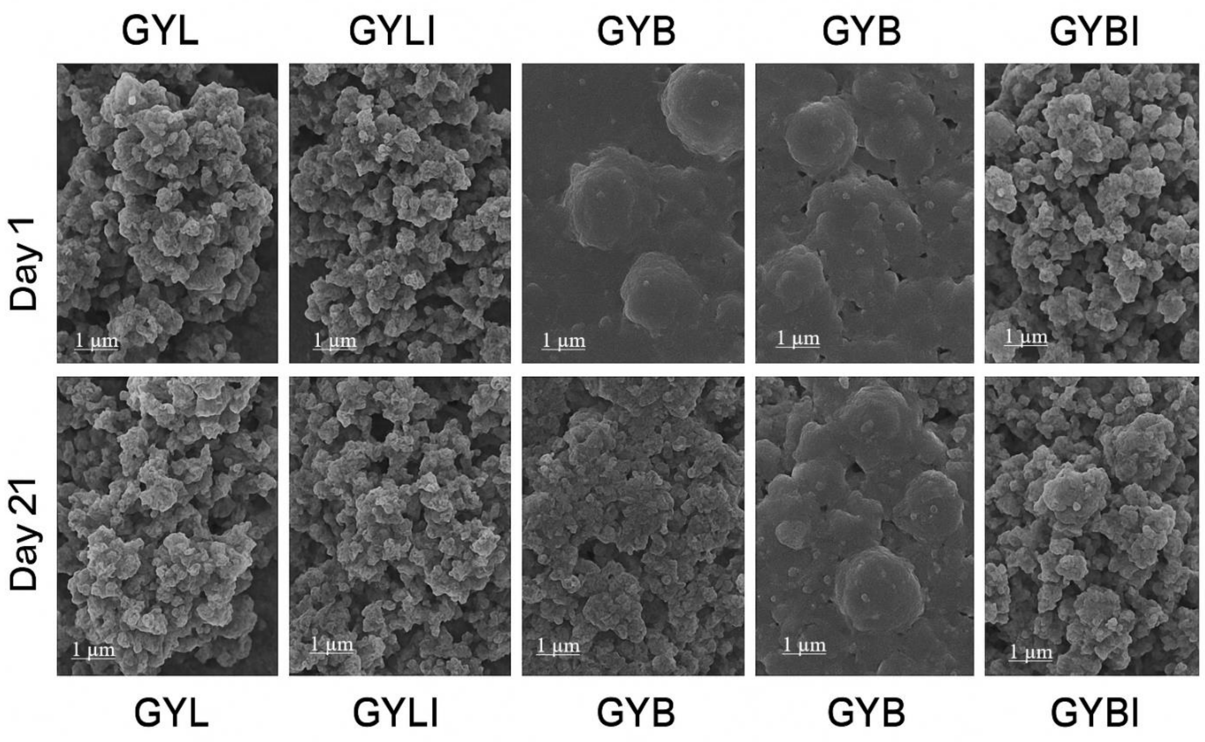

Fig. 5. SEM images of Greek yogurt samples (affected by inulin) on the 1th and 21st of shelf life Probiotic Greek yogurt containing *Lactobacillus rhamnosus* GG (GYL) and *Bifidobacterium longum* HH-BL18 (GYB) and individually with inulin as a prebiotic (GYLI and GYBI).

In SEM images, GYLI and GYBI exhibited a smoother, uniform structure with smaller pore sizes compared to those GYL and GYB. This aligned with findings from previous studies indicating that inulin reduces pore size and enhances gel cohesion due to water-binding properties (Dias *et al*., 2021; Kamel *et al*., 2021). Over a 21-day shelf life, inulin-containing yogurts maintained their structural integrity better than control. The latter showed more visible cracks and signs of syneresis, while the former retained a cohesive gel network; which, stability attributed to inulin's role as a stabilizing agent (Costa *et al*., 2022). While inulin influence more pronounced, there were also strain-specific effects observed. For instance, formulations with *L. rhamnosus* GG tended to have a more granular structure, whereas those with *B. longum* HH-BL18 displayed smoother surfaces. This suggested that the choice of probiotic strain could subtly affected yogurt microstructure, although adding prebiotics like inulin often has a dominant effect (Turgut and Diler, 2025). The denser gel networks in inulin-containing yogurts correlated with enhanced sensory attributes such as a smoother texture and creamier mouthfeel, which positively evaluated by panelists. This supported the conclusion that inulin not only improves physical parameters but also contributes to better sensory qualities (Costa *et al*., 2022; Turgut and Diler, 2025).

Conclusion

The present research outlined that Greek yogurt represented an effective vehicle for probiotic delivery, due to superior physicochemical characteristics relative to conventional samples, which enhanced survival and stability. Meanwhile, inulin had no significant influence on parameters, such as fat, protein, pH, and acidity. GYLI and GYBI significantly improved probiotic strain viability during shelf life, while simultaneously reducing syneresis, elevating WHC, and sensory qualities including flavor, texture, and overall acceptability. Additionally, considerable differences did not observe between the effects of two-probiotic strains utilized. The findings recommend that incorporating inulin alongside probiotics offer a practical approach to develop high-quality synbiotic Greek yogurt with improved functional and beneficial attributes for consumers.

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References

Akbari, M., Eskandari, M. H., Niakosari, M. & Bedeltavana, A. (2016). The effect of inulin on the physicochemical properties and sensory attributes of low-fat ice cream. *International Dairy Journal*, *57*, 52-55. https://doi.org/10.1016/j.idairyj.2016.02.040

Albay, Z., Çelebi, M. & Şimşek, B. (2025). Physicochemical, rheological, and microbiological properties of honey-fortified probiotic drinkable yogurt. *Foods and Raw Materials, 13*(2), 320-329. https://doi.org/10.21603/2308-4057-2025-2-641

AOAC. (2020). Association of official analytical chemists, In: Official methods of analysis. 19th ed. Arlington VA.USA.

Aslam, H. K. W., Saeed, M., Shakeel, A., Pasha, İ., Shabbir, M. A. & Raza, M. S. (2015). Extraction of Inulin from Cichorium intybus and its application as fat replacer in yoghurt. *Journal of Applied Biological Sciences*, *9*(3), 86-94. https://doi.org/10.1016/j.aoas.2020.02.002

Arabshahi-Delouee, S., Rahati Ghochani, S. & Mohammadi, A. (2020). Effect of flaxseed (Linum usitatissimum) mucilage on physicochemical and sensorial properties of semi-fat set yoghurt. *Journal of Food Biosciences and Technology*, *10*(2), 91-100. https://doi.org/10.32598/IJVM.17.3.1005249

Brishti, M. R., Venkatraman, G., Baba, A. S. B. H., Yajit, N. L. M. & Karsani, S. A. (2025). Natural bioactive compounds enriched functional yogurt: impact on the probiotic bacteria and its potential health benefits. *Probiotics and Antimicrobial Proteins*, 1-18. https://doi.org/10.1007/s12602-025-10461-1

Costa, M. P., Rosario, A. I. L., Silva, V. L., Vieira, C. P. & Conte-Junior, C. A. (2022). Rheological, physical and sensory evaluation of low-fat cupuassu goat milk yogurts supplemented with fat replacer. *Food Science of Animal Resources*, *42*(2), 210-224. https://doi.org/10.5851/kosfa.2021.e64

Crispín-Isidro, G., Lobato-Calleros, C., Espinosa-Andrews, H., Alvarez-Ramirez, J. & Vernon-Carter, E. J. (2015). Effect of inulin and agave fructans addition on the rheological, microstructural and sensory properties of reduced-fat stirred yogurt. *LWT-Food Science and Technology*, *62*(1), 438-444. https://doi.org/10.1016/j.lwt.2014.06.042

Dias, S. S., De Souza Vergílio, D., Pereira, A. M., Klososki, S. J., Marcolino, V. A., Da Cruz, R. M. S. & Pimentel, T. C. (2021). Probiotic Greek yogurt: Effect of the addition of prebiotic fat substitutes on the physicochemical characteristics, probiotic survival, and sensory acceptance. *Journal of Dairy Research*, *88*(1), 98-104. https://doi.org/10.1017/S0022029921000121

El-Sayed, S. M. & El-Sayed, H. S. (2020). Production of UF-soft cheese using probiotic bacteria and Aloe vera pulp as a good source of nutrients. *Annals of Agricultural Sciences*, *65*(1), 13-20. https://doi.org/10.1016/j.aoas.2020.05.002

ISIRI. (2022). Yoghurt, Specifications and test methods. Iran National Standards Organization, sixth Revision, No. 695.

Jaman, S., Islam, M. Z., Sojib, M. S. I., Hasan, M. S., Khandakar, M. M. H., Bari, M. S. & Harun-ur-Rashid, M. (2022). Physicochemical characteristics, sensory profile, probiotic, and starter culture viability of synbiotic yogurt. *Journal of Advanced Veterinary and Animal Research*, *9*(4), 694-701. https://doi.org/10.5455/javar.2022.i638

Kalkan, S., Incekara, K., Otağ, M. R. & Unal Turhan, E. (2025). The influence of hazelnut milk fortification on quality attributes of probiotic yogurt. *Food Science & Nutrition*, *13*(5), 70235. https://doi.org/10.1002/fsn3.70235

Kamarinou, C. S., Papadopoulou, O. S., Doulgeraki, A. I., Tassou, C. C., Galanis, A. & Chorianopoulos, N. G. (2022). Mapping the key technological and functional characteristics of indigenous lactic acid bacteria isolated from Greek traditional dairy products. *Microorganisms*, *10*(2), 246-250. https://doi.org/10.3390/microorganisms10020246

Kamel, D. G., Hammam, A. R., Alsaleem, K. A. & Osman, D. M. (2021). Addition of inulin to probiotic yogurt: Viability of probiotic bacteria (Bifidobacterium bifidum) and sensory characteristics. *Food Science & Nutrition*, *9*(3), 1743-1749. https://doi.org/10.1002/fsn3.2154

Kim, M. J., Song, M. H., Ji, Y. S., Park, J. W., Shin, Y. K., Kim, S. C. & Jeong, S. Y. (2025). Cell free supernatants of Bifidobacterium adolescentis and

Bifidobacterium longum suppress the tumor growth in colorectal cancer organoid model. *Scientific Reports*, *15*(1), 935-955. https://doi.org/10.1038/s41598-024-83048-5

Kumar, A. & Kumar, D. (2016). Development of antioxidant rich fruit supplemented probiotic yogurts using free and microencapsulated Lactobacillus rhamnosus culture. *Journal of Food Science and Technology*, *53*, 667-675. https://doi.org/10.1007/s13197-015-1997-7

Li, R., Ding, Q. & Zhao, X. H. (2019). Impact of milk fortification on the microbiological and physicochemical properties of set-type skimmed yoghurt using three commercial soluble prebiotics. *Foods*, *8*(6), 181-192. https://doi.org/10.3390/foods8060181

Le Ba, T., Dam, M. S., Nguyen, L. L. P., Baranyai, L. & Kaszab, T. (2025). A Review of processing techniques and rheological properties of yogurts. *Journal of Texture Studies*, *56*(1), 70006. https://doi.org/10.1111/jtxs.70006

Okur, H. H., Yıldırım, H. K., Yousefvand, A. & Saris, P. E. (2025). Production of fermented soy and soy/cow milk products with probiotic Lacticaseibacillus rhamnosus GG strain. *Food and Bioprocess Technology*, *18*(6), 5736-5748. https://doi.org/10.1007/s11947-025-03804-x

Olson, D. W. & Aryana, K. J. (2022). Probiotic incorporation into yogurt and various novel yogurt-based products. *Applied Sciences*, *12*(24), 12607-12647. https://doi.org/10.3390/app122412607

Nouri, M. (2023). The correlation of plant species and geographical regions on biological component and antioxidant potential of different honey. *Journal of Food Biosciences and Technology*, *13*(1), 61-74. https://doi.org/10.30495/JFBT.2022.66873.10288

Pascariu, O. E., Estevinho, L. M., Seixas, N. L., Dopcea, I., Boiu-Sicuia, O. A., Geicu-Cristea, M. & Israel-Roming, F. (2025). Antioxidant properties and microbiological stability of yogurt enriched with elderberry extract. *Foods*, *14*(7), 1251-1267. https://doi.org/10.3390/foods14071251

Pinto, S. S., Cavalcante, B. D., Verruck, S., Alves, L. F., Prudêncio, E. S. & Amboni, R. D. (2017). Effect of the incorporation of Bifidobacterium BB-12 microencapsulated with sweet whey and inulin on the properties of Greek-style yogurt. *Journal of Food Science and Technology*, *54*, 2804-2813. https://doi.org/10.1007/s13197-017-2717-2

Pourjavid, H., Ataee, M., Pourahmad, R., Anvar, A. A. & Behmadi, H. (2023). Changes in microbial, rheological, and sensory characteristics of probiotic yogurt sauce containing lactobacillus rhamnosus during cold storage. *Journal of Food Biosciences and Technology*, *13*(2), 27-38. https://doi.org/10.30495/jfbt.2022.69606.10298

Rezaei, R., Khomeiri, M., Aalami, M. & Kashaninejad, M. (2014). Effect of inulin on the physicochemical properties, flow behavior and probiotic survival of frozen yogurt. *Journal of Food Science and Technology*, *51*, 2809-2814. https://doi.org/10.1007/s13197-012-0751-7

Santos, C., Raymundo, A., Moreira, J. B. & Prista, C. (2025). Exploring the potential of lactic acid bacteria fermentation as a clean label alternative for use in yogurt production. *Applied Sciences*, *15*(5), 2686. https://doi.org/10.3390/app15052686

Sarwar, A., Al-Dalali, S., Aziz, T., Yang, Z., Ud Din, J., Khan, A. A. &

Dablool, A. S. (2022). Effect of chilled storage on antioxidant capacities and volatile flavors of synbiotic yogurt made with probiotic yeast Saccharomyces boulardii CNCM I-745 in combination with inulin. *Journal of Fungi*, *8*(7), 713-728. https://doi.org/10.3390/jof8070713

Son, J. K., Jo, Y. J., Jung, Y. J., Lee, Y. R., Lee, J. & Jeong, H. S. (2023). Fermentation and quality characteristics of yogurt treated with Bifidobacterium longum. *Nutrients*, *15*(15), 3490-3510. https://doi.org/10.3390/nu15153490

Tarrah, A., Da Silva Duarte, V., De Castilhos, J., Pakroo, S., Junior, W. J. F. L. & Luchese, R. H., et al. (2019). Probiotic potential and biofilm inhibitory activity of Lactobacillus casei group strains isolated from infant feces. *Journal of Functional Foods*, *54*, 489-497. https://doi.org/10.1016/j.jff.2019.02.004

Tavakoli, A., Farahmandfar, R., Motamedzadegan, A., Ariaii, P. & Asnaashari, M. (2025). Effects of hydrolyzed Spirulina platensis proteins on the physicochemical, sensory, and microbial properties of probiotic yogurt. *Journal of Food Measurement and Characterization*, 1-13. https://doi.org/10.1007/s11694-025-03377-5

Tian, H., Yu, B., Yu, H. & Chen, C. (2020). Evaluation of the synergistic olfactory effects of diacetyl, acetaldehyde, and acetoin in a yogurt matrix using odor threshold, aroma intensity, and electronic nose analyses. *Journal of Dairy Science*, *103*(9), 7957-7967. https://doi.org/10.3168/jds.2019-17495

Turgut, T. & Diler, A. (2025). The effect of Coriandrum sativum L. on yogurt: a comprehensive study of physicochemical, microbiological, sensory, and textural properties. *Frontiers in Nutrition*, *11*, 1493602. https://doi.org/10.3389/fnut.2024.1493602

Wang, X., Wang, L., Wei, X., Xu, C., Cavender, G., Lin, W. & Sun, S. (2024). Advances in yogurt development-Microbiological safety, quality, functionality, sensory evaluation, and consumer perceptions across different dairy and plant based alternative sources. *Journal of Dairy Science*, *108*(1), 33-58. https://doi.org/10.3168/jds.2024-25322

Wang, J., Wu, P., Dhital, S., Yu, A. & Chen, X. D. (2025). Impact of freezing and freeze-drying on lactobacillus rhamnosus GG survival: mechanisms of cell damage and the role of pre-freezing conditions and cryoprotectants. *Foods*, *14*(10), 1817-1829. https://doi.org/10.3390/foods14101817

Zepeda-Hernández, A., Rosales-De la Cruz, M. F., Antunes-Ricardo, M., Ortega-Hernández, E., García-García, J. D., Garcia-Amezquita, L. E. & García-Cayuela, T. (2025). Probiotic fermentation enhances the technological properties and health-promoting potential of skyr-type yogurts. *International Dairy Journal*, *9*, 106276. https://doi.org/10.1016/j.idairyj.2025.106276

Zhang, S., Niu, S., Wu, M., Li, H., Yang, C., Liu, C. & Xu, Y. (2025). Research progress on quality characteristics of yoghurt and its fermentation enhancement technology: A review. *Journal of Food Process Engineering*, *48*(1), 70042. https://doi.org/10.1111/jfpe.70042

Ziar, H., Gérard, P. & Riazi, A. (2025). CLA-producing probiotics for the development of a yogurt-type beverage. *Beverages*, *11*(2), 50-65. https://doi.org/10.3390/beverages11020050