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Evaluation of Physicochemical and Textural Properties of Synbiotic Yogurt Using *Propionibacterium freudenreichii* and Inulin during Fermentation and Cold Storage Time

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HIGHLIGHTS

- *P. freudenreichii* and inulin synergistically improved yogurt quality.
- Metabolic shift from lactic to propionic acid enhanced pH stability.
- Synbiotic yogurt had 1.76-fold higher water retention than control.
- Viscosity index peaked in synbiotic yogurt, 78% higher than control.
- The combination effectively reduces post-acidification during cold storage.

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Abbreviations
LB= *Lactobacillus delbrueckii* subsp. *Bulgaricus*
PAB= Propionic Acid Bacteria
ST= *Streptococcus thermophilus*
WHC= Water Holding Capacity

ABSTRACT

Background: Propionic acid bacteria (PAB) are probiotics that, when combined with conventional yogurt starters, can significantly influence the fermentation process and structural properties of yogurt. This study aimed to produce and evaluate a synbiotic yogurt using *Propionibacterium freudenreichii* (*P. freudenreichii*) and inulin in combination with traditional starters, focusing on their combined effect on the quality characteristics of the product during fermentation and storage at 4 °C.

Methods: In this study, three yogurt formulations were developed. The first was a control sample (YC) containing exclusively the conventional starter culture, consisting of *Streptococcus thermophilus (ST)* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (*LB*). The second formulation (YC + PS4) included both the traditional starter and *P. freudenreichii* subsp. *shermanii*. The third formulation (YC + PS4 + Inulin) was enriched with the same cultures as the second group, along with 2% (w/v) inulin as a prebiotic component. These formulations were evaluated and compared based on their physicochemical attributes, including pH, titratable acidity, water holding capacity (WHC), and textural properties during fermentation and subsequent refrigerated storage at 4 °C. All measurements were performed in triplicate, and the data were statistically analyzed using two-way ANOVA, followed by Tukey's test at a significance level of $p < 0.05$.

Results: The findings demonstrated that incorporating *P. freudenreichii* subsp. *shermanii* in combination with traditional yogurt starter cultures had a notable impact on the physicochemical and textural characteristics during refrigerated storage. The synbiotic yogurt (YC + PS4 + Inulin) exhibited the smallest decline in pH (a reduction of 0.18 units) and the lowest acidity level (111 ± 7 °D) on day 21. Additionally, viscosity reached its maximum value (25.1 g.s) in the synbiotic yogurt, whereas the

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lowest value (14.05 g.s) was observed in the control group.
Conclusion: The produced synbiotic yogurt exhibited a significantly different profile compared with the control yogurt, with the lowest pH drop, the smallest increase in acidity, the best textural properties, and the highest water-holding capacity. *Propionibacterium* and inulin can be effectively utilized as a probiotic and a prebiotic, respectively, to optimize the production of beneficial metabolites in synbiotic yogurt.

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Introduction

The development of innovative dairy products enriched with health-promoting components, such as probiotics and prebiotics, has gained increasing attention. Propionibacteria, as functional microbes, are known for their ability to synthesize bioactive metabolites, including organic acids, vitamins, and antimicrobial compounds, that enhance the quality and safety of fermented dairy products (Baker et al., 2022). These features are characteristic of a newer category of functional foods called synbiotics, which combine both probiotics and prebiotics to deliver complementary health benefits (Markowiak and Śliżewska, 2017). A wide range of food products serve as delivery vehicles for probiotics, with fermented dairy products such as yogurt and cheese representing the most prominent segment in both scientific research and commercial markets. Introducing dairy products with novel flavors and enhanced health-promoting properties has the potential to significantly boost consumer appeal and overall market sales (Zahed et al., 2021a). Yogurt is a nutrient-dense fermented dairy product that is consumed primarily for its wide range of health benefits, which extend beyond its basic nutritional value (Hadjimbei et al., 2022). The traditional yogurt starter culture is a blend of *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (LB), usually in a 1:1 ratio. In the yogurt industry, lactic acid bacteria that produce extracellular polysaccharides are also used to improve the physical stability of this product (Dan et al., 2023; Sieuwerts, 2009). To improve viscosity, avoid syneresis, increase consistency, and minimize physical damage, starter cultures that produce extracellular polysaccharides should be used in milk fermentation. Several factors, such as incubation time and temperature (Krisnaningsih et al., 2019), starter cultures, and stabilizers (Shafiee et al., 2010), influence the quality of yogurt. As a result of each factor, the pH, overall acidity, texture, nutritional value, and bacterial characteristics of the yogurt will be affected (Yu et al., 2016). The textural characteristics of yogurt during and after fermentation will be influenced by the starter culture, resulting in a variety of quality yogurts for each type of culture (Vinderola et al., 2002).

Multiple factors, such as the specific strains employed, interspecies microbial interactions, duration of fermentation, and the availability of essential nutrients, can adversely impact the viability of probiotic microorganisms (Sekhon and Jairath, 2010). The microbial composition and metabolic activity of the starter culture, along with the formulation of the base milk, fermentation conditions, and post-fermentation treatments, collectively exert a substantial influence on the texture, sensory quality, and overall flavor profile of yogurt (Mbae et al., 2023; Obadina et al., 2013). To prevent syneresis and achieve a favorable texture and stability during yogurt processing and storage, stabilizers are commonly added to yogurt. As a prebiotic, food stabilizers made from polysaccharides, such as inulin, may also play a role (Isanga and Zhang, 2009).

Propionic acid bacteria (PABs) are widely recognized as useful microorganisms in the food industry, with the ability to biosynthesize a large number of beneficial metabolites. PABs are used as potential probiotics (Zahed et al., 2021b). These bacteria are capable of producing important metabolites such as propionic acid and conjugated linoleic acid, vitamins B2, B3, B6, B7, B9, B12, and K (Van Wyk et al., 2018), as well as industrially used bacteriocins and commercial bacteriocins (Ahmadi et al., 2015). The incorporation of PAB in dairy product production, such as yogurt, enhances viscosity by producing exopolysaccharides. Additionally, PAB inhibits the growth of certain gram-positive and some gram-negative bacteria, including Pseudomonas and Salmonella, as well as fungi and yeasts. This inhibition contributes to increased shelf life by producing propionic acid and bacteriocin. Importantly, PAB growth does not interfere with the growth of LAB in dairy products (Van Wyk et al., 2018).

The objective of this study was to produce synbiotic yogurt containing *Propionibacterium freudenreichii* (*P. freudenreichii*) and inulin and to utilize specific strains of propionibacteria as adjunct cultures to investigate the physical, chemical, and textural properties of the produced yogurt during fermentation and storage.

Materials and methods

Materials

Pak Dairy Co. (Tehran, Iran) generously provided commercial skimmed milk powder and 40% (w/w) fat cream. Inulin powder was obtained from Ava Salamat Javid Co. (Tehran, Iran). All chemical reagents used in this study were of analytical or HPLC grade and were sourced from Merck (Darmstadt, Germany).

-Preparation of cultures

In this study, the yogurt starter culture utilized was YoFlex Express 1.0, which comprises *ST* and *LB*. This selection was made because of its mild acidification characteristics, which promote the growth of PAB. The culture was procured from Chr. Hansen (Hørsholm, Denmark) and prepared in accordance with the manufacturer's protocol.

The adjunct culture, *P. freudenreichii* subsp. shermanii (commercial strain PS-4), was also obtained from Chr. Hansen in freeze-dried form and stored at −18 °C until required. Prior to application, the culture was rehydrated to achieve an initial concentration of approximately 8 log CFU/ml, followed by subculturing in a sodium lactate medium composed of 1% (v/v) sodium lactate syrup, 10 g/l casein peptone, and 10 g/l yeast extract, incubated at 30 °C under microaerophilic conditions (Grinstead and Barefoot, 1992).

-Milk preparation

To prepare reconstituted milk, 13% (w/v) skim milk powder was dissolved in distilled water. The resultant mixture underwent pasteurization at 90 °C for 30 min, followed by rapid cooling in an ice-water bath to a temperature below 35 °C, thereby mitigating the risk of thermal shock to the probiotic cultures. Furthermore, the fat content was adjusted utilizing the Pearson square method.

-Fermentation

Three yogurt formulations were developed based on prior experimental experience and literature evidence. After inoculation with the respective cultures, the yogurt mixtures were transferred into 100ml sterile polypropylene cups and incubated at 43 °C until the pH reached 4.6. Fermentation was subsequently halted by immersing the cups in an ice bath, and the samples were then stored at 4 °C.

The experimental groups were:

YC: Yogurt made using only the traditional starter (ST and LB).

YC + PS4: Yogurt prepared with the traditional starter and *P. freudenreichii subsp.* shermanii.

YC + PS4 + Inulin: Yogurt containing the traditional starter, *P. freudenreichii*, and 2% (w/v) inulin.

Physicochemical analysis

In this study, physicochemical parameters were measured during fermentation and also during 21 days of storage of yogurt samples at 4 °C.

pH measurement: The pH and redox potential of yogurt samples were determined at ambient temperature utilizing a 605 pH meter (Metrohm AG, Herisau, Switzerland).

Titratable acidity: The acidity was evaluated by titrating a 10ml yogurt sample, which was diluted with 10ml of distilled water, employing 0.1 N NaOH and 0.5% phenolphthalein as an indicator, in accordance with Dave and Shah (1997). Titratable acidity was expressed in Dornic degrees (°D) and calculated as follows: $°D = V \times N \times 1000 / W$

where:

V = Volume of NaOH used (ml)

N = Normality of NaOH solution (0.1 N)

W = Weight of yogurt sample (g)

Water Holding Capacity (WHC): The WHC was evaluated by subjecting a 5 g sample of yogurt to centrifugation at 4500 rpm for a duration of 30 minutes at a temperature of 10 °C. The percentage of WHC was calculated using the following formula:

$WHC (\%) = (1 - W1/W2) \times 100$

where:

W1 = Weight of the whey collected post-centrifugation

W2 = Initial weight of the yogurt sample (Ranadheera et al., 2012).

Texture evaluation

The textural properties of the yogurt samples were assessed utilizing a Texture Analyzer TA-XT Plus (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell. Viscosity was determined through a back extrusion test, employing the following parameters: a test speed of 1 mm/s and a contact force of 10 g. Prior to measurement, the yogurt was housed in cylindrical containers with dimensions of 60 mm in depth, a 50 mm inner diameter, and a 55 mm outer diameter.

Statistical analyses

The results were subjected to statistical analysis utilizing two-way ANOVA, conducted in SPSS (IBM SPSS Statistics version 23). Tukey's test was employed to ascertain significant differences among groups, with a significance threshold established at $p < 0.05$.

Results and discussion

pH and Titratable Acidity Changes During Fermentation

The process of changes in pH and acidity during fermentation in three samples of manufactured yogurt (YC, YC + PS4, and YC + PS4 + Inulin) is shown in Figure 1. As seen in the figure, the fermentation time for YC yogurt,

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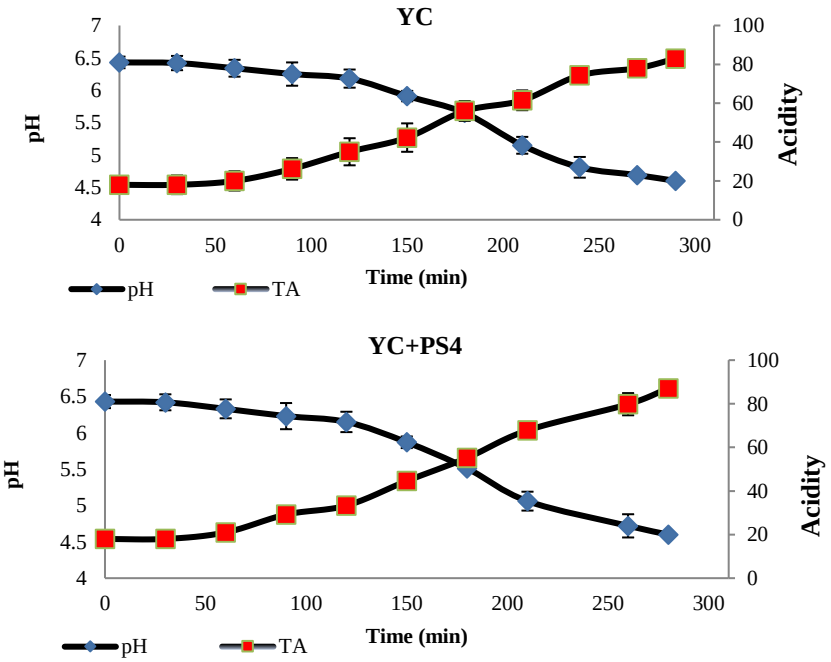
which includes traditional yogurt starters, is equal to 290 ± 5 min (when the pH reaches 4.6). Meanwhile, this time for the probiotic yogurt sample (YC + PS4) is equal to 280 ± 7 min, and for the synbiotic yogurt (YC + PS4 + Inulin), the fermentation time ends after 261 ± 7 min. Therefore, the addition of *P. freudenreichii* subsp. *shermanii* and inulin to yogurt reduced the fermentation time.

Regarding the acidity levels in the samples of produced yogurts during the fermentation time, the recorded changes were such that in the sample of YC yogurt, the acidity of this sample during the fermentation time (290 min) increased from 18 ± 0.7 °D (acidity of reconstituted milk) to 81.3 ± 1.2 °D. These final acidity levels for YC + PS4 and YC + PS4 + Inulin yogurts after the described fermentation time are equal to 83.4 and 92.1 ± 1.4 °D, respectively. Regarding acidity, significant changes ($p \leq 0.05$) were observed between the YC + PS4 + Inulin yogurt sample and the control yogurt sample (YC).

As seen in Figure 1, the fermentation duration required to achieve a pH of 4.6 for the three produced yogurt samples is between 261 and 290 min, and the lowest fermentation time is related to the synbiotic yogurt sample (YC + PS4 + Inulin). Similar to these results, it has been stated in previous studies that co-cultivation of probiotics with traditional yogurt starter bacteria partially reduced the fermentation time (Ekinci and Gurel, 2008; Saccaro et al., 2009). Figure 1 illustrates that there is no statistically significant difference in fermentation time among the three yogurt samples, which may be attributed to the heightened activity of the yogurt starter culture (ST, LB) in all three produced yogurt samples. In general, *Propionibacterium* has a slower growth rate and lower activity than the yogurt starter (ST, LB) because its peptidase activity dominates its protease activity, resulting in low proteolytic activity. In addition, *Propionibacterium* grows less in media containing a higher concentration of lactose than of lactate or lactic acid (Moslemi et al., 2016). Thus, because of the reasons mentioned above, *Propionibacterium* has little growth and activity during the fermentation time and cannot have a major and significant effect on the fermentation time. The significant increase observed in our synbiotic sample can be attributed to the specific functionalities of the added ingredients. The production of exopolysaccharides by *P. freudenreichii* and the strong water-structuring capacity of inulin created a more robust gel matrix that effectively trapped water molecules and counteracted the natural whey separation process. This represents a notable technological advantage of the developed synbiotic yogurt formulation.



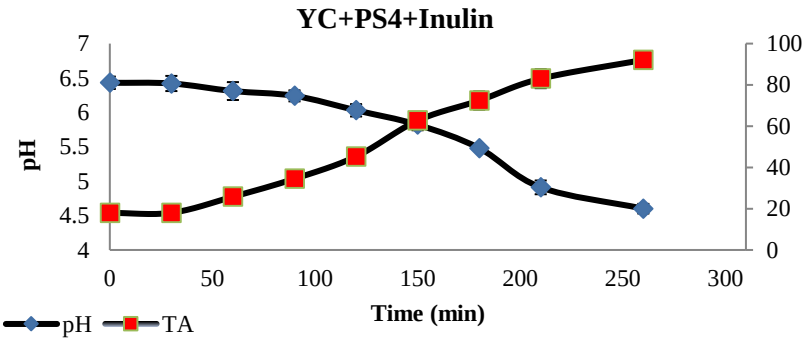


Figure 1: pH and titratable acidity changes during fermentation in three manufactured yogurts
YC = Yogurt produced with a traditional starter culture composed of *ST* and *LB.*
YC + PS4 = Yogurt made using the traditional starter along with *Propionibacterium freudenreichii* subsp. *shermanii* as an adjunct culture.
YC + PS4 + Inulin = Yogurt formulated with traditional starter, *P. freudenreichii* subsp. *shermanii*, and 2% (w/v) inulin supplementation.

Regarding Figure 1, the highest yogurt titratable acidity during fermentation among the three samples is observed in the synbiotic yogurt (YC + PS4 + Inulin), with a measurement of 92.1 ± 1.4 °D. This shows a significant difference compared with the control yogurt (YC), which has a titratable acidity of 83 °D. In addition to the production of lactic acid and acetic acid, *Propionibacterium* also produces propionic acid during fermentation when yogurt starter bacteria grow simultaneously. This contributes to increased acidity and reduced fermentation time.

Changes in pH and Titratable Acidity During Storage at 4 °C in Three Yogurt Samples

Figure 2 illustrates that the pH of all samples exhibited a gradual and continuous decline throughout the 21-day storage period at 4 °C. Comparing the control yogurt sample (YC) with the two probiotic (YC + PS4) and synbiotic (YC + PS4 + Inulin) yogurt samples, it can be concluded that the synbiotic yogurt, which contains *P. freudenreichii* subsp. *shermanii* and inulin, exhibits a lower pH decrease ratio, showing a significant difference ($p \leq 0.05$) compared with the control yogurt. Overall, after a storage period of 21 days, the most pronounced decrease in pH was noted in the YC yogurt sample (4.27 ± 0.04), whereas the lowest pH decrease was found in the YC + PS4 + Inulin yogurt (4.43 ± 0.03).

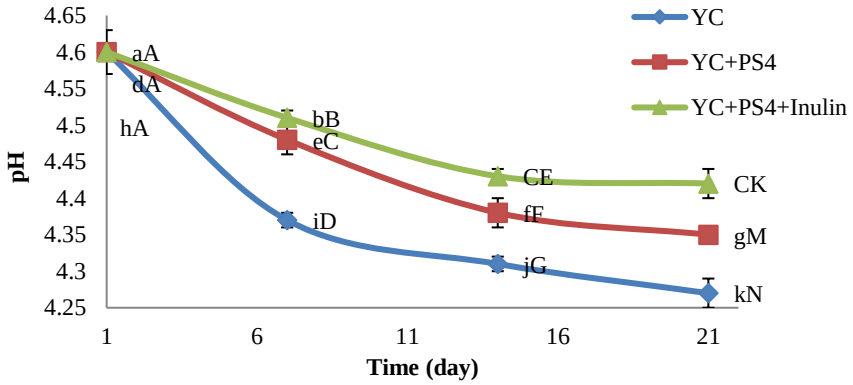


Figure 2: pH changes during the storage time at 4 °C in three manufactured yogurts

During the 21-day storage period at 4 °C, the pH of all yogurt samples decreased significantly ($p \leq 0.05$), while the titratable acidity showed a concomitant increase (Figures 2 and 3). The most pronounced changes occurred during the first week, after which the rate of acidification slowed. A key finding was the distinct behavior of the synbiotic yogurt (YC + PS4 + Inulin) compared with the control (YC). The control sample exhibited the most substantial changes, with a pH decrease of 0.33 units and an acidity increase of 45 °D, reaching a final acidity of 128 ± 5 °D. In contrast, the synbiotic yogurt demonstrated the greatest stability, with the smallest pH reduction (0.18 units) and the lowest final acidity (111 ± 7 °D). The probiotic yogurt (YC + PS4) displayed an intermediate profile, with a final acidity significantly lower than that of the control but higher than that of the synbiotic sample. This stabilizing effect in samples containing *P. freudenreichii* and inulin can be attributed to the metabolic conversion of lactic acid into weaker acids, as detailed in the subsequent discussion.

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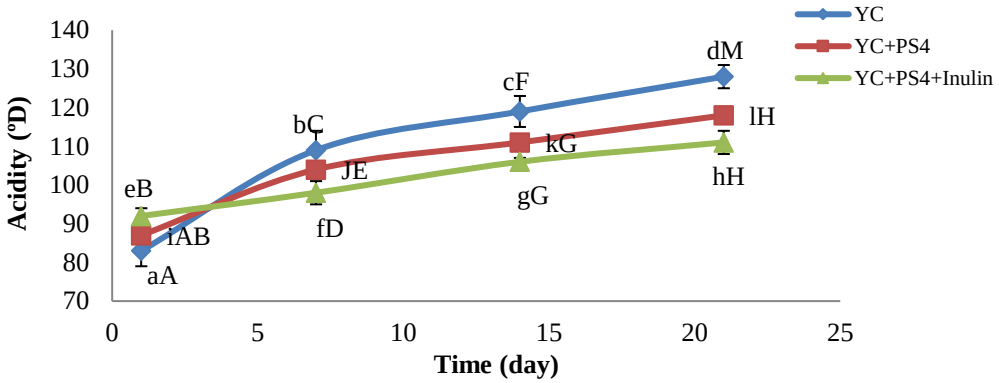

Figure 3: Changes in acidity during the storage time at 4 °C in three manufactured yogurts

A similar result was reported in a previous study that utilized *Propionibacterium* to produce a fermented drink (Farhadi et al., 2013).

There are several reasons to justify these results. As mentioned, *Propionibacterium* naturally has a long lag phase and requires more time for growth and activity than yogurt starter culture bacteria (ST and LB). Additionally, its peptidase activity is much higher than its protease activity (Moslemi et al., 2016). In contrast, *Propionibacterium* strains exhibit enhanced growth and metabolic activity when utilizing lactate-rich carbon sources, such as yogurt, compared to lactose-rich sources, such as milk (Ekinci and Gurel, 2008). Consequently, the bacteria derived from the yogurt starter culture dominate during the initial stages of storage. These bacteria utilize the lactose present in milk and generate a higher concentration of lactic acid relative to other organic acids, such as acetic and propionic acids, resulting in a reduction in the pH of the yogurt samples. During the subsequent days of storage at 4 °C, the concentration of lactate and peptides increases as a result of the proliferation of yogurt starter culture bacteria. This microbial activity also contributes to a decrease in dissolved oxygen levels within the yogurt samples (Ekinci and Gurel, 2008). Under the novel conditions of low temperature and high acidity present in yogurt samples, *Propionibacterium* demonstrates enhanced growth and metabolic activity. Prior research indicates that *Propionibacterium* in yogurt metabolizes three moles of lactic acid to yield two moles of propionic acid as the principal product, accompanied by the production of one mole of acetic acid and one mole of carbon dioxide (Zhang and Yang, 2009).

The pKa values for propionic acid, acetic acid, and lactic acid are 4.87, 4.76, and 3.86, respectively (Coban, 2020). Therefore, *Propionibacterium* consumes a strong acid (lactic acid) and produces a weaker acid (propionic acid). As a result, the acidity of the final synbiotic yogurt (YC + PS4 + Inulin) and probiotic yogurt (YC + PS4) increases less than that of the control yogurt.

The observed attenuation in pH decline and reduced titratable acidity in synbiotic and probiotic yogurts during storage can be attributed to a fundamental metabolic shift driven by the co-cultivation of *P. freudenreichii* with the traditional yogurt starters. While *ST* and *LB* rapidly ferment lactose to produce lactic acid as the primary metabolite during the initial fermentation and early storage, *P. freudenreichii* exhibits a more adaptive metabolic strategy. As the environment becomes enriched with lactate and the dissolved oxygen decreases, conditions become more favorable for propionibacteria during extended cold storage. *P. freudenreichii* initiates the metabolism of lactic acid via the Wood–Werkman cycle. In this pathway, three moles of lactate are converted into two moles of propionate, one mole of acetate, and one mole of CO_2. Given the higher pKa values of propionic acid (4.87) and acetic acid (4.76) compared to lactic acid (3.86), this metabolic conversion effectively replaces a stronger acid (lactic acid) with weaker acids (propionic and acetic acid). Consequently, this biochemical transformation mitigates the net accumulation of titratable acidity and stabilizes the pH of the product over time. The presence of inulin further amplifies this effect by selectively promoting the growth and metabolic activity of *P. freudenreichii*, thereby enhancing this pH-stabilizing mechanism in the synbiotic yogurt (YC + PS4 + Inulin) compared with its probiotic counterpart (YC + PS4). This also explains the lower pH drop in the two aforementioned yogurt samples.

As noted, the inulin present in synbiotic yogurt promotes the growth of *Propionibacterium* strains, leading to lower acidity in synbiotic yogurt (YC + PS4 + Inulin) compared with probiotic yogurt (YC + PS4).

Alterations in WHC of yogurt samples during storage at 4 °C

According to Figure 4, the WHC of the three yogurt samples demonstrated a consistent increase throughout the storage period. Notably, the synbiotic yogurt (YC + PS4 + Inulin) exhibited the highest WHC among the

samples analyzed, which increased by 1.76 times over 21 days of storage, compared with 1.3 times increase for the control yogurt (YC). Several factors, including the level of enrichment and materials used, fat content and homogenization conditions, heat treatment of milk, starter culture characteristics (type, amount of acid production, and production of exopolysaccharides), incubation temperature (affecting the growth of primary cultures), density, gel strength, and the strength of the formed bonds, influence the WHC in yogurt (Costa et al., 2015). However, the most important factors influencing the amount of WHC are the percentage of dry matter, pH, and acidity of yogurt. In general, the higher the amount of dry matter in yogurt, the higher the WHC of the product (Denin-Đurđević et al., 2001). As expected, among the three yogurt samples, the sample with a higher pH (YC + PS4 + Inulin) had a higher WHC. In synbiotic yogurt (YC + PS4 + Inulin) during the 21-day storage time, we saw the fewest changes in pH and acidity, which increased the WHC during this period. In past studies, it has been mentioned that the production of exopolysaccharides and trehalose sugar (especially under unfavorable growth conditions) is one of the prominent characteristics of *Propionibacterium* strains, which increases the percentage of dry matter in yogurt (Van Wyk et al., 2018). As a result of the increase in the percentage of dry matter in the produced synbiotic and probiotic (YC + PS4) yogurt samples, the WHC increases. Inulin, a soluble fiber, plays a crucial role in preserving water in products. Additionally, this prebiotic has the capacity to form an aggregate structure with protein gels, thereby enhancing the structural network established during fermentation and ultimately leading to the formation of a more robust gel (Costa et al., 2015). These factors and reasons are consistent with the findings of this study. In a previous study, which was carried out on the simultaneous cultivation of different percentages of *Propionibacterium* strains with traditional yogurt starters, it was stated that the amount of WHC of probiotic yogurt was increased (Ekinci and Gurel, 2008). In another study, the same results and the increase in the amount of WHC are mentioned (Torre et al., 2003).

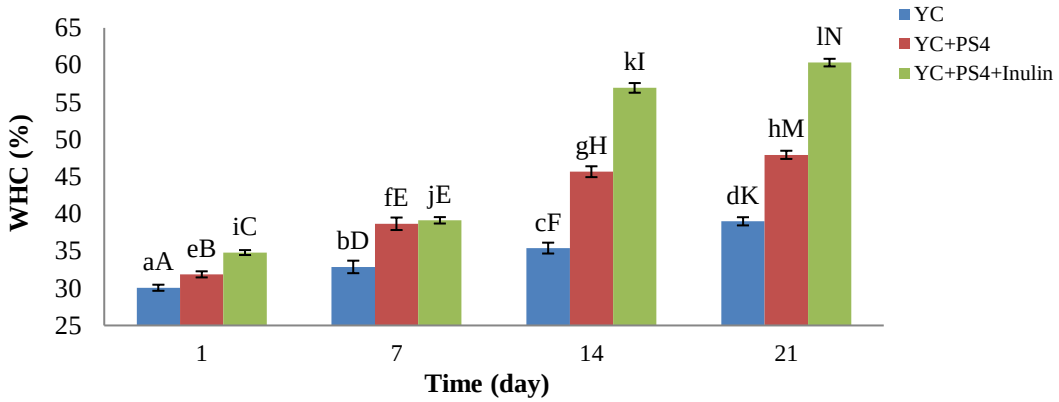

Figure 4: Changes in WHC during the storage time at 4 °C in three manufactured yogurts

Textural properties

Textural attributes, particularly the viscosity index, are summarized in Table 1. The synbiotic yogurt (YC + PS4 + Inulin) consistently exhibited the highest viscosity across all storage intervals, peaking at 25.1 ± 0.61 g.s on day 21. In contrast, the control sample showed the lowest values (e.g., 14.05 ± 0.6 g.s on day 1). Table 1 demonstrates that the refrigeration of yogurt samples at 4 °C for 21 days, in conjunction with the incorporation of prebiotic and probiotic adjuncts (PAB), has a significant impact on their viscosity index. Several elements may contribute to these textural variations, including the type of fortifying ingredients, milk fat content, homogenization parameters, thermal processing conditions, the specific starter culture used, incubation temperature, and the final pH of the product (Lucey, 2004). The elevated viscosity index observed in yogurts fortified with probiotics and inulin can be attributed to the incorporation of prebiotics, which promote the proliferation of both the traditional starter cultures and *Propionibacterium* strains. This enhanced microbial activity likely contributes to improved structural integrity and firmness of the yogurt. In the current study, the inclusion of inulin was directly associated with an increase in viscosity index values. According to Mousavi et al. (2019), increasing flaxseed concentration and extending storage duration in yogurt samples fortified with flaxseed resulted in notable enhancements in key rheological properties. In a separate study, Azari-Anpar et al. (2017) reported that the incorporation of aloe vera gel into yogurt formulations led to a decrease in product firmness, with the most pronounced softening observed at a 5% inclusion level. In contrast, inulin, a soluble dietary fiber and a recognized water-structuring agent, interacts with protein aggregates to form stable complexes. This

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interaction is essential for the establishment of the structural matrix during the fermentation and texturization processes of stirred yogurt. Although this prebiotic contributed to improved textural attributes, its functional benefits were observed only within a specific concentration range. Furthermore, the interaction between whey proteins and κ-casein helped stabilize micelles, thereby lowering their responsiveness to pH decline during the milk fermentation process (Costa et al., 2015). Comparable findings were reported by Pimentel et al. (2012), who demonstrated that the incorporation of probiotic strains and inulin into yogurt formulations enhanced textural attributes by reducing syneresis and increasing both firmness and gumminess. This study confirmed that incorporation of probiotic bacteria and inulin into yogurt formulations enhanced syneresis while simultaneously increasing both firmness and gumminess. According to Costa et al. (2015), yogurts supplemented with prebiotics exhibited higher consistency levels compared with non-supplemented samples by the conclusion of the 21-day storage period. The concentration of inulin and the duration of storage at 4 °C significantly affected the viscosity index of the samples ($p \leq 0.05$), as presented in Table 1. Notably, the incorporation of 2% w/v inulin into yogurt samples containing *P. freudenreichii* subsp. shermanii resulted in an increase in viscosity index values from 14.05 ± 0.6 to 23.81 ± 0.38 g.s.

Table 1: Textural properties of skim milk fermented over a 21-day period at 4 ºC

Textural properties	Storage Time(d)	YC	YC + PS4	YC + PS4+Inulin
Index of Viscosity (g.sec)	1	14.05 ± 0.6^{Aa}	16.69 ± 0.86^{Bd}	23.81 ± 0.38^{Cf}
	7	19.27 ± 1.01^{Db}	20.78 ± 0.73^{De}	24.38 ± 1.07^{Ef}
	14	21.51 ± 0.87^{Ec}	20.51 ± 0.51^{De}	22.78 ± 0.93^{Eg}
	21	18.74 ± 0.36^{Db}	19.67 ± 0.81^{De}	25.1 ± 0.61^{Fh}

Data are presented as mean values ± standard deviation (SD).
Lowercase letters within the same column denote statistically significant differences ($p < 0.05$).
Uppercase letters within the same row signify significant differences ($p < 0.05$).
YC = Yogurt produced with a traditional starter culture composed of *ST* and *LB*.
YC + PS4 = Yogurt made using the traditional starter along with *P. freudenreichii* subsp. *shermanii* as an adjunct culture.
YC + PS4 + Inulin = Yogurt formulated with traditional starter, *P. freudenreichii* subsp. *shermanii*, and 2% (w/v) inulin supplementation.

Conclusion

This study integrated *P. freudenreichii* subsp. shermanii and inulin with conventional yogurt starter cultures to produce synbiotic yogurt. The findings indicated that pH levels and acidity during fermentation and subsequent storage at 4 °C significantly influenced the properties of the yogurts containing *P. freudenreichii* subsp. shermanii and inulin. Importantly, the synbiotic yogurt exhibited a markedly different profile compared with the control yogurt, characterized by the lowest reduction in pH and the minimal increase in acidity. In terms of textural properties, the best results were significantly associated with the synbiotic yogurt. Moreover, regarding WHC, the highest values were recorded for the synbiotic yogurt throughout the storage period. Overall, *Propionibacterium* and inulin can be effectively utilized as a probiotic and a prebiotic, respectively, to optimize the production of beneficial metabolites in synbiotic yogurt.

Author contributions

O.Z. and K.K. designed the study; O.Z. and K.K. conducted the experimental work and analyzed the data; O.Z. authored the manuscript; all co-authors reviewed and approved the final version of the manuscript.

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Conflict of Interest

The authors report no conflicts of interest.

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Ethical consideration

This study was approved by the Ethics Committee of the National Institute for Medical Research Development (NIMAD) with ethical code [IR.NIMAD.REC.1399.179]. This article does not contain any studies with human participants or animals performed by any of the authors.

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