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Effects of Inulin Addition on Probiotic Proliferation and Physicochemical Properties in Fat-Free Synbiotic Yogurt Production

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Abstract

The aim of the current study was to investigate the effects of the addition of inulin to yoghurt produced with fat-free milk on the viability of bacteria (*Lactobacillus acidophilus*), sensory quality, and physical properties. Inulin was added at four different percentages to yoghurts (0%; IN0, 4%; IN4, 8%; IN8, and 12%; IN12). Yoghurts were produced with fat-free milk, inulin and starter cultures containing *Lactobacillus acidophilus*, *Lactobacillus delbrueckii ssp bulgaricus*, *Bifidobacterium lactis* and *Streptococcus thermophilus*. Viscosity, pH, bacterial counting (*Lactobacillus acidophilus*) and sensory evaluation were performed. Also, the sensory evaluation was performed as single-blind in terms of appearance, flavor, smell, color, creaminess, texture and consistency with 20 trained panelists. The addition of inulin increased the viscosity ($p<0.001$) and pH ($p=0.006$). Inulin, especially in IN4 and IN8, stimulated the growth of *Lactobacillus acidophilus*, and the decrease over time is less. Moreover, there were significant differences of total score in sensory evaluation ($p=0.011$). While there was no significant difference for appearance, smell and color, the highest score in terms of flavor ($p=0.002$), creaminess ($p=0.002$), texture ($p<0.001$) and consistency ($p=0.001$) was in IN8 compared to the other groups scores with respectively 64.8±5.37, 69.3±5.18, 75.0±4.35 and 68.2±5.74 ($p<0.05$). Considering the overall score, IN8 (69.3±3.64) was statistically significantly higher than IN0 (57.8±3.52), IN4 (52.6±3.99) and IN12 (56.7±3.28). The results of the current study suggest that the addition of inulin with 8% as a prebiotic may be beneficial in the production of healthy and high-quality fat-free yoghurt.

Keywords: Functional foods, inulin, prebiotic, sensory properties, synbiotic yoghurt.

Yağsız Sinbiyotik Yoğurt Üretiminde İnülin İlavesinin Probiyotik Çoğalması ve Fizikokimyasal Özellikler Üzerine Etkileri

Öz

Bu çalışmanın amacı, yağsız sütle üretilen yoğurda inülin ilavesinin bakterilerin canlılığı (*Lactobacillus acidophilus*), duyusal kalite ve fiziksel özellikler üzerine etkilerini incelemektir. Yoğurtlara farklı yüzdelerde inülin eklenmiştir (%0; IN0, %4; IN4, %8; IN8 ve %12; IN12). Yoğurtlar, yağsız süt, inülin ve *Lactobacillus acidophilus*, *Lactobacillus delbrueckii ssp bulgaricus*, *Bifidobacterium lactis* ve *Streptococcus thermophilus* içeren starter kültürler ile üretilmiştir. Viskozite, pH, bakteri sayımı (*Lactobacillus acidophilus*) ve duyusal değerlendirme incelenmiştir. Duyusal değerlendirme, görünüm, tat, koku, renk, kremsilik, doku ve kıvam açısından tek kör olarak 20 eğitimli panelist ile gerçekleştirilmiştir. Araştırmada inülin ilavesinin viskoziteyi ($p<0.001$) ve pH'ı arttırdığı görülmüştür ($p=0.006$). İnulin, özellikle IN4 ve IN8'de, *Lactobacillus acidophilus*'un büyümesini uyarmıştır ve zamanla azalma daha az olmuştur. Ayrıca duyusal değerlendirmede toplam skor açısından anlamlı farklılıklar (p=0.011) bulunmuştur. Görünüm, koku ve renk açısından anlamlı bir fark bulunmazken, tat (p=0.002), kremsilik (p=0.002), doku (p<0.001) ve kıvam (p=0.001) açısından en yüksek puan diğer gruplara göre IN8'de olmuştur, sırasıyla 64.8±5.37, 69.3±5.18, 75.0±4.35 ve 68.2±5.74. Genel puana bakıldığında IN8 (69.3±3.64); IN0 (57.8±3.52), IN4 (52.6±3.99) ve IN12'den (56.7±3.28) istatistiksel olarak anlamlı derecede yüksek bulunmuştur. Bu çalışmanın sonuçları, prebiyotik olarak %8 oranında inulin ilavesinin sağlıklı ve kaliteli yağsız yoğurt üretiminde faydalı olabileceğini göstermiştir.

Anahtar kelimeler: Fonksiyonel besinler, inülin, prebiyotik, duyusal analizler, sinbiyotik yoğurt.

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Introduction

As a functional food and having probiotic properties, yoghurt is widely consumed across the world.[1] Consumer interest in high-quality functional food products is steadily increasing, making it essential to develop innovative food options that prioritize health and well-being.[2] Fat intake is associated with increased diseases risk such as obesity, non-alcoholic fatty liver. In terms of food nutritional values and health, most consumers demand for lower fat products.[3] In recent years, there has been a significant rise in the consumption of healthy foods, such as low-fat or nonfat dairy products (particularly fat-free yogurt), driven by growing awareness of the health impacts associated with high-fat diets.[4]

It is important that the consumers prefer, like and accept. Decreasing in fat in yoghurts cause reduction in flavor quality, intensity, texture. Therefore, fat substitutes such as inulin may be ideal source for manufacturers. Because inulin is both used as fat replacer and does not cause changes in taste and texture.[5] Inulin is a high soluble dietary fiber that is used as fat replacer, and it have low viscosity. Adegoke et al. showed that the addition of inulin significantly improved the stability profile and gel strength, as evidenced by higher firmness and yield stress values.[6]

Naturally found in vegetables such as garlic, onions, asparagus, leeks, bananas, artichokes, inulin is mostly obtained from chicory plants.[7] Inulin contributes to the improvement of taste and texture, especially in low-fat or fat-free dairy products. Also, inulin has some positive effects on health such as stimulating the growth and activities of health beneficial bacteria, due to its prebiotic effects defined as food components that are resistant to gastric secretions, digestion, absorption.[8]

The prebiotic effects of inulin can also be observed on the viability of yoghurt cultures. In yogurt production, milk is fermented using lactic acid bacteria such as *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii subsp. bulgaricus*.[9] Although the viability of probiotic bacteria in yoghurt is affected by factors such as decrease in nutrients, oxygen pressure, decrease in pH due to post acidification, it has been reported that the addition of inulin may affect the viability of the yoghurt culture during the storage period. Kamel et al. stated that inulin can effectively be used as a prebiotic in probiotic yogurt production, promoting the growth of Bifidobacterium bifidum and extending the product's shelf life.[10] These effects vary based on dose of inulin.

With the increasing consumer demand for low-fat foods, yoghurt prepared from inulin added fat-free milk may has great consumption potential. The aim of this study is to compare the sensory, physicochemical and rheological properties of yoghurts produced by using different concentrations of inulin and to observe the effect of inulin on the viability of yoghurt cultures during the storage period.

Materials and Methods

Yoghurt production, rheological and sensory analysis were performed in Faculty of Health Science Food Chemistry Laboratory, Ankara University, Ankara, Türkiye. Quantitation of yoghurt cultures by qPCR Test and DNA extraction were analyzed in Diagen Laboratory, Ankara, Türkiye.

Production of Fat-free Yoghurt

Fat-free milks (<0.1%) with ultra-high temperature (UHT) were purchased from the local market in Ankara, Türkiye. Chicory inulin (Orafti®Synergy1, BENEO, Tienen, Belgium) was added at the following concentrations (w/w): 0% (IN0), 4% (IN4), 8% (IN8) and 12% (IN12).

The four batches were homogenized and heated to 43±2°C for 30min in a stainless-steel container and cooled to ambient temperature. Then, inoculated with 0.4% (w/w) traditional starter culturesthat are*Lactobacillus dellbrueckii ssp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium lactis* (containing 109 CFU/g, VİVO Industry, Ukraine). The samples were incubated at

43±1°C for 6 h until the pH was 4.6 and were stored under refrigeration at 4±1°C for 21d in sterile glass bottles for measurements and sensory tests. The pH values of the yoghurts were measured with a digital pH meter. The arithmetic mean of the triplicate results was determined to ensure accuracy and reliability of the data. Figure 1 shows that manufacturing stages for the production of yoghurts.

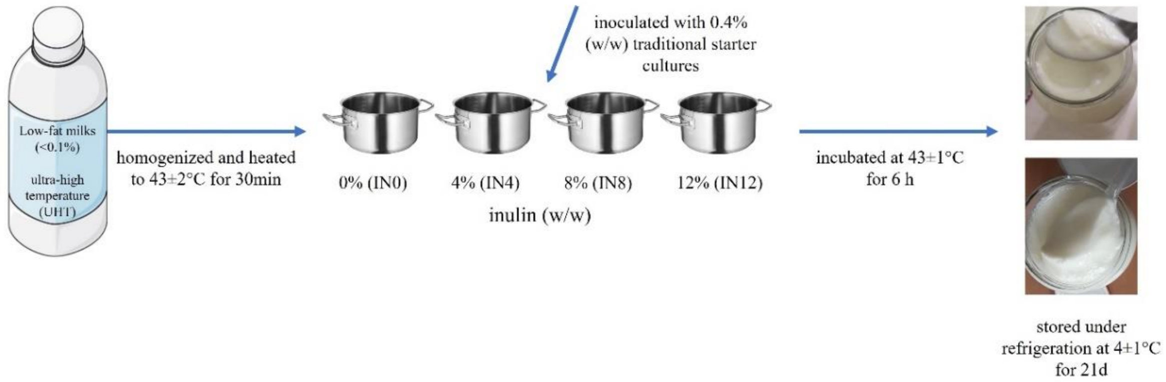

Figure 1. Manufacturing process of yoghurts. Homogenization, heating, inoculating, incubating and storing, respectively. IN: inulin.

DNA Extraction

Extraction from yoghurt samples was done with DiaRex® (Diagen) DNA extraction kit from bacteria (Cat No: BD-0865, Ankara). Firstly, 300µl Lysis solution was added to 200 mg yoghurt sample. 15mg glass and 10 pieces of zirconia beads were added on it and the application was made in the homogenizer device at 4000 rpm for 2 x 20 seconds. Then, 25µl of proteinase K was added and after a short vortex, it was left to incubate at 56°C for 60 minutes. After incubation, centrifugation was performed at 5000 g for 1 minute and the supernatant was transferred to a new tube.300µl of binding solution was added to it.After short pipetting, all contents were transferred to the spin column.The spin column was centrifuged at 8000g for 1 minute.The column was then transferred to a new tube and washes were performed according to the manufacturer's instructions.After the washes were completed, the column was treated with 50µl of elution solution and genomic DNA was recovered.

Quantitation of Yoghurt Cultures by qPCR Test

The Diagen real-time PCR kit for *Lactobacillus acidophilus* is specifically designed for the in vitro quantification of *Lactobacillus* species-specific genomes. The kit is specific to Lactobacillus species genome and has been designed to have the broadest possible detection profile.The oligonucleotide sequences in this kit have 100% homology with sequences specific to a wide variety of *Lactobacillus acidophilus* species based on extensive bioinformatics analysis.Studies were carried out in a realtime PCR device (Roche480, USA) using 0.2 microcentrifuge tubes.As a result of the realtime PCR process, the device determined how many copies were in the samples by making correlations between the samples used as the standard according to the obtained cycle threshold (Ct) values (Table 1).

Table 1.*Lactobacillus acidophilus*realtime PCR Kit (Cat No: 3010-50) contents, volumes and PCR Properties

Contents	Number of Tubes	Tube Color	Volume
Lactobacillus acidophilus specific oligonucleotide mix	1	Brown	5 µL
qPCR master mix(2X)	1	Blue	10 µL
For Positive Control-Standard Curve	1	Yellow	5 µL
Negative Control	1	White	20 µL

	PCR Cycle	Temperature	Time-Nots
PCR Properties	1	95°C	5 second, Polymerase Activation
	40	95°C	10 second, Denaturation
		50°C	30 second, Binding/Extension*

*FAM (Green Channel) channel is on.

Sensory Analysis

Firstly, perceived viscosity was measured with a spoon and evaluated with time scale, as descripted previously; i: Stir the yogurt 30 times using a coffee spoon, moving slowly and consistently in the same direction. ii: Next, take a spoonful of yogurt and lift it. iii: Allow the contents to drop and measure the flow rate. Briefly, place 1 teaspoon of the product near the lips and slurp gently to assess the liquid flow and the force required. Once the product is in the mouth, allow it to flow across the tongue and measure the rate of flow.[11]Then, the sensory evaluation of four yoghurt was performed as single-blind in terms of appearance, flavor, smell, color, creaminess, texture and consistency with 20 trained panelists regularly yoghurt consumer. Subjects aged between 20 and 32 were trained persons in Faculty of Health Sciences, Ankara University. Evaluation of yoghurts took place in individual test booths where the temperature, humidity and lighting were controlled. After each yoghurt sample was tried, the panelists were asked to drink water. Each attributes was rated with numbers ranging from 0-10 (from 0: strongly disliked to 10: strongly liked) and the scores were evaluated on 100.

Statistical Analysis

The results are presented in tables and figures as mean values with standard deviations (±SD). Statistical analysis was conducted using the SPSS software program (SPSS Inc., version 21.0), with statistical significance evaluated at the $p<0.05$ level. The Shapiro-Wilk test was used to assess normality of distributions. One-way ANOVA was used to analyze viscosity scores, pH values, and sensory evaluation results. The effects of inulin addition and storage time on yogurt culture were evaluated using Repeated Measures ANOVA. In this analysis, inulin concentrations (0%, 4%, 8%, 12%) were considered as the group factor, and storage periods (0, 7, 14, and 21 days) as the within-subjects factor. The sphericity assumption for the time factor was tested using Mauchly's Test of Sphericity, and when violated, appropriate corrections (Greenhouse-Geisser or Huynh-Feldt) were applied. When significant differences were detected in the ANOVA, pairwise comparisons between groups and time points were performed using Bonferroni post hoc tests.

Results and Discussion

As a result of this study, yoghurt containing 8% inulin was most liked by panelists. Inulin, especially 4% and 8%, stimulated the growth of *Lactobacillus acidophilus*, and the decrease over time is less. The addition of inulin increased the viscosity and pH. The results of the current study suggest that the addition of inulin may be beneficial in the production of healthy and high-quality fat-free yoghurt.

Viscosity and pH

The viscosities of the produced yoghurts were checked only on the baseline and a significant difference was found between the samples ($p<0.001$). It was concluded that as the amount of inulin increased, the viscosity enhanced. The viscosity was the highest in IN12 with 8.40±0.083 whereas the least in IN0 with 6.27±0.065. The difference between inulin added yoghurts and IN0 was statistically different ($p<0.001$). Zhou et al. explained it the decreased viscosity of yogurt with the increased addition of inulin indicated an incompatibility between the gel-forming properties of inulin and those of yogurt.[12] There are similar studies reported that because inulin is highly hygroscopic, it would bind water.[13-16] Similar observations were found in the pH. The pH, an important indicator of the production process's correctness and a key factor in research on reformulation, was found to increase with inulin supplementation.[17] The pH values of the yogurts ranged between 4.0 and 4.3 and were significantly influenced by the addition of inulin at 8% and 12%, compared to the control sample (IN0) ($p=0.006$) (Table 2).

Table 2. Mean viscosity scores and pH values of yoghurts with 0%, 4%, 8%, 12% inulin addition

	pH	Viscosity
IN0	4.01±0.011^{a}	6.27±0.065^{a}
IN4	4.07±0.015^{a,b}	6.80±0.036^{b}
IN8	4.12±0.036^{b,c}	7.53±0.108^{b}
IN12	4.16±0.065^{b,c}	8.40±0.083^{b}

The letters a, b, and c indicate mean values that show statistically significant differences ($p<0.05$). IN0: 0% inulin, IN4: 4% inulin, IN8: 8% inulin, IN12: 12% inulin (w:w).

Sensory Evaluations

Figure 2 shows that the scores of the organoleptic properties of yoghurts. Yoghurts containing inulin scored higher in organoleptic properties than yoghurt without inulin. There was no significant difference between the groups for appearance, smell and color.

The highest score in terms of flavor, creaminess, texture and consistency was in IN8 compared to the other groups scores with respectively 64.8±5.37, 69.3±5.18, 75.0±4.35 and 68.2±5.74 (for flavor p=0.002, for creaminess p=0.002, for texture p<0.001, for consistency p=0.001).Considering the overall score, IN8 (69.3±3.64) was statistically significantly higher than IN0 (57.8±3.52), IN4 (52.6±3.99) and IN12 (56.7±3.28) (p<0.05).In fat-free yoghurt, inulin may be used as a fat replacer and provides almost sensory properties like those of full-fat yoghurt.[18, 19]Swiader et al. demonstrated that the inulin addition to oolong tea yogurts resulted in a decrease in brightness, enhanced creaminess, and an increased sweet taste. The authors suggested that incorporating inulin into oolong tea-infused set yogurts could be beneficial not only as a source of prebiotic fiber in functional products but also as a factor that enhances the overall quality of these products.[20]While Pimentel et al. revealed no variance between the mean intensity values of sweet taste for full-fat and fat-free yoghurts containing 2% long-chain inulin, they still recommended that the integration of probiotics into the recipe of low-fat yoghurts could lead to similar sensory characteristics of full-fat yoghurts and the new recipe that they are trying to make would have probiotics as one of the main ingredients and it would have similar the sensory characteristics like full-fat yogurts.[21]

Overall, it can be suggested that the effect of inulin supplementation on the textural and sensory properties of yogurt depends on several factors, including the type of prebiotic added, the substitution level, the degree of polymerization, and the form in which the prebiotic is introduced (e.g., powder or gel).[22]

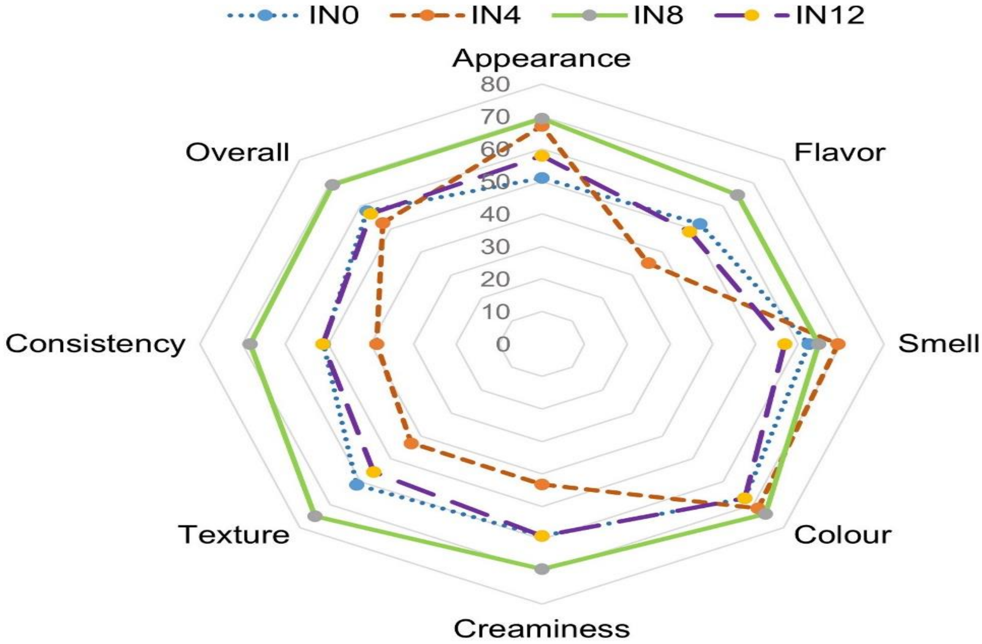

Figure 2. The view of sensory evaluation of yoghurts is depicted with different broken lines, representing the scores from aspects: appearance, flavor, smell, color, creamniess, texture, consistency and overall liking. IN: inulin.

Bacterial Assessment

Table 3 shows the effects of different inulin concentrations and storage periods on *Lactobacillus acidophilus* counts in yoghurt. The effects of different inulin concentrations (0%, 4%, 8%, and 12%) and storage time (baseline, 7, 14, and 21 days) on *Lactobacillus acidophilus* counts (log_{10} CFU/g) in yogurt were investigated using repeated measures ANOVA.The mean *Lactobacillus acidophilus* counts varied across inulin concentrations and storage days. At baseline, the highest mean count was observed in 4% inulin (6.76 log CFU/g), while the lowest was at 0% inulin (5.70 log CFU/g). Over the storage period, fluctuations in bacterial counts were noted for each group.This indicates that both storage time and inulin concentration significantly influenced bacterial counts, and their interaction was also significant.Significant main effects of inulin concentration ($F(3,8)=7522.2$, $p<0.001$, Partial $Eta^2=1.000$) and storage time ($F(3,24)=4971.4$, $p<0.001$, Partial $Eta^2=0.998$) were found, along with a significant interaction between these factors ($F(9,24)=2843.5$, $p<0.001$, Partial $Eta^2=0.999$).Bonferroni post hoc tests revealed that yoghurt samples with 4% and 8% inulin generally had higher Lactobacillus acidophilus counts than the control and 12% inulin groups across storage times. There was no significant difference between 4% and 8% inulin groups (p=0.383). These results indicate that both inulin addition and storage duration significantly affect probiotic viability in yoghurt.

Table 3. Effects of inulin addition yoghurt production and storage period on yoghurt culture (*Lactobacillus acidophilus*, CFU/g)

Groups	Storage period (day) (log_{10} CFU/g)			
	Baseline	7	14	21
IN0	5.69±0.012[a]	5.36±0.007[a]	6.22±0.002[a]	5.53±0.012[a]
IN4	6.76±0.001[b]	6.46±0.001[b]	5.86±0.002[b]	6.21±0.013[b]
IN8	6.51±0.005[b]	6.07±0.025[b]	6.17±0.017[a]	6.50±0.004[b]
IN12	6.33±0.002[b]	5.29±0.029[a]	5.15±0.003[b]	5.58±0.004[a]
	F	**df**	**p-value**	**Partial Eta²**
Inulin (Between-subjects)	7522.2	3	<0.001	1.000
Storage Time (Within-subjects)	4971.4	3	<0.001	0.998
Inulin × Storage Time Interaction	2843.5	9	<0.001	0.999

The letters a, b indicate mean values that show statistically significant differences between subjects according to repeated measures ANOVA. Pairwise comparisons between groups and time points were performed using Bonferroni post hoc tests. IN0: 0% inulin, IN 4: 4% inulin, IN8: 8% inulin, IN12: 12% inulin (w:w).

The decline in bacterial counts at the 7th day may result from freezing.[23] It can be said that the protective effect of inulin on bacterial survival and viability in the food matrix.[24-26] The amount of bacteria is important because a food product that contains sufficient doses of live probiotic microorganisms can alter intestinal microflora and plays a crucial role in maintaining the brain and nerve systems through its antioxidant functions.[27,28] Hussien et al. demonstrated that incorporating inulin into probiotic yoghurt boosts the growth of *Lactobacillus acidophilus*, *S. thermophiles,* and*Lactobacillus bulgaricus*throughout the storage period, in contrast to treatments that do not include inulin.[29] Similarly, our results demonstrated that the increase in probiotic counts in yogurt could be attributed to the role of inulin as a prebiotic substance.

According to our results, IN8 showed the highest amount of *Lactobacillus acidophilus* during storage. pH is one of the most important factors that affect the viability of bacteria. Bacterial amount in the IN8 was in accordance with the pH values. Similar results were observed in different studies.[29-31]
Increasing the inulin content in probiotic yogurt supported the growth of *Lactobacillus acidophilus* during storage, indicating that inulin serves as an effective nutrient for this probiotic strain. However, the dose of inulin supplementation also important. We observed that IN8 is more suitable. This result is consistent with previous studies that inulin increased bacterial viability and reduced viability loss during shelf life in synbiotic yoghurts containing *Bifidobacterium spp*. Pekçalışkan et al. compared the addition of 0.5%, 0.75%, 1% and 2% inulin and found that the use of inulin at different rates was effective on the viability of *Streptococcus thermophilus*, *Lactobacillus delbrueckii subsp*. *bulgaricus* and *Bifidobacterium spp*. and on physicochemical and sensory properties.[32] In a study where 1% and 2% inulin were added to yogurt production, no significant differences were observed in color, pH, titratable acidity, syneresis, or sensory evaluation on days 1, 7, and 14 of storage. Although the counts of *Lactobacillus delbrueckii ssp. bulgaricus* and *Lactobacillus acidophilus* decreased over time, the addition of inulin improved their viability during the storage of the synbiotic yogurt.[33] Krasaekoopt and Watcharapoka showed that there was no significant difference with addition of inulin (0.5%, 1% and 1.5%) in terms of *Lactobacillus acidophilus* counts in yoghurts.[34] Ergin showed that the use of inulin in increasing the dry matter of milk did not have a statistically significant effect on the microbiological properties of probiotic yoghurt samples.[35] This may be due to the added amount of 12%. In another study, inulin addition was found out to improve viscosity, viability of *Lactococcus spp*., and *Lactobacillus spp*. and sensory properties of kefir. Also, inulin addition had no effect on the pH values of the product. It was concluded that the sensorial properties of kefir can be enhanced with inulin addition as 1%.[36]

Among the main limitations of the present study are the lack of analysis of color, physical stability and textural properties. Further, evaluation of viscosity manually and only *Lactobacillus acidophilus* counting are major limitations. All of them are due to limited possibilities.

Conclusion

The current study showed that the addition of inulin in amounts of 4%, 8%, 12% w/w to the composition of natural fat-free yoghurt production showed statistically significant changes of its properties (pH, viscosity and sensory properties). The results showed that inulin addition increased the viscosity and pH. Inulin, especially in IN4 and IN8, stimulated the growth of *Lactobacillus acidophilus*, and the decrease over time is less. While there was no significant difference in appearance, smell and color, the highest score in terms of flavor, creaminess, texture and consistency was in IN8 compared to the scores of other groups. Considering the overall score, IN8 was of higher statistical significance than IN0, IN4 and IN12. The inclusion of inulin in the production of synbiotic yogurt is advisable because of its prebiotic benefits for the probiotic bacteria present in the yogurt. As a result, inulin could play a significant role in the creation of innovative dairy products. The results of the current study suggest that the addition of inulin with 8% as a prebiotic may be beneficial in the production of healthy and high-quality fat-free yoghurt.

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Peer-review: Externally independent
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Declarations:
1. Ethics Committee Approval: Due to the study methodology, an ethics committee is not required.
2. Informed Consent: Due to the study methodology, informed consent was not obtained.
3. Author Contributions: Concept-İMA; Design-İMA, FPÇ; Supervision-İMA, FPÇ; Resources-NY, İA; Materails-NY, İA; Data Collection and/or Processing-İMA, NY, İA; Analysis and/or Interpretation-İMA; Literature Search-NY, İA; Writing Manuscript-İMA, NY, İA, FPÇ; Critical Review-FPÇ.
4. Declaration of Interests: The authors declare that there is no conflict of interest.
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6. Sustainable Development Goals:

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