

Effect of *Momordica charantia* and Honey on Probiotic Yoghurt Production

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ABSTRACT

This study investigates the impact of incorporating *Momordica charantia* (bitter melon) and honey into probiotic yoghurt production, focusing on microbiological, physicochemical, and sensory properties. Bitter melon is recognized for its bioactive compounds, including antioxidants and prebiotic components, while honey is valued for its antimicrobial and nutritional benefits. Probiotic yoghurts were prepared with varying concentrations (1.5%, 3%, and 4.5%) of a honey-bitter melon mixture, and their properties were analyzed over 28 days of storage. Microbiological assessments revealed that the combination did not significantly affect the viability of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, while *Lactobacillus acidophilus* viability was significantly better preserved in groups containing the honey-bitter melon mixture compared to the control at the end of storage. Physicochemical analysis indicated that the honey-bitter melon mixture had minimal impact on pH and acidity levels. Water-holding capacity and syneresis varied among groups, with the gel structure of bitter melon contributing to reduced whey separation. Color measurements showed slight variations, suggesting the influence of bitter melon on yoghurt brightness and hue. Sensory evaluation demonstrated that honey-bitter melon mixture enhanced the overall acceptability of probiotic yoghurt, though no significant differences were observed between test groups and controls during storage. These findings suggest that incorporating bitter melon and honey into probiotic yoghurt does not compromise microbial viability, maintains desirable physicochemical properties, and improves sensory acceptance. This study highlights the potential of bitter melon and honey as functional ingredients in probiotic yoghurt, offering health benefits while preserving product quality.

Keywords: Bitter melon, Honey, Probiotic yoghurt, Sensory properties

Momordica charantia ve Balın Probiyotik Yoğurt Üretimine Etkisi

ÖZ

Bu çalışma, *Momordica charantia* (kudret narı) ve balın probiyotik yoğurt üretimine dahil edilmesinin etkisini mikrobiyolojik, fizikokimyasal ve duyuşal özelliklere odaklanarak araştırmaktadır. Kudret narı, antioksidanlar ve prebiyotik bileşenler de dahil olmak üzere biyoaktif bileşikleri ile tanınırken, bal antimikrobiyal ve besinsel faydaları nedeniyle değerlidir. Probiyotik yoğurtlar, bal-kudret narı karışımının değişen konsantrasyonlarıyla (%1,5, %3 ve %4,5) hazırlandı ve özellikleri 28 günlük depolama boyunca analiz edildi. Mikrobiyolojik değerlendirmeler, kombinasyonun *Streptococcus thermophilus* ve *Lactobacillus bulgaricus*'un canlılığını önemli ölçüde etkilemediğini, *Lactobacillus acidophilus*'un ise depolama sonunda bal-kudret narı ilaveli gruplarda kontrol grubuna göre canlılığını daha iyi koruduğu belirlendi. Fizikokimyasal analizler, bal-kudret narı karışımının pH ve asitlik seviyeleri üzerinde minimum etkiye sahip olduğunu gösterdi. Su tutma kapasitesi ve sinerez gruplar arasında değişmiştir, kudret narının jel yapısı serum ayrılmasının azalmasına katkıda bulunmuştur. Renk ölçümleri hafif farklılıklar göstermiştir ve bu da kudret narının yoğurt parlaklığı ve tonu üzerindeki etkisini göstermektedir. Duyusal değerlendirme, bal-kudret narı karışımının probiyotik yoğurdun genel kabul edilebilirliğini artırdığını göstermiştir, ancak depolama sırasında test grupları ve kontroller arasında önemli bir fark gözlenmemiştir. Bu bulgular, kudret narı ve balın probiyotik yoğurda dahil edilmesinin mikrobiyal canlılığı tehlikeye atmadığını, istenen fizikokimyasal özellikleri koruduğunu ve duyuşal kabulü iyileştirdiğini göstermektedir. Bu çalışma, kudret narı ve balın probiyotik yoğurtta işlevsel bileşenler olarak potansiyelini vurgulayarak, ürün kalitesini korurken sağlık yararları sunmaktadır.

Anahtar Kelimeler: Kudret narı, Bal, Probiyotik yoğurt, Duyusal özellikler

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INTRODUCTION

Momordica charantia, commonly referred to as bitter melon, is an annual plant belonging to the *Cucurbitaceae* family. It is mostly cultivated in tropical and subtropical locations worldwide (Li et al. 2007). The name "bitter melon" stems from its extremely bitter taste (Tuan et al. 2011). Fruits of *Momordica charantia* are rich in lutein, β -carotene, total carotenoids, α -tocopherol, and total folate, making them important sources. Bitter melon fruits are rich in α -linolenic acid, have low saturated fatty acids (SFA), and high polyunsaturated fatty acids (PUFA). Although bitter melon fruits are not particularly rich in PUFA, their high PUFA/SFA ratio may enhance their health benefits (Saini and Keum 2017). *Momordica charantia* contains several physiologically active compounds such as glycosides, saponins, alkaloids, oils, triterpenes, proteins, and steroids (Grover and Yadav 2004). This herb is commonly used in traditional medicine. The recent increase in studies evaluating its pharmacological properties is attributed to its potential biological implications (Pundir and Chandel 2021). *Momordica charantia* has antioxidant, anti-carcinogenic, and antidiabetic characteristics (Chekka and Mantipelly 2020). Moreover, it is advisable for incorporation into probiotic formulations due to its prebiotic characteristics that stimulate the proliferation of probiotic microorganisms (Fan et al. 2023).

Honey is recognized as one of the most ancient foods in the history of human civilization and serves as the main product of agricultural activities (Cilliers and Retief 2008). Honey has been extensively utilized throughout human history and served as a medical treatment in various traditions (Eteraf-Oskouei and Najafi 2013). Honey is a significant bee-derived substance that is a natural sweetener for humans (Otero and Bernolo 2020). It is produced by bees through remarkable engineering processes. The composition of its structure mostly consists of glucose, fructose, and water, along with trace quantities of proteins, minerals, organic acids, and vitamins (da Silva et al. 2016).

Yoghurt is a food that is enjoyed by many people in a variety of civilizations all over the world. Yoghurt is frequently considered as an essential component of a diet that is healthy (Mckinley 2005). Maintaining the optimum balance of the microbial population in the gut of a person is related to appropriate nutrition and overall health (Flint et al. 2012). Probiotics serve a crucial role in preserving this state of balance (Drisko et al. 2003). Yoghurt has been recognized as an essential carrier for delivering beneficial bacteria into the human digestive system (Lourens-Hattingh and Viljoen 2001; Mahmoodi Pour et al. 2002; Khorshidian et al. 2020; Moghadam et al. 2021; Abdi-Moghadam et al. 2023). Although previous studies

have investigated the individual effects of honey or bitter melon on dairy products, there is a lack of literature addressing their combined synergistic effect in a probiotic matrix. Specifically, the potential of honey to mask the intense bitterness of *Momordica charantia* while simultaneously supporting probiotic viability remains unexplored. Therefore, this study aimed to evaluate the use of a combination of bitter melon and honey in the production of probiotic yoghurt, as well as its impact on the physicochemical, microbiological, and sensory characteristics of the yoghurt.

MATERIAL and METHODS

The milk required to create probiotic yoghurt was sourced from the Farm of Burdur Mehmet Akif Ersoy University, located in Burdur. The strains Nu-trish LA5 (*Lactobacillus acidophilus* LA-5) and yoghurt culture YF-L903 (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were acquired from Chr. Hansen Laboratories in Copenhagen, Denmark. The bitter melon utilized for the experimental group research was acquired from a nearby garden in Antalya Province, Turkiye. The honey was sourced from Balparmak Company, located in Istanbul, Turkiye. The chemicals utilized for the studies were procured from Sigma-Aldrich Co. (St. Louis, USA).

Study Design

Following a heat treatment at 90°C for 5 minutes, the homogenized milk was subsequently chilled to 42°C. It was then split into four equal portions by introducing 2% of starter culture and probiotic culture. Following the separation of the first portion as the control group, a mixture of honey and bitter melon (85% honey and 15% bitter melon extract) was given to group I, group II, and group III, with concentrations of 1.5%, 3%, and 4.5% correspondingly (Table 1). Subsequently, 100 grams of polystyrene containers were filled and subjected to incubation at a temperature of 42°C for a duration of 4 hours. After the incubation period, the yoghurt samples were cooled down to a temperature of 4°C and kept at this temperature for a duration of 28 days (Tamime and Robinson 2007; Ozcan et al., 2010). The experiment was designed with three replications and analyses were conducted on days 0, 7, 14, 21, and 28.

Microbiological Analysis

The yoghurt samples analyzed for *S. thermophilus* were cultured on M-17 agar (Oxoid CM785) (Chr Hansen 2002a). The present study adopted MRS 5.4 Agar (De Man Ragoza Sharpe, Difco 288210) as the medium for investigating *L. bulgaricus* (Chr Hansen 2002b). The

ISO 20128/IDF192 technique was employed to identify the presence of probiotic *L. acidophilus* LA-5 (ISO 2006).

Table 1. Experimental study groups

	Honey (%85)/Bitter Melon (%15) Mix	<i>L. acidophilus</i> LA-5
Control I	0	-
Control II	0	+
Group I	1.5	+
Group II	3	+
Group III	4.5	+

+: Added, -: Not added

Physicochemical Analysis

The Official Methods of Analyses were applied in order to evaluate the physicochemical properties of yoghurt products (AOAC 2016). These parameters included pH, acidity, the serum separation (syneresis), and water holding capacity. The approach that was presented by Wu et al. (2000) was utilized in order to look at syneresis.

Color Analysis

A colorimeter (Konika Minolta, CR 400, available in Osaka, Japan) was utilized in order to carry out the color study. In the course of the investigation, the parameters of L^* (lightness), a^* (red/green), and b^* (yellow/blueness) were evaluated in accordance with the Hunter scale.

Sensory Analysis

The study was carried out to assess the sensory attributes of yoghurt samples throughout a 28-day duration while being stored in cold settings. A panel of 10 trained experts in dairy product evaluation (aged between 25 and 45 years, comprising academic staff and graduate students with prior experience in sensory testing) conducted the sensory analysis, following the methodology described by Canbulat and Özcan (2015). Panelists evaluated the samples for color, appearance, odor, texture, flavor, and overall acceptability using a defined scoring scale.

Statistical Analysis

The research was replicated three times, and three measurements were taken for each parameter on the 1st, 7th, 14th, 21st, and 28th day of storage. The data was subjected to statistical analysis using SPSS 25.0 software (SPSS Inc., USA). One-way Analysis of Variance (ANOVA) was applied to determine the effects of treatments and storage time. To identify

significant differences between the means of the experimental groups, Tukey's post-hoc test was utilized. Results were considered statistically significant at a confidence level of 95% ($p < 0.05$).

RESULTS

Microbiological Analysis

This study investigated the total count of *Streptococcus thermophilus* bacteria. The results indicate that the microbial count remained over 8 log cfu g⁻¹ on the 21st and 28th days in the yoghurt groups, with microbial activity continuing throughout the storage duration. Although statistical differences were observed on specific storage days (e.g., day 21), the microbial count generally remained above 8 log cfu g⁻¹ for all groups. The count of *Lactobacillus bulgaricus* bacteria was assessed in yoghurt trial groups, with the group II exhibiting the highest quantity. Despite the hypothesis that a bitter melon and honey mixture could enhance the multiplication of this bacterium, significant fluctuations were observed in *L. bulgaricus* counts depending on the storage day and concentration ($p < 0.05$), although no consistent linear increase was identified. The storage duration was shown to have no significant impact on the quantity of *Lactobacillus bulgaricus*. The bacterial counts of *Lactobacillus acidophilus* were analyzed in yoghurt trial groups, showing that the greatest values were predominantly recorded during the initial two days. Statistical analysis revealed significant differences by day 28 ($p < 0.05$). While *L. acidophilus* counts in the control group decreased to 5.78 log cfu/g, the honey-bitter melon supplemented groups maintained significantly higher viability (>6.6 log cfu/g), indicating a protective effect on probiotic survival (Table 2).

Table 2. Microbiological analysis results of the experimental study (\log_{10} cfu g^{-1}).

<i>S. thermophilus</i>	0. day	7. day	14. day	21. day	28. day
Control I	8.71±0.11 ^a	8.36±0.09 ^{ab}	8.45±0.15 ^a	8.77±0.16 ^{bc}	8.64±0.15 ^a
Control II	8.54±0.15 ^a	8.31±0.12 ^b	8.41±0.15 ^a	8.58±0.13 ^c	8.85±0.18 ^a
Group I	8.68±0.14 ^a	8.32±0.17 ^{ab}	8.70±0.19 ^a	8.99±0.15 ^{ab}	8.89±0.15 ^a
Group II	8.77±0.14 ^a	8.11±0.16 ^c	8.55±0.14 ^a	9.09±0.11 ^a	8.93±0.15 ^a
Group III	8.92±0.16 ^a	8.04±0.19 ^c	8.49±0.16 ^a	8.72±0.17 ^{bc}	8.83±0.11 ^a
<i>L. bulgaricus</i>	0. day	7. day	14. day	21. day	28. day
Control I	7.61±0.15 ^{bc}	7.54±0.15 ^b	7.64±0.18 ^{ab}	5.90±0.14 ^d	6.00±0.15 ^c
Control II	8.01±0.27 ^{ab}	8.10±0.15 ^a	7.05±0.09 ^c	6.62±0.14 ^c	6.01±0.14 ^c
Group I	7.16±0.04 ^c	7.91±0.10 ^{ab}	7.17±0.16 ^c	6.99±0.02 ^b	7.20±0.15 ^b
Group II	8.13±0.16 ^a	7.84±0.12 ^{ab}	7.75±0.16 ^a	7.01±0.13 ^b	6.89±0.15 ^b
Group III	7.78±0.07 ^{abc}	7.60±0.12 ^b	6.77±0.17 ^d	7.49±0.10 ^a	7.61±0.18 ^a
<i>L. acidophilus</i>	0. day	7. day	14. day	21. day	28. day
Control I	-	-	-	-	-
Control II	7.63±0.13 ^a	6.93±0.14 ^b	6.63±0.11 ^b	6.18±0.15 ^c	5.78±0.02 ^c
Group I	7.13±0.15 ^b	7.02±0.15 ^b	7.30±1.06 ^{ab}	6.98±0.12 ^b	6.13±0.12 ^b
Group II	7.67±0.15 ^a	7.62±0.16 ^a	6.98±0.13 ^{ab}	6.78±0.16 ^b	6.68±0.15 ^a
Group III	7.44±0.13 ^{ab}	7.52±0.05 ^a	7.34±0.11 ^a	6.97±0.53 ^a	6.74±0.11 ^a

Values are presented as mean \pm standard deviation. ^{a-d} Different lowercase letters in the same column indicate statistically significant differences between groups ($p < 0.05$) according to Tukey's post-hoc test.

Physicochemical Analysis

Table 3 presents the physicochemical results of the experimental groups. The pH change data were analyzed in the yoghurt experimental groups. Throughout the storage duration (7, 14, 21, and 28 days), a general increase in pH values was observed. Although pH values increased over time across all groups, no significant differences were detected across them. The pH values generally exhibited a comparable trend across all groups, with the exception of the group II. The pH value of 4.71 in the group II was slightly higher numerically from that of the other groups. During the last storage periods (21 and 28 days), a notable increase in pH values was noticed. This increase did not result in a substantial difference between the experimental groups. The addition of the bitter melon honey mixture to the probiotic yoghurt had a negligible influence on the pH value. Despite the general increase in pH values over the storage period, no statistically significant differences were noted among the groups. The results demonstrate that the bitter melon honey combination

did not significantly influence the pH value in the manufacturing of probiotic yoghurt. Analysis of acidity determination results in the yoghurt trial groups revealed fluctuations in acidity levels over the course of storage (7, 14, 21, and 28 days). Nonetheless, the cohorts exhibiting statistically significant changes were restricted. The addition of the bitter melon honey mixture to the probiotic yoghurt had a negligible impact on acidity. The water holding capacity of the experimental groups varies during the storage duration. Nevertheless, no clear pattern was identified among the groups. Consequently, differences in holding capacity are observed among the experimental groups. The syneresis values of the experimental groups vary over the storage period. No consistent trend was identified among the groups. Consequently, differences in syneresis values are observed among the experimental groups. Group III exhibits the highest syneresis value. The gel structure of bitter melon appears to mitigate syneresis formation by effectively retaining the serum phase.

Table 3. Physicochemical results of the experimental groups.

pH	0. day	7. day	14. day	21. day	28. day
Control I	4.39±0.15 ^{ns}	4.36±0.18 ^{ns}	4.60±0.23 ^{ns}	4.63±0.16 ^{ns}	4.66±0.14 ^{ns}
Control II	4.36±0.16 ^{ns}	4.38±0.14 ^{ns}	4.56±0.21 ^{ns}	4.54±0.21 ^{ns}	4.63±0.29 ^{ns}
Group I	4.25±0.15 ^{ns}	4.20±0.10 ^{ns}	4.44±0.32 ^{ns}	4.38±0.30 ^{ns}	4.43±0.30 ^{ns}
Group II	4.29±0.10 ^{ns}	4.24±0.11 ^{ns}	4.55±0.20 ^{ns}	4.54±0.18 ^{ns}	4.71±0.19 ^{ns}
Group III	4.31±0.07 ^{ns}	4.24±0.11 ^{ns}	4.68±0.24 ^{ns}	4.63±0.28 ^{ns}	4.58±0.27 ^{ns}
Acidity (%LA)	0. day	7. day	14. day	21. day	28. day
Control I	1.04±0.04 ^{ns}	1.15±0.06 ^{ns}	1.08±0.01 ^{ns}	1.13±0.01 ^{ns}	1.18±0.02 ^{ns}
Control II	1.10±0.05 ^{ns}	1.12±0.04 ^{ns}	1.10±0.02 ^{ns}	1.11±0.01 ^{ns}	1.11±0.06 ^{ns}
Group I	1.04±0.01 ^{ns}	1.13±0.04 ^{ns}	1.10±0.01 ^{ns}	1.09±0.04 ^{ns}	1.12±0.10 ^{ns}
Group II	1.01±0.03 ^{ns}	1.11±0.03 ^{ns}	1.03±0.04 ^{ns}	1.01±0.01 ^{ns}	1.09±0.06 ^{ns}
Group III	1.03±0.04 ^{ns}	1.11±0.03 ^{ns}	1.06±0.02 ^{ns}	1.13±0.01 ^{ns}	1.14±0.06 ^{ns}
Water holding capacity (%)	0. day	7. day	14. day	21. day	28. day
Control I	61.76±1.2 ^b	68.11±1.5 ^{ab}	66.75±1.4 ^a	67.56±1.1 ^a	67.11±1.3 ^a
Control II	67.91±1.4 ^a	68.88±1.3 ^{ab}	67.39±1.6 ^a	64.59±1.5 ^b	67.02±1.2 ^a
Group I	59.64±1.1 ^b	71.89±1.6 ^a	62.30±1.5 ^b	65.05±1.4 ^{ab}	64.04±1.1 ^{ab}
Group II	58.43±1.5 ^b	65.93±1.4 ^b	62.33±1.2 ^b	63.96±1.3 ^b	67.55±1.4 ^a
Group III	58.14±1.3 ^b	72.61±1.7 ^a	64.49±1.8 ^{ab}	68.98±1.6 ^a	67.75±1.5 ^a
Syneresis (%)	0. day	7. day	14. day	21. day	28. day
Control I	24.29±1.2 ^b	23.70±1.5 ^b	34.29±2.1 ^a	23.49±1.1 ^c	21.53±1.4 ^c
Control II	23.61±1.4 ^b	25.52±1.3 ^b	28.02±1.8 ^b	24.38±1.2 ^{bc}	23.69±1.5 ^{bc}
Group I	24.13±1.1 ^b	25.92±1.6 ^b	30.82±2.0 ^{ab}	23.70±1.0 ^c	29.57±1.8 ^a
Group II	30.26±1.8 ^a	28.42±1.4 ^a	28.91±1.5 ^b	30.82±1.9 ^a	30.28±1.6 ^a
Group III	27.79±1.5 ^{ab}	31.33±1.7 ^a	36.57±2.2 ^a	28.21±1.4 ^{ab}	22.39±1.3 ^c

Values are presented as mean ± standard deviation. ^{a-d} Different lowercase letters in the same column indicate statistically significant differences between groups ($p < 0.05$) according to Tukey's post-hoc test. 'ns' indicates no significant difference.

Color Analysis

In color analysis, the L^* value indicates an indicator characteristic of color brightness. In groups I, II, and III, the L^* values are typically marginally lower than in other groups, indicating that the colors are darker and less vibrant. A notable difference in L value is evident between groups I and II, but group III has a slightly increased L^* value compared to the other groups. Variations in the proportions of bitter melon and honey within yoghurt samples can result in variable measurement findings. Variations in light intensity can affect color perception and potentially influence the results of color analysis. Variations in light intensity can lead to discrepancies in color perception and, consequently, in color measures such as the L^* value. Alterations in the ratios of bitter melon and honey may influence the composition of yoghurt, resulting in variations in attributes such as color, viscosity, aroma, and flavor. This may also be evident in the outcomes of color analysis. The a^*

value defines the color's position on the scale, which is between green and red. Groups I, II, and III showed a medium a^* values. In these groups, the bitter melon honey mixture may have provided a balance of green and red tones. The b^* value indicates the blue-green (negative) and yellow (positive) color components. These values can indicate, for instance, the color variations of food goods or the color disparity between distinct samples. Overall, the b^* values were elevated on the final analysis day compared to the initial analysis day. This implies that the products experienced a color alteration during storage, exhibiting a more noticeable blue-green or yellow color. Such color changes may result from ripening or other physicochemical transformations.

The minimum b^* value was observed in the control groups on the initial day of analysis. This indicates that the control groups possess fewer blue-green or yellow color components compared to the other groups (Table 4).

Table 4. Results of color analysis for the experimental groups

<i>L*</i> value	0. day	7. day	14. day	21. day	28. day
Control I	89.33±4.14 ^{ab}	92.83±4.10 ^a	96.24±0.16 ^a	91.88±0.74 ^a	91.93±0.67 ^a
Control II	89.57±3.87 ^{ab}	85.39±11.55 ^{ab}	94.25±0.89 ^a	93.60±0.25 ^a	92.88±1.85 ^a
Group I	94.37±0.56 ^a	85.96±12.29 ^{ab}	90.93±2.70 ^b	89.25±2.39 ^{ab}	91.54±1.97 ^a
Group II	92.94±0.50 ^a	94.66±1.52 ^a	95.26±0.78 ^a	87.67±3.93 ^b	91.55±0.61 ^a
Group III	86.94±3.00 ^b	93.57±1.54 ^a	93.12±2.79 ^{ab}	91.08±0.91 ^a	89.23±3.02 ^a
<i>a*</i> value	0. day	7. day	14. day	21. day	28. day
Control I	1.46±0.11 ^d	1.16±0.14 ^c	1.61±0.04 ^b	0.82±0.15 ^d	1.20±0.14 ^d
Control II	1.40±0.01 ^d	1.07±0.02 ^c	1.05±0.01 ^d	0.93±0.08 ^d	1.49±0.07 ^c
Group I	2.36±0.25 ^c	1.89±0.01 ^b	1.32±0.03 ^c	1.52±0.09 ^c	1.40±0.05 ^{cd}
Group II	2.81±0.06 ^a	1.80±0.10 ^b	2.46±0.23 ^a	1.95±0.06 ^b	1.87±0.11 ^b
Group III	2.59±0.01 ^b	2.45±0.16 ^a	1.74±0.18 ^b	3.68±0.23 ^a	2.50±0.01 ^a
<i>b*</i> value	0. day	7. day	14. day	21. day	28. day
Control I	3.40±0.21 ^c	3.69±0.17 ^c	5.07±1.04 ^b	8.07±1.35 ^b	4.60±0.33 ^d
Control II	3.02±0.02 ^c	13.13±2.55 ^a	11.10±0.27 ^a	9.87±0.12 ^a	5.82±0.30 ^{cd}
Group I	5.45±0.11 ^b	2.42±0.20 ^c	3.15±0.16 ^c	5.58±0.49 ^c	10.42±0.37 ^a
Group II	5.29±0.08 ^b	6.22±0.10 ^b	4.52±0.29 ^b	7.12±0.37 ^b	6.49±0.77 ^c
Group III	7.06±0.04 ^a	3.16±0.70 ^c	4.34±0.06 ^b	7.27±0.27 ^b	8.42±0.35 ^b

Values are presented as mean ± standard deviation. ^{a-d} Different lowercase letters in the same column indicate statistically significant differences between groups ($p < 0.05$) according to Tukey's post-hoc test. 'ns' indicates no significant difference.

Sensory Analysis

Figure 1 shows the sensory analysis values of the yoghurt experimental groups. The assessment of the general acceptability parameter indicates that the scores for groups I, II, and III are predominantly above 6.5. The data indicates that groups I, II, and III

were predominantly more favorable in the initial day analyses and were preferred over the control groups and other groups containing bitter melon-honey. No statistically significant change was noted in certain groups throughout the storage period.

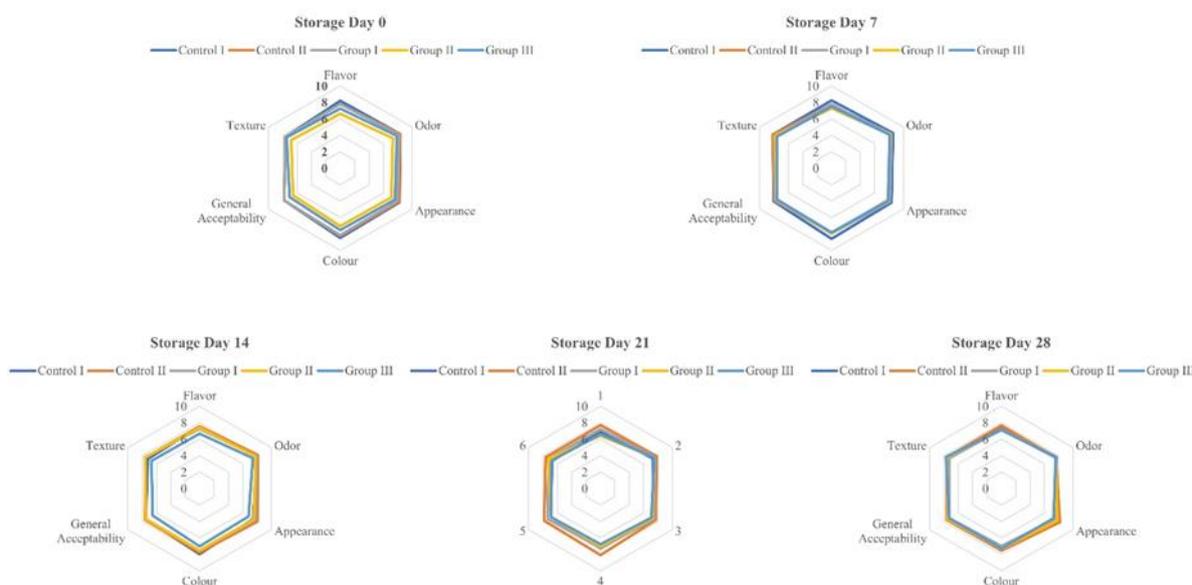


Figure 1: Sensory analysis of experimental groups.

DISCUSSION

The research by Coskun and Dirican (2019) concluded that the addition of pine honey did not significantly affect the pH values in yoghurts, in line with the findings of this investigation. Another investigation indicated that differences

in pH levels in yoghurts may arise based on the ratios of certain fruits.

Probiotic bacteria can generate organic acids, thus decreasing pH levels. The results indicate that the pH levels of yoghurts with fruit additions may vary depending on the fruit type, amount, and

time of storage (Costa et al. 2015; Albayati et al. 2024). This study concluded that the addition of a bitter melon honey mixture to probiotic yoghurt had a negligible influence on pH, with just a slight rise in pH values noted over the storage period. The results indicate that the combination of bitter melon and honey does not significantly influence the pH value in the preparation of probiotic yoghurt. The impact of fruit incorporation on pH values was more distinctly noted in the research conducted by Albayati et al. (2024). This disparity indicates that the interaction of fruit kind, ratio, and content with yoghurt cultures varies in relation to the bitter melon honey mixture in the research. The impact of fruit-added yoghurts on pH values may fluctuate, influenced by parameters such as fruit type, proportion, composition, and storage duration.

Water holding capacity (WHC) is a critical quality attribute in yoghurt production, influencing texture, mouthfeel, and overall consumer acceptance. The incorporation of various ingredients and probiotic strains can significantly affect the WHC of yoghurt. Several studies have demonstrated the impact of different additives on the WHC of probiotic yoghurt. The use of oat flour in synbiotic yoghurt formulations has been reported to reduce syneresis and improve WHC, contributing to a more desirable texture (Reshma et al. 2022). The type of probiotic strains used can also influence WHC. For example, probiotic soy yoghurt has been observed to exhibit better WHC compared to plain soy yoghurt, suggesting that the presence of probiotics can enhance the structural integrity of the yoghurt matrix (Kanchana et al. 2016). Additionally, the fortification of yoghurt with whey protein concentrate (WPC) has been found to enhance WHC more effectively than sodium calcium caseinate, due to the finer protein network formed by WPC (Akalin et al. 2012). Coskun and Karabulut Dirican (2019) completed a study to determine the water holding capacities of samples during storage, aiming to evaluate changes in these capacities over time. The study's results indicated a reduction in water holding capacity across all samples with extended storage duration. The water holding capacity values varied between the control group and the other groups with distinct components. This study assessed the varying water holding capacities among groups in the bitter melon and honey mixture study. Variations in water retaining capacity were noted among the groups during the storage duration. Discrepancies may have arisen in comparison to

the published research due to the inclusion of additional components.

A key quality criterion influencing the texture and customer acceptance of probiotic yoghurts is syneresis, the expulsion of whey from yoghurt. Different chemicals and circumstances have been investigated in many research on syneresis in probiotic yoghurt. Prebiotics like inulin, lactulose, and β -glucan have been proven to greatly affect syneresis in probiotic yoghurt. For probiotic yoghurt formulations, for example, 1.5% Hi-maize or β -glucan produced lower syneresis, therefore improving the general texture and sensory qualities of the yoghurt (Heydari et al. 2018). Likewise, although it somewhat lowered the sensory ratings compared to plain yoghurt, the addition of rice bran reduced syneresis and offered antioxidative effects (Demirci et al. 2017). It has been noted that adding fruit purees like sloe berries and elderberry into probiotic yoghurts boosts syneresis. The high water content and the structural alterations brought about by the fruit components most certainly have this effect. These fruit additions improved the antioxidant potential of the yoghurts, ascribed to their high polyphenol content, even if syneresis increased (Najgebauer-Lejko et al. 2021). But the kind of fruit utilized affected the stability of various components, including anthocyanins; elderberry showed better stability than sloe berries (Najgebauer-Lejko et al. 2021). A study analyzed the syneresis parameters of probiotic yoghurt samples with almond milk additions throughout storage. Statistically significant differences were identified among all samples. Owing to the water retention characteristics of milk proteins and milk fat globules, serum separation was found to be less pronounced in the control group sample compared to the other samples. It was established that serum separation values diminished with prolonged storage duration and were categorized into statistically distinct categories (Yılmaz Ersan and Topcuoglu 2019). Consequently, serum separation significantly influences yoghurt quality. This study shows variations in syneresis values among the groups. No statistically significant differences were reported between the groups. The syneresis values of the experimental groups varied throughout the storage period; however, no consistent trend was seen among the groups. Syneresis readings can be influenced by factors including components, additions, and duration of storage. Additives such bitter melon and almond milk can enhance the gel structure and minimize syneresis development. The duration of storage

results in variations in syneresis values throughout time.

The incorporation of prickly pear influences the whiteness value of probiotic yoghurts, and the duration of storage significantly impacts color attributes (Albayati et al. 2024). In the current study demonstrates that bitter melon influences the color tone and brightness of yoghurts, and that the ratios of bitter melon and honey significantly impact color alteration. Manufacturers must carefully choose and modify the additional components and their quantities to ensure the color attributes of yoghurt conform to the specified criteria. Color analysis is a crucial factor influencing the aesthetic attractiveness of a product and consumer preferences (Pathare et al. 2013). In a study, the control yoghurt sample exhibited the lowest a^* and b^* values at the onset of storage (without fruit powder addition) compared to yoghurts including fruit powder. The a^* and b^* values increased with the fruit ratio in all samples during the storage period (Tarakçı et al. 2016). A separate study indicated that including prickly pear into probiotic yoghurts elevated the a^* values, with a corresponding intensification of the reddish-orange hue as the fruit proportion grew. The a^* values diminished during storage, attributed to the yoghurt containing only 10% prickly pear addition. The inclusion of prickly pear in yoghurts resulted in elevated a^* values, attributed to the presence of natural colors like carotenoids in the fruit and the influence of lipid oxidation. The incorporation of prickly pear fruit pulp at varying proportions elevated the b^* values of yoghurts, resulting in a more pronounced yellow hue with the increased fruit ratio. Albayati et al. (2024). This investigation indicated that the a^* values on the color scale represented the spectrum between red and green tones. This suggests that the a^* values were often reasonable in the bitter melon-honey combo, potentially creating a balance between green and red colors in these groups.

The yoghurt fermentation process is mainly dependent on *S. thermophilus* and *L. bulgaricus*. In combination, these bacteria enhance the fermentation process, which is essential for the development of yoghurt's texture and flavor. Research indicates that the proportion of prickly pear fruit incorporated into yoghurt may influence the viability of *S. thermophilus* (Albayati et al. 2024). In the current study found that the bitter melon and honey combination did not significantly impact the viability of *S. thermophilus*. Conversely, the research by Albayati et al. (2025) demonstrated that the incorporation of honey

enhanced the population of *S. thermophilus* in drinkable yoghurt throughout storage. In the study of Yılmaz Ersan and Topcuoglu (2019), the amounts of *L. acidophilus* and *L. bulgaricus* were examined in probiotic yoghurt samples enriched with almond milk. It was observed that the number of *L. bulgaricus* decreased with the storage period, but increased in the sample containing 100% almond milk. It was also determined that the number of *L. acidophilus* increased during storage. This study shows that almond milk can promote the proliferation of *L. bulgaricus* and *L. acidophilus* bacteria in probiotic yoghurt. In another study, the effect of clove on probiotic yoghurt was examined. According to the results, the number of *L. acidophilus* in yoghurts with clove addition was found to be significantly lower than in yoghurts with clove addition in the control group probiotic yoghurt. In addition, it was determined that the number of this bacteria decreased with the increase in the clove concentration (Bayır and Bilgin 2019). In this study, the numbers of *L. bulgaricus* and *L. acidophilus* bacteria were examined. Although the mixture did not initially accelerate proliferation, it significantly supported the survival of *L. acidophilus* towards the end of storage compared to the control ($p < 0.05$). This protective effect may be attributed to the prebiotic potential of bitter melon and honey, which helps maintain probiotic viability.

Honey added to probiotic yoghurt has demonstrated to improve taste, texture, and general acceptability among other sensory qualities. For goat yoghurt including probiotic *L. acidophilus*, honey has been demonstrated to improve color, viscosity, and sensory acceptability, hence raising customer purchase intention (Machado et al. 2017). The addition of manuka honey to yoghurt has been linked to a beneficial fermentation metabolite profile, which improves sensory attributes like flavor and aroma (Mohan et al. 2020). Studies of consumer perception show that consumer preferences can be much influenced by the sensory qualities of probiotic yoghurt. For instance, low-fat choices are chosen because of their firmness and texture, hence yoghurt with additional sugars and low milk fat have been proven to influence consumer acceptance (Dias et al. 2020). Furthermore, the inclusion of honey in yoghurt formulations has been welcomed; formulations with 5% honey earned highest ratings in sensory tests (Rashid and Thakur 2012). Honey's presence influences not only the sensory qualities but also the yoghurt's probiotic cultures' survivability. Particularly at

specific dosages, studies have shown that honey can improve the growth of probiotic bacteria including *L. bulgaricus* and *S. thermophilus* (Rashid and Thakur 2012). This implies that honey not only enhances the sensual appeal but also promotes the functional advantages of probiotic yoghurt. Honey improves sensory qualities, but its concentration should be balanced to keep probiotics viable and the intended sensory profile. As several formulations where increased honey concentrations produced less pleasant sensory outcomes (Rashid and Thakur 2012) show, excessive honey can cause unwelcome changes in flavor and texture. Thus, designing a probiotic yoghurt that satisfies customer expectations and maintains its health advantages depends on optimizing the levels of honey and other components such as *Momordica charantia*. The evaluation of the general acceptance parameter reveals in this study that the groups including the bitter melon-honey mixture and the control groups are more agreeable than the others. Honey added to the yoghurt increased its general acceptance, it was found; in some groups, acceptability did not change statistically significantly throughout storage. These results indicate that the combination of honey and bitter melon could influence the yoghurt's general acceptance qualities.

CONCLUSION

This research demonstrates that the combination of bitter melon and honey positively influences the physicochemical, microbiological, and sensory attributes of probiotic yoghurt. The research findings indicate that the incorporation of a bitter melon and honey mixture in probiotic yoghurt manufacture enhanced the yoghurt's nutritional content. The sensory study results indicate that the incorporation of bitter melon and honey enhanced the taste, aroma, consistency, and overall acceptability of probiotic yoghurt. The findings indicate that bitter melon and honey enhance the quality of probiotic yoghurt for consumers and elevate the overall acceptability of the product. The manufacture of probiotic yoghurt through the incorporation of natural components presents an intriguing opportunity for the food sector as a healthful alternative product. This study's findings enhance studies on functional food items and healthy nutrition, introducing a novel method for probiotic yoghurt production. However, this study has certain limitations, including the production being conducted on a pilot scale and

the distinct bitterness of *Momordica charantia* which may still be perceptible to sensitive consumers despite the addition of honey. Innovative research is required to mitigate the unpleasant flavor of bitter melon through combining it with other components. Future studies should focus on microencapsulation technologies to better mask the bitterness and controlled clinical trials to validate the in vivo health benefits of this functional yoghurt.

Authors' Contributions: The experiments were designed and organized by EK. EK and DK executed the experiments. EK and DK designed and implemented the simulations. DK and EK participated in the analysis of the findings. EK coordinated the manuscript's composition. All writers offered helpful criticism and contributed to the development of the study, analysis, and manuscript.

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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Explanation: We will present as a (oral) presentation at the 8th International Health Sciences and Life Congress (IHLSC) (2025).

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