



## Effect of Perga Addition on The Bioactivity and Physicochemical Characteristics of Yogurt During Storage

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### Abstract

This study investigated the effects of adding bee bread (perga) on the bioactivity and shelf life of yogurt. Raw milk sourced from the Kayseri-Gesi region was fermented using a yogurt starter culture with traditional methods, and perga was added to the formulations at ratios of 0.01%, 0.02%, 0.03%, and 0.04%. The resulting yogurts were analyzed on days 1, 4, 7, 14, and 21; pH, titration acidity, water-holding capacity, color ( $L^*$ ,  $a^*$ ,  $b^*$ ), total phenolic content, and DPPH free radical scavenging capacity were determined. According to the results, as the perga concentration increased, the pH value of the yogurts decreased slightly compared to the control group, but the pH values of all samples remained within an acceptable range during storage. Titration acidity ranged from 0.91 to 1.22 g lactic acid/100 g at all concentrations and was found to be consistent with standard yogurt values. A significant increase in water-holding capacity was observed with the addition of perga and increased storage time, with perga-containing samples showing significantly higher water-holding values than the control group, particularly on days 14 and 21. Although there was a slight tendency toward darkening and yellowing at high perga levels at the beginning in the color parameters, these differences decreased during storage, and no undesirable color defects occurred. Total phenolic content and DPPH antioxidant capacity values fluctuated between days, but no significant and dose-dependent increase was detected with low-dose perga addition compared to the control group. In general, it was concluded that adding up to 0.04% perga did not negatively affect the physicochemical quality and shelf life of yogurt; in fact, it could contribute to the structural stability of the product by improving its water-holding capacity.

## Perga İlavesinin Depolama Boyunca Yoğurdun Biyoaktivitesi ve Fiziko-Kimyasal Özellikleri Üzerindeki Etkisi

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### Özet

Bu çalışmada, arı ekmeği (perga) ilavesinin yoğurdun biyoaktivitesi ve raf ömrü üzerindeki etkileri araştırılmıştır. Kayseri-Gesi bölgesinden temin edilen çiğ süt, geleneksel yöntemlerle yoğurt starter kültürü kullanılarak fermente edilmiş ve formülasyonlara %0,01, %0,02, %0,03 ve %0,04 oranlarında perga ilave edilmiştir. Elde edilen yoğurtlar 1., 4., 7., 14. ve 21. günlerde analiz edilmiş; pH, titrasyon asiditesi, su tutma kapasitesi, renk (L\*, a\*, b\*), toplam fenolik içerik ve DPPH serbest radikal temizleme kapasitesi belirlenmiştir. Sonuçlara göre, perga konsantrasyonu arttıkça, yoğurtların pH değeri kontrol grubuna kıyasla hafifçe azalmış, ancak tüm numunelerin pH değerleri depolama sırasında kabul edilebilir bir aralıkta kalmıştır. Titrasyon asitliği tüm konsantrasyonlarda 0,91 ila 1,22 g laktik asit/100 g arasında değişmiş ve standart yoğurt değerleriyle tutarlı olduğu görülmüştür. Perga ilavesi ve saklama süresinin uzamasıyla su tutma kapasitesinde önemli bir artış gözlemlenmiştir; perga içeren numuneler, özellikle 14. ve 21. günlerde kontrol grubuna göre önemli ölçüde daha yüksek su tutma değerleri göstermiştir. Renk parametrelerinde başlangıçta yüksek perga seviyelerinde hafif bir koyulaşma ve sararma eğilimi görülse de, bu farklılıklar saklama süresince azalmış ve istenmeyen renk kusurları meydana gelmemiştir. Toplam fenolik içerik ve DPPH antiradikal kapasite değerleri günler arasında dalgalanma göstermiş, ancak kontrol grubuna kıyasla düşük dozda perga ilavesi ile önemli ve doza bağlı bir artış tespit edilmemiştir. Genel olarak, %0,04'e kadar perga ilavesinin yoğurdun fizikokimyasal kalitesini ve raf ömrünü olumsuz etkilemediği, aksine su tutma kapasitesini artırarak ürünün yapısal stabilitesine katkıda bulunabileceği sonucuna varılmıştır.

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### Introduction

Perga, popularly known as bee bread, is a valuable bee product. Perga, a mixture of pollen, honey, and honeybee enzymes, is fermented and stored in the honeycomb cells. Honeybees meet their nutritional needs from pollen, nectar, and water. Nectar is the primary carbohydrate source, while perga provides protein, lipid, vitamin, and mineral needs. It has been reported to contain approximately 35% sugar, 20-22% protein, 3.5% lactic acid, 2.43% minerals, and 1.6% lipids [1]. It is also rich in vitamins A, B complex, C, P, E, D, K, and H, numerous minerals, essential amino acids, and phenolic substances

such as campferol, coumaric acid, apigenin, chrysin, caffeic, and ferulic acid [2]. Perga has been reported to improve lipid peroxidation caused by obesity, and to have anticarcinogen, antimicrobial and constipation-fighting properties [3-6].

Yogurt is one of the fermented foods obtained from milk by lactic acid fermentation. Yogurts fermented with natural additives aim to preserve the traditional yogurt structure while enhancing its functional properties. These can include prebiotics and functional fibers (inulin, oligofructose, beta-glucan, etc.), plant-derived stabilizers (pectin, natural gums, etc.), fruit and plant extracts (pomegranate, grape seed, turmeric, cinnamon), natural colorants (spirulina, betanin, paprika extract), and natural protein sources (whey protein, milk powder) [7-15].

Numerous factors affect the physical, chemical, and sensory properties of yogurt. These include raw material quality (total dry matter content, protein content, casein and non-casein protein ratio, acidity), additives, homogenization, heat treatment practices, denatured serum proteins, cultures used in fermentation, inoculum amount, incubation temperature, cooling, and storage conditions [16]. Yogurt generally contains 14-20% dry matter, 2-8% fat, 4-8% protein, and 0.8-1.2% minerals. Furthermore, its amino acid and conjugated acid content, iron, and vitamin D, phosphorus, riboflavin (B2), cobalamin (B12), folate (B9), niacin (B3), and vitamin A also contribute to its nutritional value [17]. The water-retention capacity of yogurt indicates how well it can retain water molecules within its structure and is a crucial characteristic for yogurt quality. A high water-holding capacity minimizes water release during storage and consumption and maintains its consistency. Yogurt's water-holding capacity is optimal at a pH of 4.3-4.6 [18].

The phenolic content (gallic acid, caffeic acid, ferulic acid, kaempferol, apigenin, etc.) is responsible for the antioxidant activity of bee bread. Scientific studies have shown that although the total phenolic content of Turkish bee bread varies, it ranges from 8.4 mg GE/g to 26.38 mg GAE [18]. This variability is attributed to factors such as plant source, geography, climatic conditions, and the fermentation process. Lactic acid fermentation, which occurs during the conversion of pollen into bee bread, increases the bioavailability of phenolic compounds. As a matter of fact, the fermentation process can positively affect general biological activity by releasing some phenolic compounds [19].

This study aimed to investigate the potential changes in the shelf life and bioactivity of yogurt, a fermented food, when perga, a fermented and probiotic ingredient, is used in its fermentation process.

## **Material and method**

### **Materials**

Perga, obtained from a migratory beekeeper, was used in the study after being stored at -20°C for one week. The milk used in the study was obtained raw from a dairy farmer who raises livestock in the Kayseri-Gesi region. Vivo brand starter culture was chosen as the yogurt starter. Commercial yogurt starter culture, containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii ssp. bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium lactis* was purchased from Turkish supermarket.

### **Methods**

Perga concentrations were determined as 0.01, 0.02, 0.03, and 0.04% in the experiments. The commercial yogurt starter was added to the milk at 0.4% (w/v). A total of 25 different jars were used to ferment 500 ml of yogurt. The fermented yogurts were analysed at 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> days of storage

### **pH Analysis**

pH measurements were taken at  $25\pm 1^\circ\text{C}$  using a pH meter (WTW InolabpH Level1, Germany). Before measurement, the samples were thoroughly crushed and mixed thoroughly with distilled water at a 1:1 ratio to achieve homogeneity. The yogurt samples and distilled water were used in a ratio of 1:1 (w:v). The pH meter electrode was immersed in the prepared samples for reading values [20].

### **Titration Acidity Analysis**

One g of yogurt samples was taken, 10ml of distilled water was added, and 3-4 drops of phenolphthalein (Merck Millipore, 100074, Germany) were added as an indicator. The mixture was titrated with a 0.1N NaOH (NAOH, Tekkim, TK.400298, Turkey) solution until the desired pink color was achieved. Results were calculated in % lactic acid [21].

### **Color Analysis**

The color of the samples was measured using a Minolta colorimeter (CR-300, Japan) according to the Hunter system. Before measurement, the device was calibrated using a standard white plate ( $L^*$ : 96.97,  $a^*$ : 0.16,  $b^*$ : 1.86). Three replicate readings were taken from different points of the yogurt samples, and the averages were taken.  $L^*$  (brightness) values were determined [22].

### **Water Holding Capacity**

Twenty g of each yogurt sample was placed in 50 ml liquefaction tubes and centrifuged at 3500 g for 10 minutes at  $4^\circ\text{C}$ . After centrifugation, the amount of serum separated was divided by the amount of yogurt, and the water retention capacity was determined as a percentage [23]. Water holding capacity =  $[1 - (\text{Amount of serum separated from the centrifuge/sample amount})] * 100$

### **Total Phenolic Content**

The total phenolic content of samples was determined using a modified Folin-Ciocalteu colorimetric method [24]. 0.5 ml of Perga yogurt extract was taken, and 2.5 ml of Folin-Ciocalteu reagent (10%, v/v) was added. After three minutes, 2 ml of  $\text{Na}_2\text{CO}_3$  solution (7.5% (w/v)) was added. After mixing thoroughly, the yogurt was left in the dark at room temperature for 1 hour. At the end of 1 hour, the absorbance of the sample against the blank was measured at 765 nm using a UV-Visible Spectrophotometer (Hach DR 6000 Germany). Calculations were made using a standard curve drawn with gallic acid, and the results were expressed as mg GAE/g of gallic acid equivalents.

### **DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Analysis**

One g of yogurt sample was placed in 50 ml falcons, a 70% methanol solution was added, and the sample was stirred for 1 hour. The total antiradical capacity of the yogurts with Perga was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method [25]. Perga yogurt extract was placed in 100  $\mu\text{L}$  tubes, and 3.9 ml of DPPH radical (0.025 g/L) prepared with methanol was added. The tubes were mixed using a vortex mixer and left to stand at room temperature in the dark for 30 minutes. After 30 minutes, the absorbance of the samples was measured at 517 nm using a UV-Visible Spectrophotometer.

### **Statistical Analysis**

Statistical analysis was performed using a two-way ANOVA in SAS software (Ver. 5.0.1, SAS Institute Inc., Cary, NC, USA). When a significant effect was detected, mean comparisons among groups were carried out using Duncan's multiple range test at a significance level of  $\alpha = 0.05$ .

## Result and discussion

As a result of the analyses, the differences observed depending on the concentration and storage days were found to be statistically significant ( $p < 0.05$ ). The pH values of all samples decreased throughout the storage period (Table 1). This may be due to the fact that bacterial activity persists even at low storage temperatures. It was observed that pH values decreased more rapidly on the first day of storage than on the other days. This may be due to the starter bacteria continuing their activity until the internal temperature of the yogurts dropped below 5°C after incubation, thus continuing the acidity development [26].

Table 1. pH values of yogurt samples

Storage period (day)	Concentrations				
	0 (control)	0.01	0.02	0.03	0.04
1	4.16 <sup>Aa</sup>	4.17 <sup>Aa</sup>	4.07 <sup>Ad</sup>	4.11 <sup>Ac</sup>	4.13 <sup>Ab</sup>
4	3.96 <sup>Cc</sup>	3.98 <sup>Cb</sup>	4.06 <sup>Aa</sup>	3.95 <sup>Dd</sup>	3.93 <sup>Ce</sup>
7	4.07 <sup>Bab</sup>	4.06 <sup>Bc</sup>	4.07 <sup>Abc</sup>	4.08 <sup>Ba</sup>	4.02 <sup>Bd</sup>
14	4.05 <sup>Bb</sup>	4.08 <sup>Ba</sup>	4.05 <sup>Bb</sup>	4.05 <sup>Cb</sup>	4.03 <sup>Bc</sup>
21	3.85 <sup>Dc</sup>	3.96 <sup>Ca</sup>	3.97 <sup>Ca</sup>	3.89 <sup>Eb</sup>	3.92 <sup>Cb</sup>

Capital letters in the same column indicate statistical differences between storage days ( $p < 0.05$ ). Lowercase letters in the same row indicate statistical differences between concentrations ( $p < 0.05$ ).

According to Table 2, the water-holding capacities of the yoghurt samples generally showed an increasing trend throughout storage, and this increase became more pronounced depending on the perga concentration. While the water-holding capacity in the control samples was approximately 49% on 1<sup>st</sup> day, it rose to around 60% at the end of storage, meaning that the time factor alone provided a partial improvement. The addition of perga significantly increased the water-holding capacity above that of the control, particularly in the later days of storage; on day 21, the water-holding capacity of samples containing 0.01–0.04 perga reached approximately 69–72%, and these values were statistically higher than those of the control on the same day ( $p < 0.05$ ). Although a lower water-holding capacity was initially observed at low perga levels (especially at a concentration of 0.04%), these samples rapidly recovered as storage progressed, yielding the highest values on days 14 and 21. When the letters in the columns and rows are evaluated together, it can be said that both storage time and perga concentration, as well as the interaction of these two factors, have a statistically significant improving effect on the water-holding capacity of yoghurts.

Table 2. Water holding capacities of yogurt samples

Concentrations					
Storage period (day)	0 (control)	0.01	0.02	0.03	0.04
1	49.27 <sup>Ba</sup>	48.66 <sup>Ca</sup>	52.43 <sup>Ba</sup>	50.37 <sup>Da</sup>	36.16 <sup>Ba</sup>
4	56.26 <sup>AAb</sup>	52.82 <sup>BCb</sup>	51.20 <sup>Bb</sup>	61.43 <sup>Ba</sup>	54.33 <sup>Abb</sup>
7	55.18 <sup>Aa</sup>	55.71 <sup>Bca</sup>	56.93 <sup>Ba</sup>	55.01 <sup>Ca</sup>	55.00 <sup>Aba</sup>
14	59.95 <sup>Aa</sup>	59.58 <sup>Ba</sup>	58.33 <sup>Ba</sup>	61.47 <sup>Ba</sup>	61.96 <sup>Aa</sup>
21	59.95 <sup>Ac</sup>	68.97 <sup>Ab</sup>	72.47 <sup>Aa</sup>	71.44 <sup>Aa</sup>	71.08 <sup>Aa</sup>

Capital letters in the same column indicate statistical differences between storage days ( $p < 0.05$ ). Lowercase letters in the same row indicate statistical differences between concentration ( $p < 0.05$ ).

Titrateable acidity is one of the factors that directly affects shelf life of yogurt [26]. Therefore, it is crucial that it does not exceed the standard limits. Titrateable acidity values of yogurts fermented with Perga according to storage days are shown in Table 3. According to Table 3, the titration acidity of the yoghurt samples showed very limited variation in terms of both storage time and perga concentration, with values remaining in the range of approximately 0.91–1.22 g lactic acid/100 g across all groups. While the acidity of the control sample was at its highest level (1.220) on the first day, significantly lower acidity values were determined, particularly in yoghurts containing 0.03% and 0.04% perga, meaning that the addition of perga initially reduced the titration acidity to some extent. In the following days of storage, acidity values fluctuated slightly in both the control and perga-added groups, but by day 21, all samples reached similar levels and the differences between concentrations decreased significantly. Considering the letters in the columns and rows, although statistical differences were found in some day and concentration combinations, it can be said that, in general, the addition of perga did not have a significant increasing or decreasing effect on the titration acidity of the yoghurt, and the storage period only slightly affected the acidity.

Table 3. Titrateable acidity values of yogurt samples

Concentrations					
Storage period (day)	0 (Control)	0.01	0.02	0.03	0.04
1	1.220 <sup>Aa</sup>	1.000 <sup>Ab</sup>	1.006 <sup>BCb</sup>	0.941 <sup>Cb</sup>	0.923 <sup>Bb</sup>
4	1.015 <sup>Bab</sup>	1.008 <sup>Aab</sup>	0.915 <sup>Db</sup>	1.019 <sup>Bab</sup>	1.065 <sup>Aa</sup>
7	1.119 <sup>Aba</sup>	1.076 <sup>Aa</sup>	0.975 <sup>Ca</sup>	1.021 <sup>Ba</sup>	1.079 <sup>Aa</sup>

14	1.114 <sup>Aba</sup>	1.039 <sup>Ab</sup>	1.077 <sup>Aab</sup>	1.089 <sup>Abab</sup>	1.114 <sup>Aa</sup>
21	1.172 <sup>Aa</sup>	1.059 <sup>Aab</sup>	1.030 <sup>Bb</sup>	1.127 <sup>Aab</sup>	1.146 <sup>Aab</sup>

Capital letters in the same column indicate statistical differences between storage days ( $p < 0.05$ ). Lowercase letters in the same row indicate statistical differences between concentrations ( $p < 0.05$ ).

Color in yogurt is a criterion that directly influences consumer preferences. Table 4 shows the  $L^*$  value of the color determination results for yogurt samples. In the CIE system,  $L^*$  represents the lightness (lightness; on a scale from 0 to 100, 0 = black, 100 = white) and the whiteness of the yogurt color. On 1<sup>st</sup> day, the control sample showed the highest lightness (90.40), while especially the 0.02 and 0.03% perga treatments had markedly lower  $L^*$  values (62.18 and 70.07, respectively), indicating a darker appearance; the different lowercase letters in this row confirm that these treatments differed significantly from the control and from each other ( $p < 0.05$ ). At day 4<sup>th</sup>, however, the  $L^*$  values of all formulations converged (all marked with lowercase “a”), and no significant difference remained between control and perga-added yogurts. During the later storage period (days 7–21),  $L^*$  values fluctuated slightly but generally increased for the perga-containing samples, and in several cases (e.g., 0.02% on days 7, 14 and 21, and 0.01% on day 14) the perga treatments even reached or exceeded the control in lightness. Capital letters within the same column indicate that, except for some changes between the first and subsequent days (particularly in 0.02 and 0.04% treatments), storage time did not cause a pronounced or systematic decrease in  $L^*$  for any formulation. These results suggest that perga addition initially tended to darken the yogurt matrix at higher concentrations, but this effect diminished during cold storage and was not associated with a progressive loss of lightness over time.

Table 4.  $L^*$  values of yogurt samples

Storage period (day)	Concentrations				
	0 (control)	0.01	0.02	0.03	0.04
1	90.40 <sup>Aa</sup>	87.06 <sup>Ba</sup>	62.18 <sup>Cc</sup>	70.07 <sup>Cbc</sup>	75.83 <sup>BCb</sup>
4	90.32 <sup>Aa</sup>	83.29 <sup>Ba</sup>	82.92 <sup>Ba</sup>	85.83 <sup>Aba</sup>	90.84 <sup>Aa</sup>
7	96.16 <sup>Aab</sup>	85.44 <sup>Bc</sup>	98.99 <sup>Aa</sup>	81.12 <sup>Bc</sup>	87.70 <sup>Abbc</sup>
14	94.42 <sup>Aa</sup>	98.84 <sup>Aa</sup>	98.36 <sup>Aa</sup>	93.68 <sup>Aa</sup>	66.60 <sup>Cb</sup>
21	96.54 <sup>Aa</sup>	94.74 <sup>ABa</sup>	99.99 <sup>Aa</sup>	81.18 <sup>Bb</sup>	99.10 <sup>Aa</sup>

Capital letters in the same column indicate statistical differences between storage days ( $p < 0.05$ ). Lowercase letters in the same row indicate statistical differences between concentrations ( $p < 0.05$ ).

According to Table 5, the  $a^*$  values remained negative (approximately between -1.48 and -4.19) in all yoghurt samples, indicating that the products retained a slightly greenish tone throughout storage and did not show a significant shift towards red. While the  $a^*$  values in the control and 0.01% perga samples fluctuated slightly between -2.3 and -3.3, the 0.02% perga yoghurts had the lowest  $a^*$  values, particularly on 7<sup>th</sup> and 21<sup>st</sup> days, exhibiting a greener appearance compared to other concentrations. At 0.03% perga, the values formed a moderately stable greenish tone between -2.44 and -3.21, while 0.04% perga provided the least green (relatively most reddish) appearance, giving the closest  $a^*$  value to zero among all samples, particularly on 14<sup>th</sup> day with a value of -1.48. returning to more negative values on other

days. Overall, it can be stated that both the perga concentration and the storage period, as well as their interaction, had a statistically significant but more limited effect on the a\* values compared to the L\* values.

Table 5. a\* values of yogurt samples

Concentrations					
Storage period (day)	0 (control)	0.01	0.02	0.03	0.04
1	-2.97 <sup>Ac</sup>	-2.98 <sup>Ac</sup>	-2.25 <sup>Aa</sup>	-2.77 <sup>Abbc</sup>	-2.41 <sup>Bab</sup>
4	-2.92 <sup>Aab</sup>	-3.00 <sup>Aab</sup>	-2.79 <sup>Aba</sup>	-3.21 <sup>Bab</sup>	-3.30 <sup>Cb</sup>
7	-3.28 <sup>Aab</sup>	-2.89 <sup>Aa</sup>	-4.05 <sup>Cb</sup>	-2.44 <sup>Aa</sup>	-2.90 <sup>Bca</sup>
14	-3.30 <sup>Abc</sup>	-2.33 <sup>Aab</sup>	-3.67 <sup>BCc</sup>	-2.69 <sup>Aabc</sup>	-1.48 <sup>Aa</sup>
21	-3.15 <sup>Ab</sup>	-2.96 <sup>Ab</sup>	-4.19 <sup>Cc</sup>	-2.47 <sup>Aa</sup>	-3.20 <sup>Cb</sup>

Capital letters in the same column indicate statistical differences between storage days ( $p < 0.05$ ). Lowercase letters in the same row indicate statistical differences between concentrations ( $p < 0.05$ ).

According to Table 6, the b\* values were positive in all yoghurt samples (ranging from approximately 13.4 to 19.0), indicating that the samples had a predominantly yellow appearance throughout storage. In the control samples, b\* values increased significantly between days 1 and 14 (from 13.65 to 17.83), indicating a marked increase in yellowness, which then decreased again on 21<sup>st</sup> day. The addition of perga generally increased the b\* values above those of the control and caused the yoghurts to appear more yellow; in particular, samples containing 0.02 and 0.03% perga yielded the highest b\* values (around 18) on most storage days, increasing the intensity of yellowness. The 0.04% perga, while initially similar to the control, showed the highest b\* value of 19.02 among all samples on the 14<sup>th</sup> day, thus creating the most intense yellow tone at this point.

Table 6. b\* values of yogurt samples

Concentrations					
Storage period (day)	0 (Control)	0.01	0.02	0.03	0.04
1	13.65 <sup>Dc</sup>	15.23 <sup>Bbc</sup>	18.06 <sup>Aa</sup>	18.33 <sup>Aa</sup>	16.12 <sup>BCb</sup>
4	15.03 <sup>Cb</sup>	16.90 <sup>Aa</sup>	17.09 <sup>Aa</sup>	17.54 <sup>Aa</sup>	14.02 <sup>Db</sup>
7	16.69 <sup>Bb</sup>	17.46 <sup>Aab</sup>	14.92 <sup>Bc</sup>	18.38 <sup>Aa</sup>	17.35 <sup>Bb</sup>
14	17.83 <sup>Ac</sup>	15.20 <sup>Bb</sup>	16.79 <sup>Ab</sup>	15.84 <sup>Ba</sup>	19.02 <sup>Aa</sup>
21	13.42 <sup>Dc</sup>	14.00 <sup>Cb</sup>	13.92 <sup>Bb</sup>	14.92 <sup>Ba</sup>	15.06 <sup>CDa</sup>

Capital letters in the same column indicate statistical differences between storage days ( $p < 0.05$ ). Lowercase letters in the same row indicate statistical differences between concentrations ( $p < 0.05$ ).

According to Table 7, the total phenolic content of the yoghurt samples showed significant fluctuations during storage. In all groups, phenolic levels were quite low on days 1 and 4 (approximately 0.13–0.76 mg GAE/g), and generally no significant difference was observed between these two days. In contrast, on 7<sup>th</sup> day, there was a very sharp increase, particularly in the control, 0.01% and 0.04% perga-containing samples, and these groups yielded the highest phenolic values on the same day (6.61, 6.76 and 4.32 mg GAE/g, respectively;  $p < 0.05$ ). In the 0.02% and 0.03% perga applications, the increase on the 7<sup>th</sup> day was more limited, and the 0.03% level was even found to be significantly lower than the other concentrations. On the 14<sup>th</sup> day of storage, the phenolic content decreased in all samples but remained at high levels compared to the initial levels, and this time the highest value was determined in the 0.02% perga addition (4.11 mg GAE/g). On the 21<sup>st</sup> day, the phenolic content continued to decline in all concentrations but still remained at higher levels compared to 1<sup>st</sup> and 4<sup>th</sup> days. Considering the letters in the rows and columns, it is seen that both the perga concentration and the storage period, as well as their interaction, have a statistically significant effect on the total phenolic content, with noticeable differences depending on the perga level, particularly during the middle storage period (days 7–14).

Table 7. Total phenolic content of yoghurt samples

Storage period (day)	Concentrations				
	0 (Control)	0.01	0.02	0.03	0.04
1	0.756 <sup>Ca</sup>	0.446 <sup>Cb</sup>	0.240 <sup>Dc</sup>	0.669 <sup>Da</sup>	0.443 <sup>Dcb</sup>
4	0.151 <sup>Cb</sup>	0.133 <sup>Cb</sup>	0.218 <sup>Db</sup>	0.322 <sup>Ea</sup>	0.213 <sup>Db</sup>
7	6.614 <sup>Aa</sup>	6.760 <sup>Aa</sup>	2.515 <sup>Bc</sup>	0.267 <sup>Bc</sup>	4.321 <sup>Ab</sup>
14	2.964 <sup>Bc</sup>	3.129 <sup>Bc</sup>	4.106 <sup>Aa</sup>	3.590 <sup>Ab</sup>	3.029 <sup>Bc</sup>
21	1.144 <sup>Cab</sup>	0.959 <sup>Cbc</sup>	1.401 <sup>Ca</sup>	1.312 <sup>Cab</sup>	0.767 <sup>Cc</sup>

Capital letters in the same column indicate statistical differences between storage days ( $p < 0.05$ ). Lowercase letters in the same row indicate statistical differences between concentrations ( $p < 0.05$ ).

According to Table 8, the DPPH antiradical capacity of yoghurts is generally low (inhibition  $\approx 0.10$ –4.96) but shows a fluctuating trend depending on both perga concentration and storage time. On the first day of storage, the DPPH inhibition of all samples was very similar ( $\sim 1.3$ –1.45%), and the addition of perga did not create a significant difference compared to the control. On the fourth day, the activity increased slightly, especially in yoghurts containing 0.01% and 0.03% perga, while lower values were obtained at the 0.04% level. During the middle storage period (7<sup>th</sup> day), yoghurts prepared with 0.03 and 0.04% perga addition exhibited the highest antiradical capacity with inhibition values above 3%, while the control and low perga levels remained significantly lower. On day 14, a sharp decline was observed in all concentrations; in particular, the activity of samples containing 0.01% and 0.04% perga approached zero. At the end of storage (21<sup>st</sup> day), DPPH inhibition increased again in all groups, with the highest value measured at 0.04% perga addition (4.96%), while other concentrations were at similar levels in the range of 2.3–3.2%.

Table 8. DPPH antiradical capacity (%inhibition) values of yoghurt samples

Storage period (day)	Concentrations				
	0 (Control)	0.01	0.02	0.03	0.04
1	1.37 <sup>Aa</sup>	1.45 <sup>Aa</sup>	1.38 <sup>Ba</sup>	1.38 <sup>Bca</sup>	1.38 <sup>Bca</sup>
4	1.55 <sup>Aabc</sup>	1.98 <sup>Aab</sup>	1.35 <sup>Bbc</sup>	2.12 <sup>ABCa</sup>	1.08 <sup>Bcc</sup>
7	0.88 <sup>Ab</sup>	2.34 <sup>Aab</sup>	1.66 <sup>Bab</sup>	3.22 <sup>Aa</sup>	2.44 <sup>bBa</sup>
14	0.84 <sup>Ab</sup>	0.10 <sup>Ac</sup>	1.21 <sup>Ba</sup>	0.68 <sup>Cb</sup>	0.16 <sup>Cc</sup>
21	2.79 <sup>Aa</sup>	2.33 <sup>Aa</sup>	3.22 <sup>Aa</sup>	2.69 <sup>ABa</sup>	4.96 <sup>Aa</sup>

Capital letters in the same column indicate statistical differences between storage days ( $p < 0.05$ ). Lowercase letters in the same row indicate statistical differences between concentrations ( $p < 0.05$ ).

There are numerous studies on the nutrients added to yogurt [7-15]. In one of these, Citta et al. (2017) investigated the physical and chemical properties of yogurts fermented with purees obtained from cherry and peach fruits and observed their results for 9 weeks [28]. When the total phenolic content they obtained was examined, an increase was observed in the total phenolic content of yogurts with fruit puree added, while no significant change was observed during storage. Keyvan and Yurdakul (2024) investigated higher bee bread concentrations on probiotic yogurts and stated that bee bread did not affect physicochemical parameters such as pH, acidity, dry matter, ash, milk fat ( $P > 0.05$ ) but affected protein and syneresis ( $P < 0.05$ ) they also stated that there was a decrease in syneresis as the storage period increased ( $P < 0.05$ ) as similarly to our study [29]. In our study the addition of perga increased WHC while decreasing syneresis values. Similarly, it was concluded that the addition of natural nutraceuticals increases WHC and viscosity during storage and reduces syneresis [30]. In another study, the addition of bee pollen significantly increases the total phenolic content and antioxidant capacity in yogurt, with effects depending on pollen quantity and milk type [31]. But in our low-dose perga applications, phenolic and antioxidant activity rising was limited.

## Conclusion

In this work, yogurt was fermented with progressively elevated concentrations of perga (0.01–0.04%) to assess its impact on bioactivity and shelf life. Adding perga accelerated the acidification process a little bit at the start of storage, as seen by the lower pH values compared to the control. However, the titratable acidity in all treatments stayed within the typical range for yogurt for the whole 21-day period. The most consistent technological effect of perga was that it made the gel structure tighter and possibly reduced syneresis over shelf life. This happened more when the perga concentration and storage period rose. On the other hand, color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) were only slightly impacted. Perga merely changed the lightness and yellowness a little bit and did not produce any unwanted color flaws. The total phenolic content and DPPH antiradical capability showed significant day-to-day variations but did not demonstrate a distinct dose-dependent increase relative to the control, and the overall bioactive profile of the products remained consistent between treatments. In conclusion, these results show that adding

small amounts of perga ( $\leq 0.04\%$ ) to yogurt doesn't change its physicochemical quality, color, or oxidative stability, and it may even help it hold more water. Future studies should investigate elevated perga concentrations, alternative formats (such as extracts or encapsulated forms), and sensory acceptance to optimize the health benefits and functional attributes of this fermented bee product within dairy matrices.

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