

An Investigation of Effects of Whey Protein Hydrolysate on Yogurt Starter Cultures and Probiotic Bacteria in Ayran

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ABSTRACT

This study aims to monitor the effect of whey protein hydrolysate (WPH) on the growth and activity of probiotic bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*) in ayran, and also to enhance the functionality of ayran by addition of the probiotics. Effects of WPH and probiotics addition as 0%, 0.25%, 0.5% and 1% on the growth of probiotic bacteria were studied before and after the fermentation and 7th, 14th, and 21st days of the storage. WPH and probiotics had a significant effect ($p<0.05$) on the growth of target microorganisms and the biochemical variables. The addition of WPH and probiotics significantly ($p<0.05$) increased the growth of probiotics compared to the control after the fermentation up to the 21st day of storage. The samples containing different WPH levels had lower ($p<0.05$) pH levels compared to the control during the study time. The mixing of WPH at 1% had the highest total solids and protein content compared to the control samples. The WPH decreased ($p<0.05$) the viscosity and Hunter color parameters in added the samples. Results indicated that WPH had great potential for enhancing the growth of probiotic bacteria and the nutritional of ayran.

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Peynir Altı Suyu Proteini Hidrolizatının Ayranda Yoğurt Starter Kültürleri ve Probiyotik Bakteriler Üzerine Etkilerinin İncelenmesi

ÖZET

Bu çalışmanın amacı, peynir altı suyu (whey) protein hidrolizatın (WPH) ayrana eklenmesinin probiyotik bakterilerinin (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Lactobacillus acidophilus* ve *Bifidobacterium lactis*) aktivitesinin ve gelişimlerinin arttırılmasının araştırılmasıdır. Fermentasyon öncesi ve sonrasında 7., 14. ve 21. saklama süresince, WPH ve probiyotiklerin %0, %0.25, %0.5 ve %1 konsantrasyonlarında probiyotik bakteri büyümesi üzerine etkileri çalışılmıştır. WPH ve probiyotiklerin hedef mikroorganizmalar ve biyokimyasal değişkenler üzerinde önemli etkilere ($p<0.05$) sahip olduğu belirlenmiştir. WPH ve probiyotiklerin eklenmesi fermantasyondan sonra 21'inci saklama gününe kadar kontrole kıyasla önemli ölçüde ($p<0.05$), probiyotiklerin büyümesini arttırdığı görülmüştür. Farklı WPH düzeylerini içeren numuneler çalışma süresi boyunca kontrole kıyasla anlamlı derecede ($p<0,05$) daha yüksek titre edilebilir asitlik seviyesine sahip olduğu belirlenmiştir. WPH %1 düzeyinde karıştırılması, kontrol numunelerine kıyasla en yüksek toplam katı ve protein içeriğine sahip olduğu tespit edilmiştir. WPH içeren numuneler viskoziteyi ve Hunter renk parametrelerini azaltmıştır ($p<0.05$). Sonuçlar WPH ayran probiyotik bakterilerin büyümesini ve besinselliğini arttırmada büyük bir potansiyele sahip olduğu görülmüştür.

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INTRODUCTION

The growing request for a healthy diet is caused by developing innovation and new product stimulation in the food industry (Champagne et al., 2018). Nowadays fermented milk and products participate in health with the natural food and improve the intestinal flora with existing lactic acid bacteria. Fermented milk products such as yogurt, ayran, kefir, etc. are supplement-strong foods that are considered to be one of the most popular fermented milk products around the world (Shangpliang et al., 2018, Turkmen et al., 2019). The general utilization of milk products, especially, probiotic milk products achieves perfect evaluation of food long-term because of the great impacts over the health authenticated by the records of nutritionists and specialists (Kerry et al., 2018). Probiotics are live microorganisms food supplements, can benefit the health of users by preserving, or beneficent their intestinal microorganism balances (Kerry et al., 2018, Şanlıer et al., 2019). As well as the increase rate of probiotics in fermented milk products is a typical and common issue with a mercantile importance (Korbekandi et al., 2011).

Currently, the species of *Lactobacillus* and *Bifidobacterium* are extremely utilized as a part of probiotic fermented milk products and commonly connected with the gastrointestinal tract (Turkmen et al., 2019). Lactic acid bacteria (basically *Lactobacillus*) and *Bifidobacteria* are the essential operators of the probiotics in the practical nourishment industry (Kerry et al., 2018, Turkmen et al., 2019). They provide for the human body pioneer benefits such as energizing of the immune system (Perdigon et al., 1992), protection from many infections (De Macias et al., 1993), improving digestion and absorption (Michael et al., 2010). Probiotic bacteria are related to various health advantages and the most important issue for probiotic is viability and survival in a specific concentration in the gastrointestinal tract, stay survive in the different conditions (Gerez et al., 2012, Turkmen et al., 2019) and improve the microbial balance of the intestinal medium.

Whey protein (WP) is a group of globular protein isolated from whey that can be used as a nutritional supplement (Wirunsawanya et al., 2018). Whey protein hydrolysate (WPH) is utilized generally by dairies, bread shops, confectionaries, meat preparing, canned products, and refreshment foundations for their different capacities in sustenance quality and solidness (Wirunsawanya et al., 2018). The WPH is predigested, that's mean the tall chains of amino acids organized in isolates and concentrates, will be

solved to a simple amino acid. Also, WPH has the fastest absorption capacity; release of amino acid through the bloodstream and may carry to greater muscle proteins when intake after the exercises than other kinds of WP. So, the WPH is utilized in the multiplication of body-building. Many studies proved the possibility of WP can promote the survival of probiotic and culture bacteria (Wirunsawanya et al., 2018, Turkmen et al., 2019). Whey protein hydrolysate is added into yoghurt to improve the growth of yoghurt cultures and probiotics in milk supplemented (McComas Jr and Gilliland, 2003, Krunić et al., 2019).

Ayran is one of the most important fermented milk drinks and common in many countries in Asia, the Middle East, and known especially as a traditional drink in Turkey. Ayran usually prepared in-home by adding water to yoghurt or industrially by adding yoghurt starter culture like *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* into milk (TFC, 2009). Whenever, ayran is prepared from yoghurt, is produced by mixing of yoghurt with water (between 30-50%) and is salt (0-1%) (Köksoy and Kılıç, 2003).

Effect of supplement with nutrient addition on the viability of probiotic bacteria in fermented milk is an important issue through the fermentation and storage. Considering this, the addition of WPH and probiotics to the fermented milk will promote the ability of these probiotics, will increase their functional characteristic and will increase the effectiveness of the product by promoting the viability of the probiotics. Due to containing a high level of nutrients, WPH and probiotics added into ayran can improve functionality. The present study was aimed (i) to investigate enhancement probability of growth and activity of probiotic bacteria (*Lactobacillus acidophilus*, *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus* and *Bifidobacterium lactis*) by adding of WPH and probiotics into ayran before and after the fermentation and 7th, 14th and 21th days of fermentation; (ii) to improve the chemical characteristics of ayran such as pH and acidity, reduce the decline in pH values through the incubation time and at the end of storage time; (iii) to enhance protein content, and (iv) to follow color and viscosity changes were studied during the storage period of ayran.

MATERIALS and METHODS

Experimental design

Fresh milk was heated at 95 °C for 15 min. Heat treated milk was cooled down to the fermentation

temperature of 40°C. Then yoghurt starter was inoculated as 4% (v/v) and divided into 10 groups (control, 3 groups for WPH (0.25 %, 0.5 %, and 1.0% (w/v)), 3 groups for probiotics (0.25 %, 0.5 %, and 1.0% (w/v)) and 3 groups for mix of WPH and probiotics). WPH and probiotics (*Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* contain 2.5×10^9 cfu/10g) were added in concentrations 0 (control), 0.25, 0.5, and 1.0% (w/v). Samples were produced by adding sterile water in 50% (v/v) and salt in the concentration of 0.5% (w/v) into the mixtures. All samples were mixed very well then were incubated at 40 °C. The fermentation process continued until reaching to pH of 4.4 ± 0.02 . After the fermentation, ayran samples were cooled down and keep at 4°C for 21 days in a refrigerator. The biochemical parameters such as the changes in the pH and acidity were measured through the fermentation, after fermentation and during the storage time after 7th, 14th and 21st days. Probiotic bacteria were counted before and after the fermentation, and through 7th, 14th and 21st days of the storage. For clear measurements, viscosity and Hunter Color Analyses were recorded after one-day of sampling.

Microbiological analysis

The numbers of bacteria were counted and expressed as log CFU/ml. Enumeration of bacteria in ayran containing 0, 0.25, 0.5, 1% (w/v) of WPH and/or probiotics were recorded before and after the fermentation and after 7th, 14th and 21st days of the storage. All the samples were diluted by tenfold with peptone water (0.1% v/v) then pour or spread to agar after that were incubated. Finally, the colonies were counted. Growth of bacteria was recorded by the following method of enumeration of *L. acidophilus* (Dave and Shah, 1998) was completed by spreading on to MRS agar by adding 1% of maltose. The inoculated petri dishes were incubated at 37°C for 3 days after that the colonies were counted. *S. thermophilus* colonies were enumerated by the pour plate technique inoculation of appropriate dilution on M17 agar with lactose 1%. The Petri dishes were incubated at 45°C for 24 hour and colonies were counted. *Lactobacillus bulgaricus* colonies was counted by the pour-plate technique into MRS agar with fructose at 1%, petri dishes were incubated at 45°C for 3 days and counted. Enumeration of *B. lactis* was done by the pour technique in to MRS agar. Then petri dishes were incubated for 45°C- 3 days and the colonies were counted.

Chemical analyses

Values of pH were measured using a pH meter every one hour through fermentation of samples containing 0.25, 0.5, 1% (w/v) of WPH and/or probiotics during

the storage.

Total solid of samples were determined using an oven method proposed by TS1330 (Anonymous, 2006). Briefly, the samples were put into a beaker on a boiling water bath up to the excess water were removed from the samples then were cooled at room temperature. The empty dishes were heated in oven for one hour at 102-103°C. The 5 mL of sample was added and recorded the weight before drying, then put it in the oven at 102-103°C up to constant weight reached.

Protein determination was carried out according to a Turkish standard method TS1330 (Anonymous, 2006). Briefly, 10 ml of milk, 0.5 ml of phenolphthalein, and 0.4 ml saturated potassium oxalate solution was mixed in a conical flask and waited for 2 min. After that, the mixture was neutralized with 0.1 M NaOH of a burette until getting a faint color. Then 2 ml of 40% formaldehyde solution was added and completed the titration until getting the same pink color like previously color and determine the level of 0.1 M sodium hydroxide wanted for the second titration.

The viscosity was followed for all ayran samples after 24 h of sampling time by a Brookfield viscometer (DV3T™ viscometer, USA) at 20 rpm in 150 mL beaker to taking the measurements of viscosity.

The color of ayran samples was recorded after the fermentation and through 7th, 14th and 21st days of storage time by Hunter color equipment (Colorflex color measurement spectrophotometer, Hunter Associates Lab. Inc. Mumbai). For each sample was taken 10 mL in a special class and put on the equipment, then L*, a* and b* values were recorded.

Statistical Analyses

A one-way ANOVA (Analysis of Variance) was used to determine significant differences at $\alpha=0.05$ level in the number of probiotic bacteria, pH, colors, and viscosity as a function of time and among products, using the SPSS version 19.0 (SPSS Inc., Chicago, USA). For the identification of the homogeneous groups of time and samples' variables, Duncan multiple comparison test was performed. Statistical analyses were applied to find how samples of ayran and storage time affect the probiotic bacteria and other parameters.

RESULTS and DISCUSSION

Growth of *Streptococcus thermophilus*

Use of 0.25, 0.5 and 1% of WPH significantly ($p<0.05$) increased the growth of *S. thermophilus* compared to the control before the fermentation. The incubation and storage time had a significant ($p<0.05$) effect on the growth of *S. thermophilus* (Figures 1a-c). The highest growth of *S. thermophilus* (8.86 log CFU) was

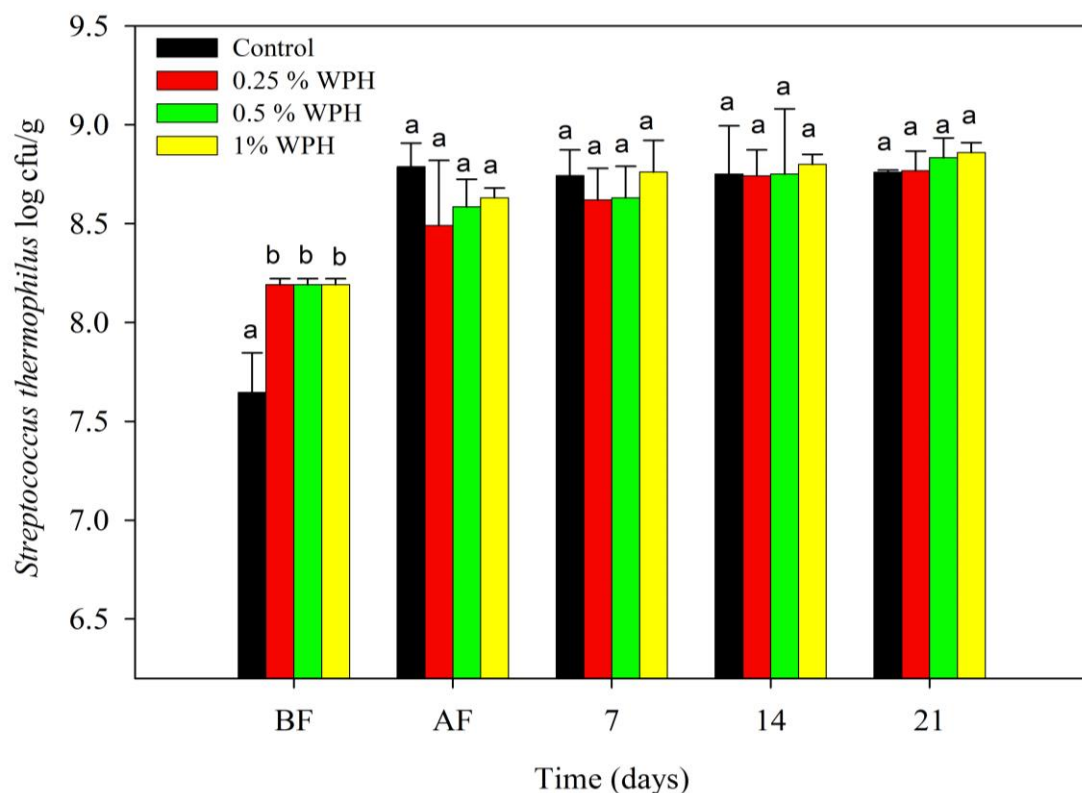
observed by the addition of 1% WPH at the end of the storage among the only WPH containing samples (Figure 1a). This increment could be due to WPH containing the branched-chain amino acid, which could play an important role in the growth of probiotic bacteria (Güler-Akın and Akın, 2007). Also, whey protein contains milk proteins about 20% (Hoffman and Falvo, 2004). High amounts of amino acids, minerals, and beneficial compounds could stimulate the growth of this bacteria, in agreement with the report of Fox (1986). Akalin et al. (2007) reported that the counts of *S. thermophilus* in low-fat yoghurt containing 1.5% WPC [(8.56 log CFU/mL) was higher than in the low-fat yoghurt without the addition of WPC (8.08 log CFU/mL)] after the 28 days of storage time.

Addition of probiotics had no significant effect ($p>0.05$) on the growth of *S. thermophilus* compared to the control till the end of the storage (Figure 1b). However, the highest growth of *S. thermophilus* (8.93 log CFU/mL) was determined by the addition of both WPH and probiotics (Figure 1c) at 0.25% into ayran ($p<0.05$). Probiotics are sensible to environmental conditions as well as had nutritional requirements

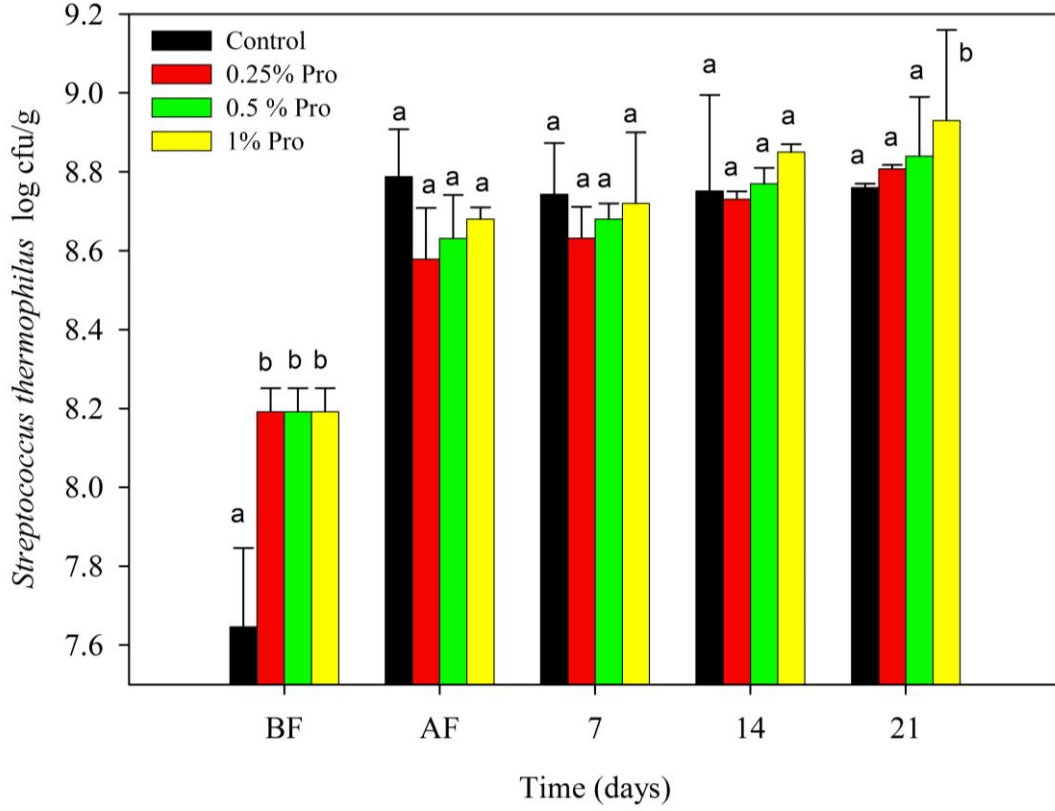
more than the other kind of bacteria. That the growth of probiotics may effect an increase/decrease by the other addition on a media (Gardiner et al., 2002).

Growth of *Lactobacillus delbrueckii* ssp. *bulgaricus*

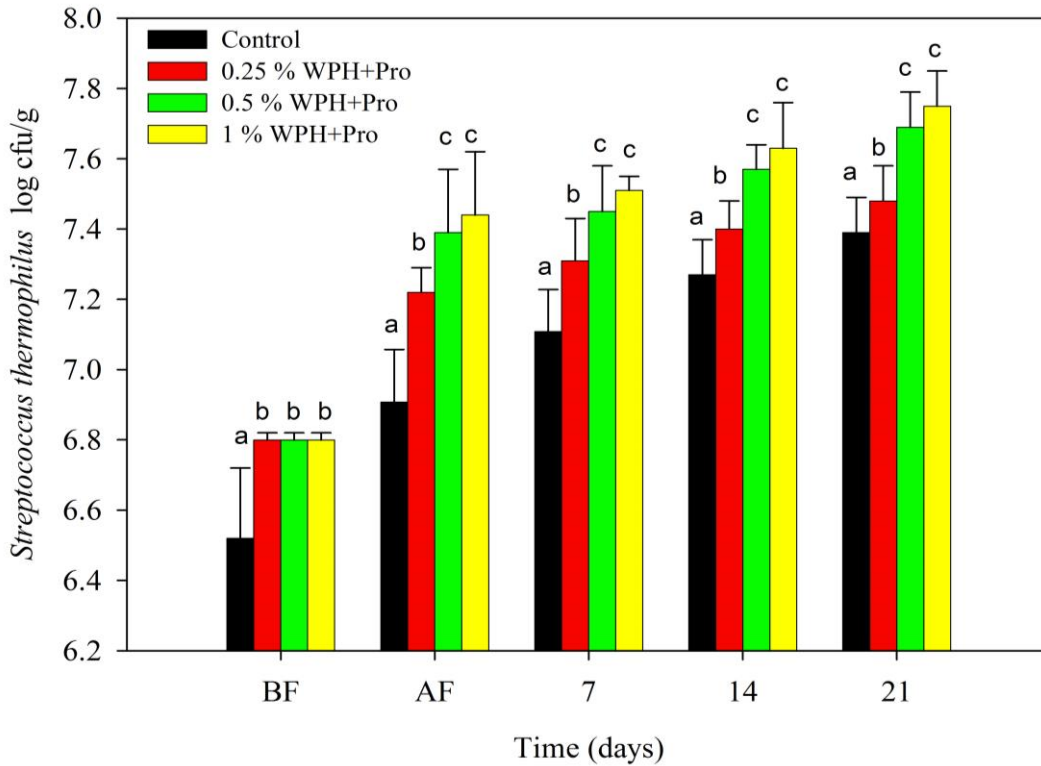
Statistical analyses indicated that the increase in the concentrations of WPH caused to increase ($p<0.05$) the growth of *L. delbrueckii* ssp. *bulgaricus* (Figures 2a-c). The highest growth of *L. bulgaricus* was found in the samples containing 1% WPH at 21st days of storage (Figure 2a). The addition of WPH worked as a buffering agent to prevent the changes in the acidity and to avoid the lethal pH value that may destroy the growth of *L. delbrueckii* ssp. *bulgaricus* (Dave and Shah, 1998). The decrease in pH caused by the post-fermentation acidification as a result of the metabolic activity of *L. bulgaricus* which interpreted the changes of pH value through the storage time of the product (Almeida et al., 2009). Glušac et al. (2015) reported that the addition of whey protein concentrates at 1% and honey at 2% and 4% improved the growth and the viability of lactic acid bacteria such as *L. delbrueckii* ssp. *bulgaricus* in yoghurt through 21st of storage.



a)



b)



c)

Figure 1. Effect of addition (a) WPH, (b) probiotics, and (c) WPH+ probiotics on the growth of *Streptococcus thermophilus*, through fermentation and storage time. BF and AF are before and after fermentation, respectively. A7, A14, and A21 indicate after 7, 14, and 21 days, respectively.

Şekil 1. (a) WPH, (b) probiyotikler ve (c) WPH+probiyotiklerin fermantasyon ve saklama süresi boyunca *Streptococcus thermophilus*'un büyümesi üzerindeki etkisi. BF ve AF sırasıyla fermantasyondan önce ve sonradır. A7, A14 ve A21 sırasıyla fermantasyondan sonra 7., 14. ve 21. günü göstermektedir.

Use of 0.25 and 0.5 % of probiotics significantly ($p < 0.05$) increased the growth of *L. bulgaricus* (Figure 2b). Sarvari et al. (2014) reported that the viability of probiotic bacteria such as *L. delbrueckii ssp. bulgaricus* influenced by the associative groups of yoghurt bacteria after the storage at 4°C for 21 days.

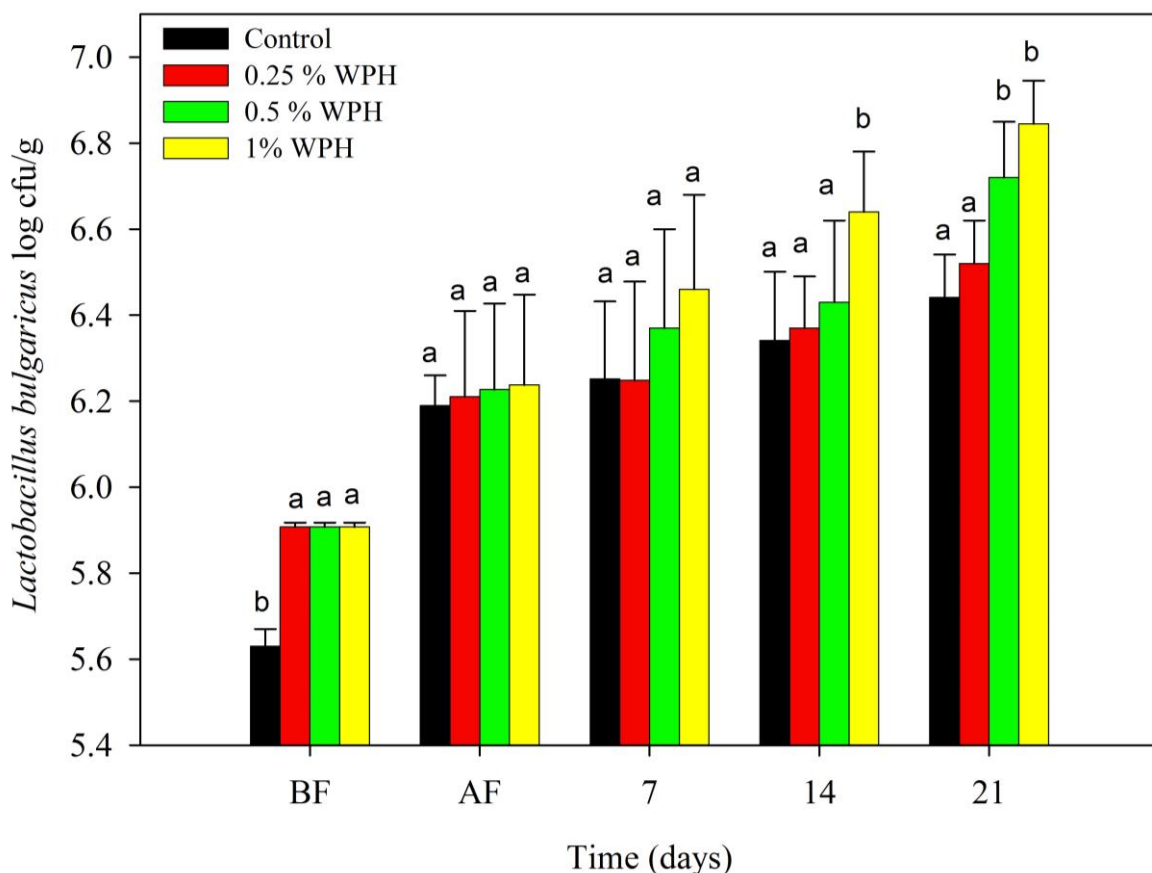
Changes in the growth of *L. bulgaricus* in samples prepared the addition of 0.25, 0.5 and 1% WPH with probiotics (Figure 2c) into ayran were not significant ($p > 0.05$). Vargas et al. (2015) keynoted that the addition of 3% whey protein isolate (WPI) significantly ($p < 0.05$) increased the counts of *L. delbrueckii ssp. bulgaricus* than those of 1 and 2% WPI after the end of storage time.

Growth of *Lactobacillus acidophilus*

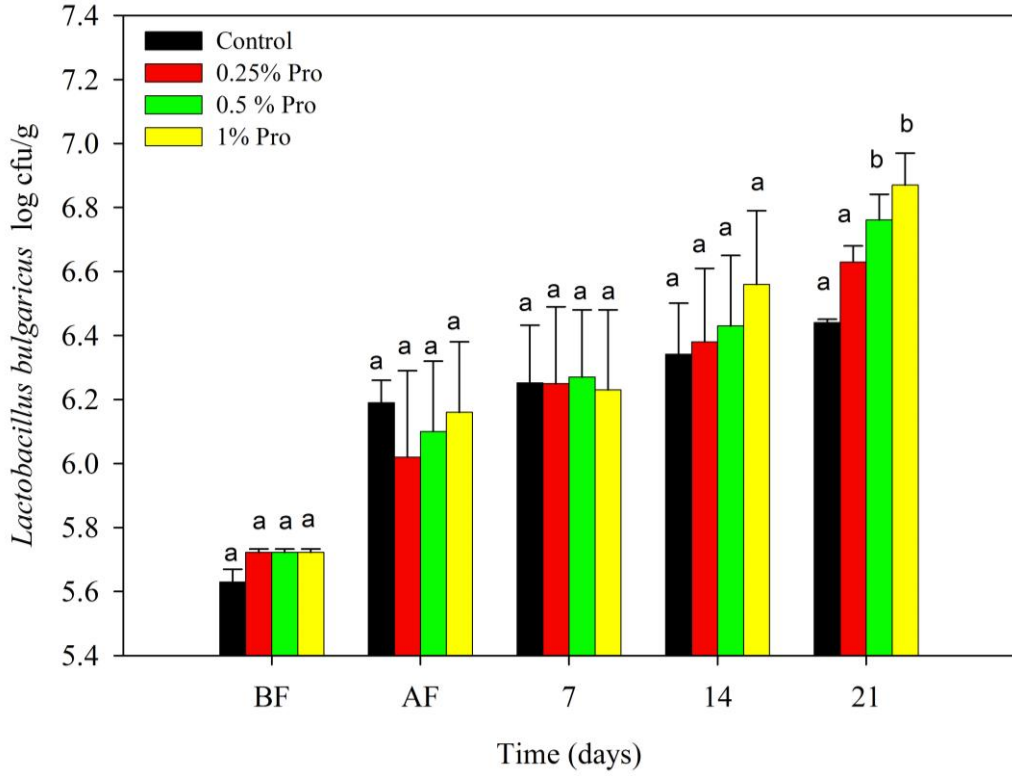
Changes in the growth of *L. acidophilus* in the samples were recorded before and after the fermentation and during the storage time (Figures 3a-c). The addition of WPH had a significant effect ($p < 0.05$) on the growth of *L. acidophilus* after the fermentation, and at 21st days of storage compared to the control (Figure 3a). Use of 0.5 and 1% WPH

caused significantly ($p < 0.05$) increase in the growth of *L. acidophilus* compared to the control samples. This could be due to that WPH could increase viability of lactic acid bacteria because of promoting the buffering ability of culture (Kailasapathy and Supriadi, 1996) as a unique source of nutrients and amino acid in WPH. The addition of lactic acid bacteria into milk may improve the production of free fatty acids by lipolysis of milk fat that caused to support the growth of *L. acidophilus* (Öndül, 2004, Yadav et al., 2007). The nutrients supplementation of ayran by WPH had positive impacts on the promoting probiotics and lactic acid bacteria growth.

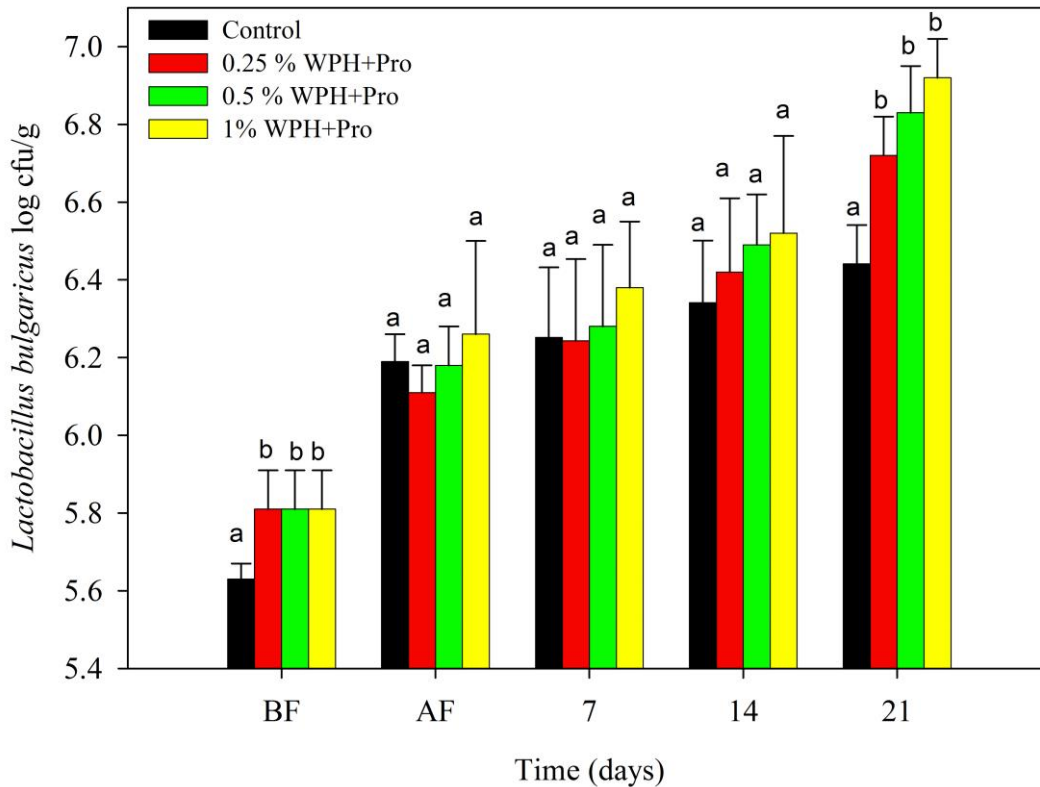
Use of 0.25, 0.5 and 1% probiotics significantly ($p < 0.05$) enhanced the growth of *L. acidophilus* at 21st days of storage (Figure 3b). Güler-Akın and Akın (2007) reported similar behavior that the viability of probiotic bacteria like *L. acidophilus* could be influenced by the addition of a pure cysteine into yoghurt samples. The samples including WPH and probiotics did not a significant ($p > 0.05$) effect on the growth of *L. acidophilus* compared to the control at end of the storage except 1% (Figure 3c).



a)



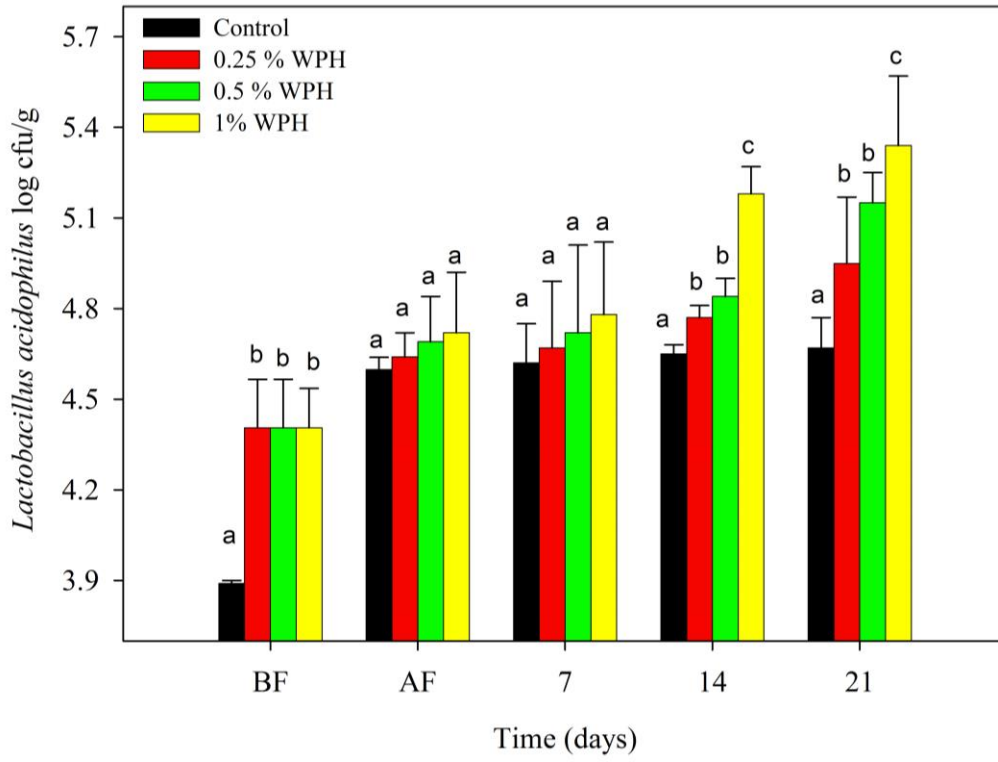
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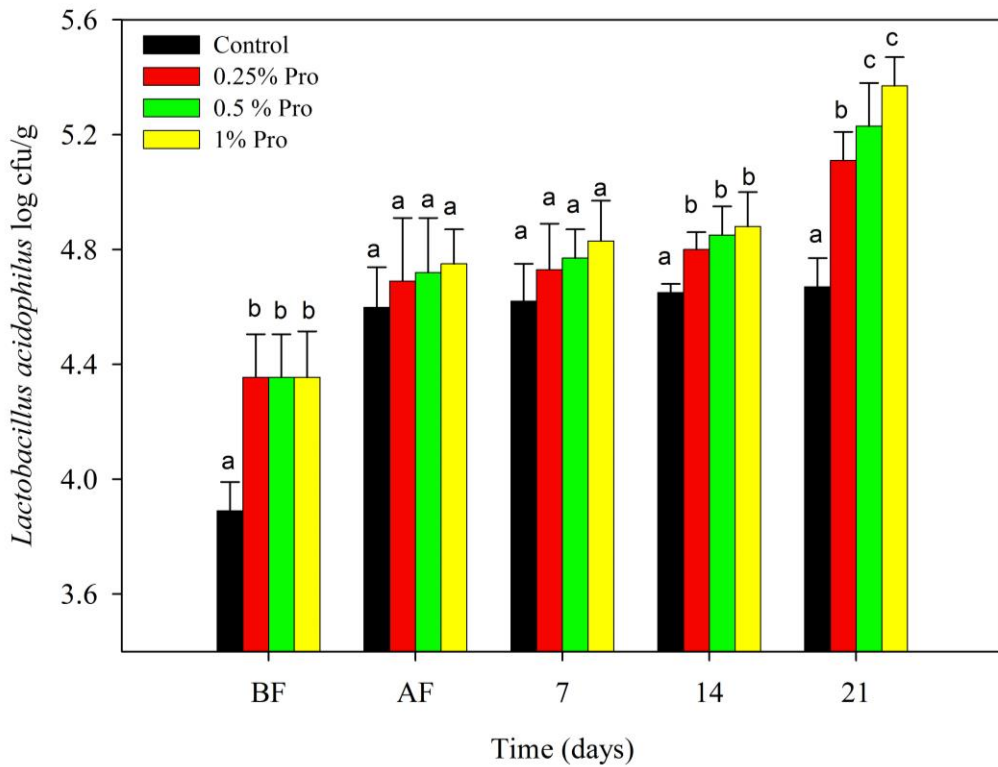
c)

Figure 2. Effect of addition (a) WPH, (b) probiotics, and (c) WPH+probiotics on the growth of *Lactobacillus bulgaricus*, through fermentation and storage time. BF and AF are before and after fermentation, respectively. 7, 14, and 21 indicate after the fermentation 7, 14, and 21 days, respectively.

Şekil 2. (a) WPH, (b) probiyotikler ve (c) WPH+probiyotiklerin fermantasyon ve saklama süresi boyunca *Lactobacillus bulgaricus*'un büyümesi üzerindeki etkisi. BF ve AF sırasıyla fermantasyondan önce ve sonradır. A7, A14 ve A21 sırasıyla fermantasyondan sonra 7., 14. ve 21. günü göstermektedir.



a)



b)

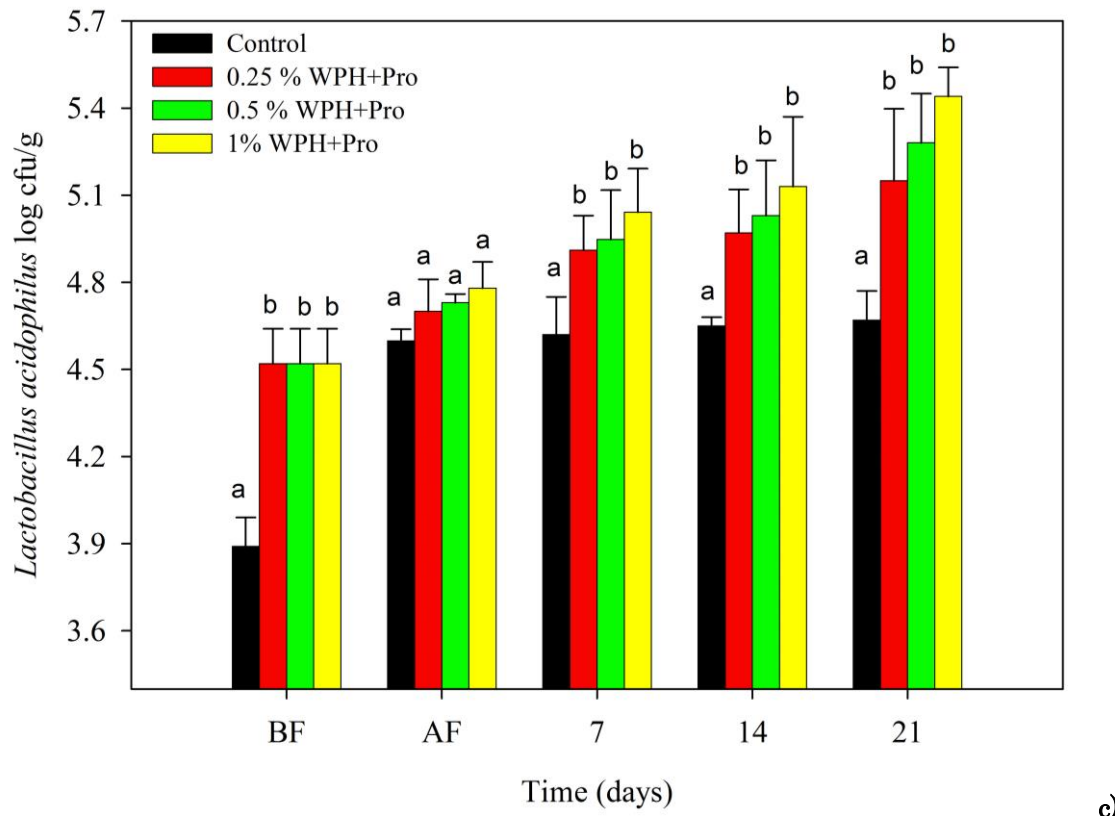


Figure 3. Effect of addition (a) WPH, (b) probiotics, and (c) WPH+probiotics on the growth of *Lactobacillus acidophilus*, through fermentation and storage time. BF and AF are before and after fermentation, respectively. 7, 14, and 21 indicate after the fermentation 7, 14, and 21 days, respectively.

Şekil 3. (a) WPH, (b) probiyotikler ve (c) WPH+probiyotiklerin fermentasyon ve saklama süresi boyunca *Lactobacillus acidophilus*'ün büyümesi üzerindeki etkisi. BF ve AF sırasıyla fermentasyondan önce ve sonradır. A7, A14 ve A21 sırasıyla fermentasyondan sonra 7., 14. ve 21. günü göstermektedir.

Growth of *Bifidobacterium lactis*

Statistical analyses indicated that an increase in WPH amount from 0.25% to 1% caused to increase ($p<0.05$) the growth of *B. lactis* compared to the control group (Figures 4a). McComas Jr and Gilliland (2003) recorded similar results in the growth of probiotics in milk supplemented by adding WPH that improved the growth of *B. longum*. However, Glušac et al., (2015) recorded that the addition of 1% Whey protein concentrate did not enhance the viability of lactic acid bacteria during the storage for 21 days.

After the fermentation, addition of 0.25, 0.5 and 1% of probiotics significantly ($p<0.05$) increased the count of *B. lactis* compared to the control (Figure 4b). While no more effect ($p>0.05$) of adding 0.25% of probiotic appeared after 7th days of fermentation on the growth of *B. lactis*, but the addition of 0.5 % of probiotics into ayran samples significantly ($p<0.05$) decreased the growth of *B. lactis* at the same time of storage. At 14th days of storage, addition of 0.25, 0.5 and 1 % of probiotics significantly ($p<0.05$) decreased the growth of *B. lactis* compared to control but remarkable ($p<0.05$) increase in the growth of *B. lactis* was seen after 21st of storage time (Figure 4b). Sarvari et al.,

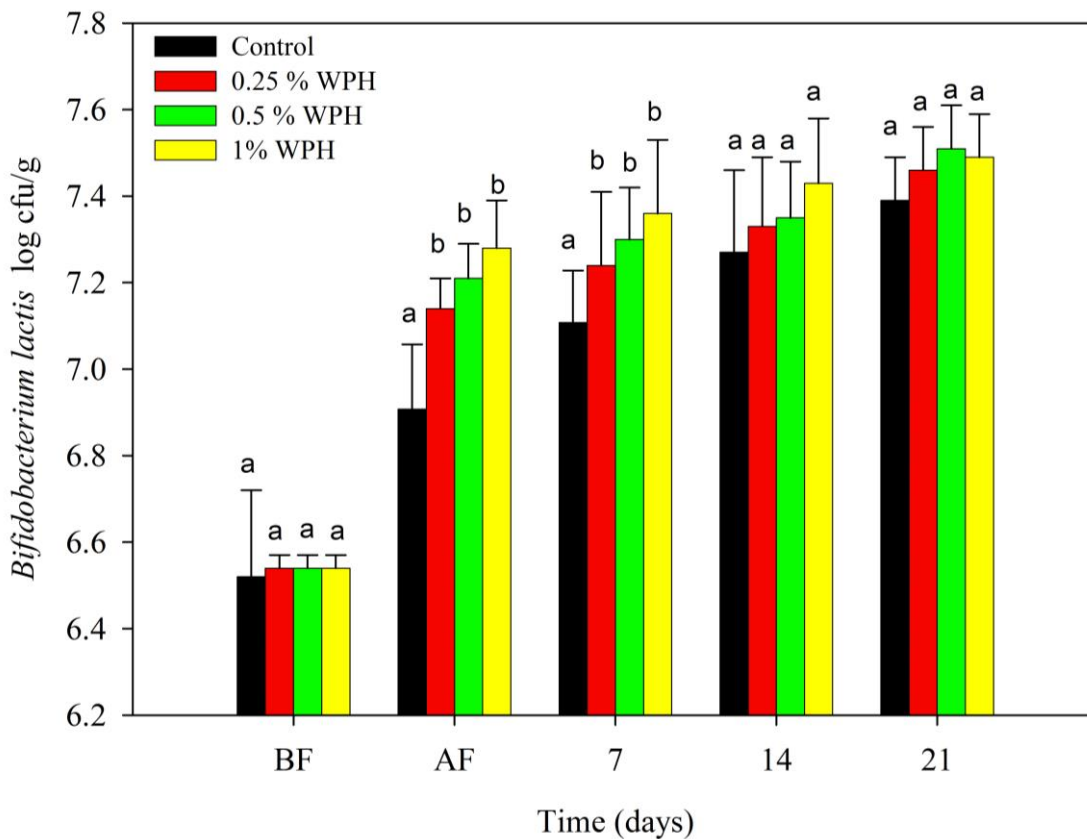
(2014) found the similar behavior that ability of *Bifidobacteria* was slow and fixed through the storage time in the yoghurt. WPH and probiotics at 1% had a significant ($p>0.05$) effect on the growth of *L. acidophilus* compared to the control during the storage time (Figure 4c).

Value of pH

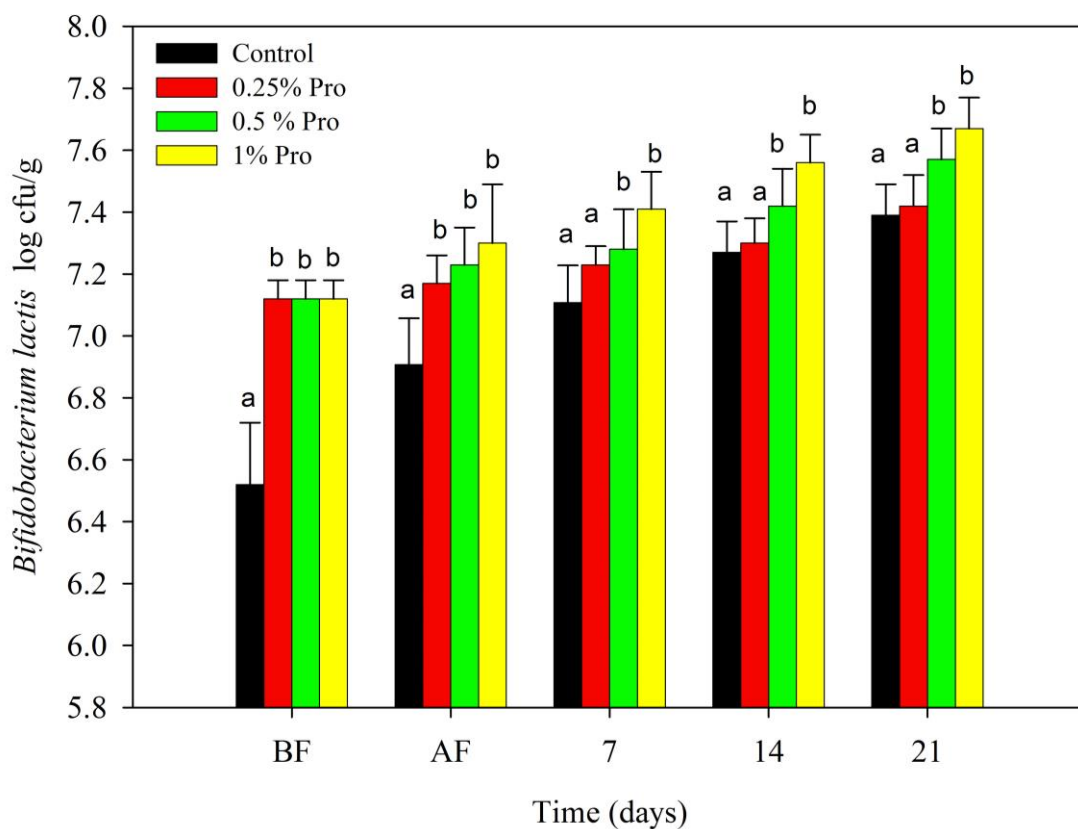
Addition of 0.25, 0.5 and 1% of WPH had significant ($p<0.05$) effect on pH value after fermentation and 7th, 14th and 21st days of storage compared to control (Table 1). The WPH containing samples at 0.25, 0.5 and 1% had significantly ($p<0.05$) lower pH levels compared to the control after fermentation at 7th and 14th of storage. However, the samples containing 0.25, 0.5 and 1% of WPH had significantly ($p<0.05$) higher pH levels compared to the control after 21st of storage (Table 1). Nadal et al., (2010) reported that the addition of WP decreased the acidification value at the end of storage of product. Moreover, Almeida et al., (2009) reported that the decrease in pH caused by post-fermentation acidification through storage appeared as a result of the metabolic effect of *S. thermophilus* and *L. bulgaricus*.

The samples prepared by addition of 0.5 and 1% of probiotics had significant ($p<0.05$) increase in pH levels compared to the control at 21st days of storage

time. Use of WPH and probiotics together at 0.25, 0.5 and 1% showed significant ($p<0.05$) increase in pH values compared to the control at 21st days.



a)



b)

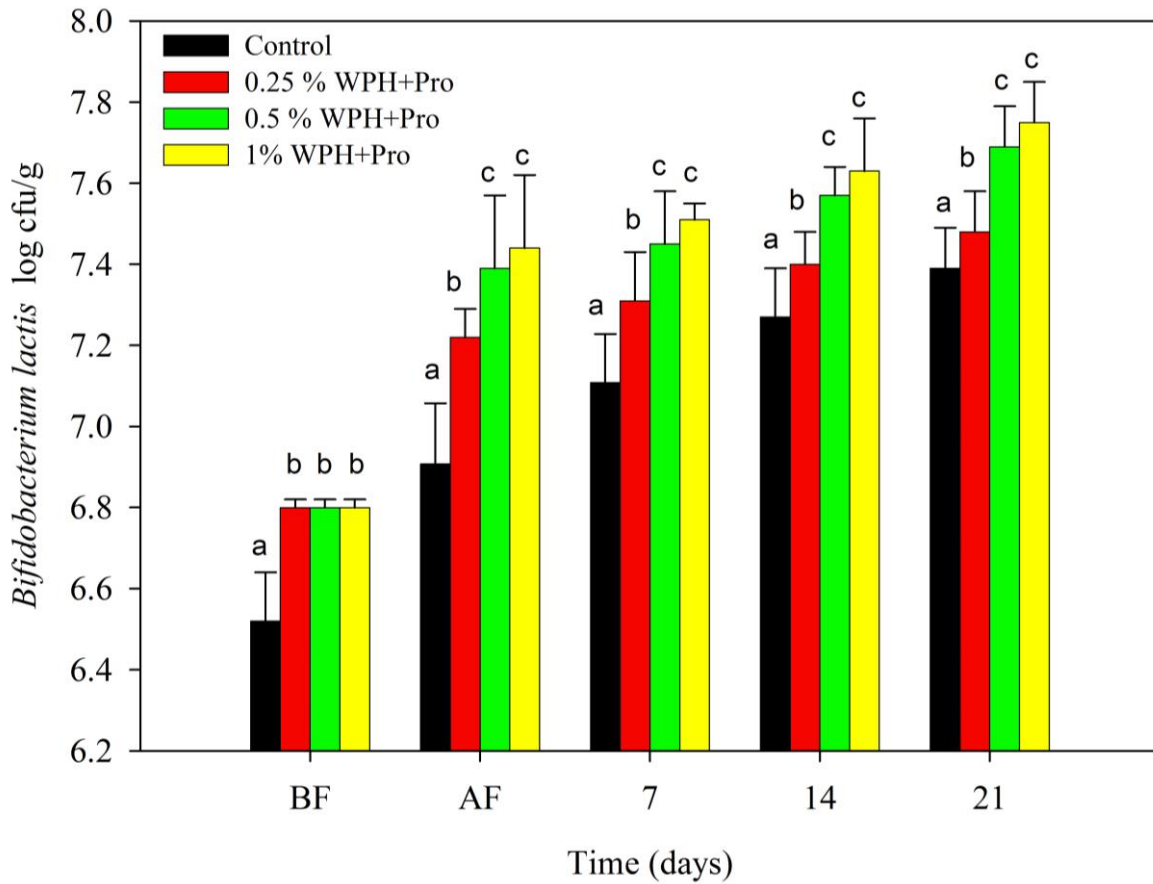


Figure 4. Effect of addition (a) WPH, (b) probiotics, and (c) WPH+probiotics on the growth of *Bifidobacterium lactis*, through fermentation and storage time. BF and AF are before and after fermentation, respectively. 7, 14, and 21 indicate after the fermentation 7, 14, and 21 days, respectively.

Şekil 4. (a) WPH, (b) probiyotikler ve (c) WPH+probiyotiklerin fermentasyon ve saklama süresi boyunca *Bifidobacterium lactis*'ün büyümesi üzerindeki etkisi. BF ve AF sırasıyla fermentasyondan önce ve sonradır. A7, A14 ve A21 sırasıyla fermentasyondan sonra 7., 14. ve 21. günü göstermektedir.

Table 1. Effect of addition WPH, probiotics, and WPH+probiotics on the pH value through the fermentation and the storage time.

Çizelge 1. WPH, probiyotikler ve WPH+probiyotiklerinin fermentasyon ve saklama süresi boyunca pH değeri üzerine etkisi.

Sample	%	Before fermentation	After fermentation	After 7 days	After 14 days	After 21 days
(Örnek)	(%)	(Fermentasyon öncesi)	(Fermentasyon sonrası)	(7 gün sonra)	(14 gün sonra)	(21 gün sonra)
WPH (VPH)	0	6.14 ^{aF} ±0.29	4.69 ^{dD} ±0.29	4.33 ^{dC} ±0.29	4.06 ^{aA} ±0.29	4.13 ^{aB} ±0.29
	0.25	6.23 ^{bF} ±0.29	4.63 ^{aD} ±0.2	4.23 ^{cB} ±0.29	4.07 ^{bA} ±0.2	4.27 ^{bC} ±0.29
	0.5	6.23 ^{bF} ±0.29	4.65 ^{bD} ±0.2	4.15 ^{aB} ±0.29	4.08 ^{bA} ±0.2	4.30 ^{cC} ±0.29
	1	6.25 ^{cF} ±0.29	4.66 ^{cD} ±0.2	4.21 ^{bB} ±0.29	4.13 ^{cA} ±0.2	4.33 ^{dC} ±0.29
Probiotics (Probiyotikler)	0.25	6.34 ^{bF} ±0.29	4.34 ^{aD} ±0.2	4.24 ^{cC} ±0.29	4.06 ^{aA} ±0.2	4.13 ^{aB} ±0.29
	0.5	6.42 ^{cF} ±0.29	4.36 ^{bD} ±0.2	4.14 ^{aB} ±0.29	4.10 ^{bC} ±0.2	4.23 ^{bC} ±0.29
	1	6.57 ^{dF} ±0.29	4.47 ^{cD} ±0.2	4.21 ^{bB} ±0.29	4.08 ^{bA} ±0.2	4.30 ^{cC} ±0.29
WPH+ Probiotics (VPH+ Probiyotikler)	0.25	6.32 ^{bD} ±0.2	4.32 ^{aC} ±0.28	4.22 ^{cB} ±0.28	4.02 ^{aA} ±0.28	4.23 ^{bB} ±0.28
	0.5	6.42 ^{cF} ±0.2	4.38 ^{bD} ±0.29	4.14 ^{aB} ±0.29	4.07 ^{cA} ±0.29	4.27 ^{cC} ±0.29
	1	6.53 ^{dF} ±0.28	4.49 ^{cD} ±0.28	4.20 ^{bB} ±0.28	4.17 ^{dA} ±0.28	4.35 ^{dC} ±0.28

Different capital letters; A, B, C, D, F indicate statistical difference between the times at $\alpha=0.05$ level among products at each time obtained from One-Way ANOVA. Different small letters; a, b, c, d, f indicate statistical difference between the concentrations at $\alpha=0.05$ level among products at each time obtained from One-Way ANOVA.

Dave and Shah (1998) reported that addition of WPH can increase lactic acid, that causes to reduce pH level. They recorded that WPH could be used to control the pH levels. The increase in buffering capacity led to decrease pH slowly and promoted acidification level by starter bacteria (Shafiee et al., 2010).

Total solid and protein measurements

Total solid and protein contents of control and WPH containing samples (0.25, 0.50, and 1%) were measured after the end of the storage time (Table 2). At 0.5% of WPH containing samples had higher total solid level than that of the control, 0.25% and 1% of WPH. WPH contains about 45-50% of milk solids (Hoffman and Falvo, 2004).

Table 2. *Effect of addition WPH, probiotics, and WPH+probiotics on the total solid value after the storage time.*
 Çizelge 2. *WPH, probiyotikler ve WPH+probiyotiklerinin saklama süresinden sonra toplam katı değeri üzerine etkisi.*

Addition % (Eklenen %)	Total solid (Toplam katı)			Protein (Protein)		
	WPH (VPH)	Probiotics (Probiyotikler)	WPH+ Probiotics (VPH + Probiyotikler)	WPH (VPH)	Probiotics (Probiyotikler)	WPH + Probiotics (VPH + Probiyotikler)
0	0.082	0.082	0.082	17.17	17.17	17.17
0.25	0.084	0.083	0.084	16.49	17.68	18.70
0.5	0.086	0.084	0.087	19.38	19.24	19.21
1	0.085	0.088	0.091	19.51	22.1	20.57

Use of probiotics at 0.5 and 1% concentrations showed a higher value of total solid compared to control. Besides, samples containing WPH and probiotics had higher total solid content compared to the control.

When the use of WPH increased from 0.25 % to 1%, protein content of ayran increased from 16.49 to 19.51 mg/L. Incorporation of 0.5 and 1 % WPH into ayran significantly increased protein content compared to the control. The highest protein content of ayran was measured with the addition of 1% probiotics, followed

by 1% of WPH+probiotics. The increase of protein content in the sample could come from the rich amount of protein in WPH (Vargas Lopez, 2013).

The viscosity

Addition of WPH, probiotics, and WPH+probiotics showed a significant ($p<0.05$) effect on the viscosity compared to the control samples after fermentation and 7th, 14th and 21st days of storage time (Table 3).

Table 3. *Effect of addition WPH, probiotics, and WPH+probiotics on the viscosity value (cp) through fermentation and storage time.*

Çizelge 3. *WPH, probiyotikler ve WPH+probiyotiklerinin fermantasyon ve saklama süresi boyunca viskosite değeri (cp) üzerine etkisi.*

Sample (Örnek)	% (%)	After 1 day (1 gün sonra)	After 7 days (7 gün sonra)	After 14 days (14 gün sonra)	After 21 days (21 gün sonra)
Control (kontrol)	0	42.24 ^{dB} ±0.01	50.76 ^{dC} ±0.01	57.81 ^{De} ±0.01	40.95 ^{cA} ±0.01
WPH (VPH)	0.25	17.86 ^{aA} ±0.01	39.87 ^{Bc} ±0.01	66.27 ^{Dd} ±0.01	40.12 ^{bC} ±0.01
	0.5	18.98 ^{bA} ±0.01	38.77 ^{bB} ±0.01	55.46 ^{Db} ±0.01	45.11 ^{dC} ±0.01
	1	19.25 ^{cA} ±0.01	32.72 ^{aB} ±0.01	42.23 ^{Da} ±0.01	35.41 ^{aC} ±0.01
Probiotics (Probiyotikler)	0.25	29.85 ^{aC} ±0.01	30.07 ^{aD} ±0.01	18.86 ^{Ba} ±0.01	17.07 ^{aA} ±0.01
	0.5	30.03 ^{bC} ±0.01	50.35 ^{cD} ±0.01	21.12 ^{Bc} ±0.01	18.56 ^{cA} ±0.01
	1	30.77 ^{cC} ±0.01	49.28 ^{bD} ±0.01	16.1 ^{Ba} ±0.01	14.08 ^{aA} ±0.01
WPH + Probiotics (VPH+ Probiyotikler)	0.25	20.97 ^{cA} ±0.01	29.97 ^{cB} ±0.01	38.82 ^{Dc} ±0.01	32.22 ^{cC} ±0.01
	0.5	19.41 ^{bA} ±0.01	21.32 ^{bB} ±0.01	33.71 ^{Db} ±0.01	29.45 ^{bC} ±0.01
	1	19.12 ^{aA} ±0.01	21.01 ^{aC} ±0.01	33.07 ^{Da} ±0.01	19.62 ^{aB} ±0.01

Different capital letters; A, B, C, D, F indicate statistical difference between the times at $\alpha=0.05$ level among products at each time obtained from One-Way ANOVA. Different small letters; a, b, c, d, f indicate statistical difference between the concentrations at $\alpha=0.05$ level among products at each time obtained from One-Way ANOVA.

After the 21st days, use of these supplements significantly decreased ($p<0.05$) the viscosity value of

ayran except the sample containing 0.5 % of WPH compared to the control group. Patocka et al. (2006)

recorded even though the increased level of addition WPH with 1-3%, the viscosity value did not affect after 35 days of storage. The effects of these parameters on viscosity may relate to different value physicochemical characteristics of exopolysaccharides produced by the cultures of the samples. Vargas et al. (2015) reported that the addition of 1% WPI increased the apparent viscosity compared to the control after the first day of storage. The increase of the viscosity could be due to the ability of the new style globules of

wehey protein to bind a higher amount of water, while the hydrophilic section of amino acids is put on the outer section of protein globule (Ipsen et al., 2000).

Hunter color parameters

Hunter color parameters; a* redness-greenness, b* yellowness-blueness, L* lightness-darkness have been previously recorded to describe visual colors (Garza et al., 1999).

Table 4. Effect of addition WPH, probiotics, and WPH+probiotics on Hunter color parameters through the fermentation and the storage time.

Çizelge 4. WPH, probiyotikler ve WPH+probiyotiklerinin fermantasyon ve saklama süresi boyunca Hunter renk parametreleri üzerine etkisi.

	Sample (Örnek)	% (%)	After 1 day (1 gün sonra)	After 7 days (7 gün sonra)	After 14 days (14 gün sonra)	After 21 days (21 gün sonra)	
lightness L* (parıllık L*)	WPH (VPH)	0	88.56 ^{dB} ±0.01	85.42 ^{dA} ±0.01	89.77 ^{dC} ±0.01	90.26 ^{dD} ±0.01	
		0.25	84.88 ^{aA} ±0.16	86.82 ^{cB} ±0.01	89.75 ^{eD} ±0.01	89.52 ^{aC} ±0.01	
		0.5	89.13 ^{cB} ±0.01	89.69 ^{aA} ±0.01	89.56 ^{bC} ±0.001	89.75 ^{eD} ±0.01	
		1	89.84 ^{dD} ±0.01	89.66 ^{dB} ±0.0	89.32 ^{aA} ±0.03	89.70 ^{bC} ±0.01	
	Probiotics (Probiyotikler)	0.25	67.83 ^{cA} ±0.01	69.53 ^{cB} ±0.01	69.56 ^{cB} ±0.001	69.33 ^{cB} ±0.01	
		0.5	61.04 ^{bC} ±0.01	61.05 ^{bC} ±0.01	60.26 ^{bA} ±0.01	60.80 ^{bB} ±0.01	
		1	49.10 ^{aA} ±0.01	49.76 ^{aB} ±0.03	50.49 ^{aC} ±0.06	50.95 ^{aD} ±0.01	
	WPH+ Probiotics (VPH+ Probiyotikler)	0.25	68.27 ^{cA} ±0.06	61.74 ^{bC} ±0.01	69.73 ^{cC} ±0.01	69.27 ^{cB} ±0.01	
		0.5	61.85 ^{bC} ±0.01	69.77 ^{cB} ±0.01	61.45 ^{bA} ±0.01	61.84 ^{bC} ±0.01	
		1	49.86 ^{aB} ±0.01	49.42 ^{dB} ±0.01	49.96 ^{aB} ±0.01	61.47 ^{aA} ±0.01	
	redness a* (kırmızılık a*)	WPH (VPH)	0	-2.58 ^{dA} ±0.01	-1.54 ^{dB} ±0.01	-1.24 ^{dC} ±0.06	-1.03 ^{dD} ±0.01
			0.25	-1.68 ^{cA} ±0.04	-1.45 ^{bB} ±0.04	-1.13 ^{cC} ±0.01	-1.32 ^{aD} ±0.25
0.5			-3.10 ^{aA} ±0.04	-1.35 ^{cB} ±0.01	-1.15 ^{cD} ±0.01	-1.25 ^{abC} ±0.15	
1			-1.66 ^{cA} ±0.01	-1.46 ^{bB} ±0.01	-1.17 ^{bC} ±0.01	-1.16 ^{bcC} ±0.11	
Probiotics (Probiyotikler)		0.25	-8.16 ^{cA} ±0.01	-7.92 ^{cB} ±0.01	-7.11 ^{cC} ±0.01	-6.78 ^{cD} ±0.25	
		0.5	-10.17 ^{bA} ±0.01	-10.07 ^{bB} ±0.02	-9.63 ^{bC} ±0.01	-8.38 ^{bD} ±0.5	
		1	-11.57 ^{aB} ±0.01	-11.77 ^{aA} ±0.01	-10.44 ^{aC} ±0.01	-9.35 ^{aD} ±0.11	
WPH+ Probiotics (VPH+ Probiyotikler)		0.25	-7.85 ^{cC} ±0.01	-7.93 ^{cB} ±0.01	-7.99 ^{cA} ±0.01	-7.23 ^{cD} ±0.25	
		0.5	-9.83 ^{bB} ±0.01	-9.86 ^{bA} ±0.01	-9.63 ^{bC} ±0.01	-8.59 ^{bD} ±0.15	
		1	-12.16 ^{aA} ±0.01	-12.16 ^{aA} ±0.01	-12.06 ^{aB} ±0.01	-11.13 ^{aC} ±0.01	
yellowness b* (sarılık b*)		WPH (VPH)	0	3.95 ^{bA} ±0.01	4.84 ^{bB} ±0.01	5.82 ^{dC} ±0.01	7.98 ^{dD} ±0.01
			0.25	5.05 ^{cA} ±0.01	6.97 ^{cC} ±0.01	6.45 ^{bB} ±0.01	7.17 ^{eD} ±0.25
	0.5		4.74 ^{bA} ±0.01	7.77 ^{dD} ±0.01	7.15 ^{dC} ±0.01	6.3 ^{bB} ±0.05	
	1		5.37 ^{dA} ±0.01	6.83 ^{bD} ±0.01	6.73 ^{cC} ±0.01	5.42 ^{aB} ±0.01	
	Probiotics (Probiyotikler)	0.25	4.17 ^{bA} ±0.01	5.17 ^{bB} ±0.01	6.45 ^{bC} ±0.01	6.45 ^{bC} ±0.25	
		0.5	5.16 ^{cA} ±0.01	6.54 ^{dC} ±0.01	7.57 ^{eD} ±0.01	6.18 ^{aB} ±0.05	
		1	5.44 ^{dA} ±0.01	6.43 ^{cB} ±0.01	7.62 ^{dD} ±0.01	6.92 ^{cC} ±0.01	
	WPH+ Probiotics (VPH+ Probiyotikler)	0.25	3.75 ^{aA} ±0.01	7.11 ^{cC} ±0.01	7.58 ^{bD} ±0.01	6.83 ^{aB} ±0.25	
		0.5	6.15 ^{cA} ±0.01	6.55 ^{bB} ±0.01	7.62 ^{bD} ±0.01	7.25 ^{bC} ±0.05	
		1	7.34 ^{dA} ±0.02	7.77 ^{dB} ±0.01	8.47 ^{eD} ±0.01	8.38 ^{dC} ±0.01	

Different capital letters; A, B, C, D, F indicate statistical difference between the times at α= 0.05 level among products at each time obtained from One-Way ANOVA. Different small letters; a, b, c, d, f indicate statistical difference between the concentrations at α= 0.05 level among products at each time obtained from One-Way ANOVA.

It can be stated that the color of fermented products is an important issue for consumer preferences as well as for the shelf life of products. Its measurement can supply reliable information about food quality

characteristics.

Samples containing 0.25, 0.50, and 1% of WPH showed a significant effect on the lightness value compared to the control after the fermentation, 14th

and 21st of the storage (Table 4). In WPH added samples at 0.25, 0.5 and 1%, Hunter's a* values changed significantly during the storage.

WPH having samples at 0.25, 0.5 and 1% recorded (p<0.05) increase in b* level after the fermentation and 14th days of storage compared to the control. The samples having 0.25, 0.5 and 1% of WPH had lower (p<0.05) in the b* level at 21st days of fermentation.

CONCLUSIONS

In this study, addition of WPH and probiotics at different concentrations (0, 0.25, 0.5 and 1%) into the ayran effected growth of *S. thermophilus*, *L. delbrueckii* spp. *bulgaricus*, *L. acidophilus*, and *B. lactis*. Besides, addition of these supplements could affect pH, total solid value, protein value, viscosity, and Hunter color values of ayran through the fermentation and the storage time. WPH and probiotics had great potentials for enhancing the growth of probiotic bacteria and nutritional of ayran.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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