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The effect of probiotic strain of *Lactobacillus fermentum* on growth of *Escherichia coli* O111 during yogurt storage

Mohammad Mehdi Hassanpour [1], Anousheh Sharifan [1*], Afshin Akhondzadeh basti [2]

[1] Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

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ABSTRACT

The World Health Organization's Food Safety Unit has given high priority to study fermentation as a technique for food preparation and preservation because in developing countries one-tenth of under-five children die from dehydration. Loss of water is mainly due to the spreading of diarrhea, and the cause of diarrhea are foods that do not meet the standards of hygiene, the health standard of a food is based on the process and conditions of the raw material, and lactic fermentation of food as a standard process has been known to reduce the risk of growth of foodborne pathogens. In this study, the effect of a probiotic strain of *Lactobacillus fermentum* on the growth of *Escherichia coli* O111 during yogurt storage was evaluated. Different conditions were used in this study: concentration of *L. fermentum* at three levels and *E. coli* O111 at one level. In probiotic yogurt, the *L. fermentum* count and *E. coli* O111 count, pH, acidity, and syneresis were evaluated. The results showed that the total count of *E. coli* O111 in the control sample was higher than the probiotic samples. Probiotic bacteria also decreased during the storage period. The results indicate that probiotic yogurt had antimicrobial properties during storage. Syneresis characteristics also showed that the control sample had more syneresis than the other samples. The results of this study showed that *L. fermentum* has antimicrobial potential in dairy products.

1. Introduction

The World Health Organization's Food Safety Unit has given high priority to study fermentation as a technique for food preparation and preservation because in developing countries one-tenth of under-five children die from dehydration. Loss of water is mainly due to the spreading of diarrhea, and the cause of diarrhea are foods that do not meet the standards of hygiene, the health standard of a food is based on the process and conditions of the raw material, and lactic fermentation of food as a standard process has been known to reduce the risk of growth of foodborne pathogens (1). Yogurt is one of the most consumed fermented dairy products in the world due to its beneficial health effects (2). It has long been prescribed for the treatment of certain diseases and poisonings and has been recognized as a beneficial food because of its beneficial bacteria. Due to the activity of the lactase enzyme, lactose content in yogurt is lower than milk which helps to its digestion in the small intestine and is useful for people who have lactose intolerance. Yogurt calcium is better absorbed than other forms of calcium. The high content of dry mass in yogurt has increased its protein content in equal volumes compared to milk. Due to the effect of yogurt bacterial enzymes on milk proteins, the digestibility of yogurt proteins becomes higher and easier. Also, milk fat is easily digestible due to pre-digestion reactions during fermentation (3). Bacteria used for the fermentation of Yogurt were *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* that have synergic effects on each other during the fermentation of milk through the production of useful compounds and molecules. *L. bulgaricus* produces essential amino acids for the growth of *S. thermophilus*, and *S. thermophilus* also provides growth stimulating factors of *L. bulgaricus*. As the two bacteria grow together, the rate of acid production increases compared to independent growth (4). The ingredients produced during

*Corresponding author: Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
E-mail address: anousheh.sharifan@yahoo.com (Anousheh Sharifan).

fermentation, in addition to aid in growth, cause tissue formation and aroma and flavoring. Antibiotic-like compounds of yogurt include acidoline, acidophin, lactocidin, and bacteriocins produced by *Lactobacillus* (5). In 2001, the World Health Organization (WHO) and the World Food and Agriculture Organization (FAO) introduced a comprehensive definition of probiotics. According to this definition, probiotics are living microorganisms that, if consumed in certain amounts, will have a health effect on the host. According to the above definitions, it is understood that probiotics are living microorganisms that have health effects on the body. Now it is necessary to know first how many of these microscopic organisms can have health effects on the body, secondly, what foods can cause the stimulating and growth of these microorganisms and their health benefits. (6). Probiotics improve the digestive process. These bacteria, by their activity and enzyme secretion, make food better digestible. They also help the immune system by stimulating it. Probiotics make up 85% of the intestinal flora. This level of bacteria indicates that how much these microorganisms play a vital role in the digestive system. At present, these microorganisms reach the human body either by dietary supplements or by fermented foods. Various mechanisms have been suggested for the function and activity of probiotics that can prevent various damage to the host body. (a): production of prophylactics: Probiotics can prevent some infectious diseases by producing substances that can inhibit microorganisms such as lactic acid, bacteriocin, hydrogen peroxide, etc.; (b): blocking adhesion sites in pathogens: Probiotics prevent colonization and growth by placing and covering the adhesion site of pathogenic microorganisms; (c): competition for nutrition: Probiotics use existing foods before being consumed by pathogenic microorganisms; (d): Immune system stimulation: Probiotics can stimulate both specific and non-specific immunity against intestinal diseases. For example, *Lactobacillus casei* in viral diarrhea enhances the immune response (6). Among the probiotic products, probiotic yogurt is the most popular of these products. Probiotics yogurt is useful for the treatment of intestinal infections, especially diarrhea, bacterial, and yeast infections of the reproductive system. In addition to the therapeutic effects of this product, less acidification during storage (mild taste) and higher levels of lactic acid L (+) than D (-), unlike traditional yogurt, are effective in their popularity. Yogurt is produced using *Streptococcus thermophilus* and *Lactobacillus bulgaricus* as starter cultures. The consumption of these two bacteria has a health-promoting effect, but neither of them is intestinal flora microbes. Their beneficial effects are mainly related to the presence of specific enzymes and the production of their metabolites. Probiotic bacteria (therapeutic initiators) have the ability to tolerate gastric acid and bile salts and are capable of being replaced in the gut (7). *Lactobacillus fermentum* is one of the most important genera of the Lactobacillus family, which is widely found in its sourdough and fermented dairy products, which is a major part of the human intestinal flora and its probiotic potential has proven. This bacterium is used as a commercial probiotic in the production of probiotic products due to its good resistance to heat and physical stress and gastrointestinal stress. The ability of lactic acid bacteria to produce antimicrobial compounds is well known. The fermentation process results in a decrease in the carbohydrate content of the food product and the formation of low molecular weight organic molecules with antimicrobial properties, most commonly acetic, lactic, and propionic acid (8). In addition, other antimicrobial compounds are produced by lactic acid bacteria, which are not necessarily produced to improve human health, and the biological effects of these compounds predominate in one species over other symbiotic bacteria. This can be done by acidifying the environment and strict growth conditions and by producing toxic compounds such as hydrogen peroxide, bacteriocin, and carbon dioxide against competing bacteria (9). *Escherichia coli* is a gram-negative bacterium of the rod-shaped *Enterobacteriaceae* family. Most *E. coli* bacteria naturally live in the human intestine without problems. Pathogen strains of *E. coli* from natural and non-pathogenic species are separated by the production of toxic compounds by pathogenic species. Enteric pathogenic *E. coli* bacteria for humans include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC). Enterohemorrhagic *E. coli* can lead to mild diarrhea, inflammation associated with bleeding, and sometimes acute brain disease. O157 is the most common bacterium in this bacterial branch, accounting for approximately 70-60% of reported EHEC infections after that is O26 with 20-25% of EHEC infections and then are subgroups of *E. coli* including O111, O121, and O103 (10). The aim of this study was to investigate the effect of probiotic strain *Lactobacillus fermentum* at different concentrations on the growth of *E. coli* O111 during yogurt storage at refrigeration temperature.

2. Material and methods

2.1. Research Outline

In this study, *Lactobacillus fermentum* was used as a probiotic agent and *Escherichia coli* O111 as a pathogen. Preparation were prepared in three different concentrations of probiotic *L. fermentum* and added to the samples during the preparation of the constant concentration of *E. coli*. Finally, 4 Preparations were evaluated for investigating the factors discussed in this study:
(*i*)-Control: yogurt sample without probiotic containing *E. coli*
(*ii*)-YF-1%: yogurt sample containing 1% of *L. fermentum* and *E. coli*; (*iii*)-YF-2%: yogurt sample containing 2% of *L. fermentum* and *E. coli*; (*iv*)-YF-3%: yogurt sample containing concentration three of *L. fermentum* and *E. coli.* Then the tests were evaluated six times (0, 12 h, 24 h, day 7, day 14, and day 21).

2.2. Method of preparation of yogurts studied

L. fermentum (PTCC 1744) was obtained from the Industrial Fungi and Bacteria Collection Center of Iran. The milk dry matter was first adjusted by adding 2% lean milk powder and

then pasteurized in a warm water bath at gentle stirring for 30 minutes. It was then cooled to 43˚C and the yogurt fermentation starter was added to the milk and divided into different sections for inoculation with *L. fermentum* and E. coli O111 isolate (10^6 CFU/ml). Samples were incubated in sterile containers until a pH of 4.6 with a temperature of 43 ° C was reached. After this period, the yogurt samples were kept in the refrigerator at 4˚C until the tests were carried out. Specimens containing yogurt starter bacteria and *E. coli* were also prepared as controls (11).

2.3. Measuring pH

The pH of the samples was measured at pH 25c (according to the AOAC 2002 method) using a pH meter (Kalimatic Model 766 Merck, Germany).

2.4. Measurement of acidity

Acidity was measured by AOAC, 2002 based on titration with 1.0 normal sodium hydroxide in the presence of phenolphthalein reagent until purple was obtained and acidity percentage was measured based on lactic acid.

2.5. Measurement of syneresis

For measuring syneresis, 25 g of yogurt samples were centrifuged at centrifuge tubes at 1500 rpm for 15 minutes at room temperature. The extracted liquid was removed from the top of the sample tubes and the tubes were weighed again. The syneresis was reported as the water weight lost per 100 g of yogurt.

2.6. Statistical analysis

In this study, all experiments were performed in 3 replications and the results were analyzed in a completely randomized simple design using SAS 9.1 software. Duncan's multiple range test was used to compare the means at the significant level $p<0.05$.

3. Results and discussion

3.1. Survival results of L. fermentum during storage

The results of changes in the survival of the probiotic bacterium *L. fermentum* are reported in Table 1. The results showed that bacterial survival in all preparations decreased significantly at $p<0.05$ during the storage period. *L. fermentum* of YF-1% at the end of the storage period was lower than 10^6 CFU / g of yogurt which, according to the standard probiotic yogurt, does not have probiotic yogurt properties at the end of the survival period. However, the two Preparations F-2% and YF-3% had a higher count of *Lactobacillus* than the minimum probiotic bacterium levels criteria for probiotic yogurts until the end of the storage period. On day 1 and at time 0 of storage, it was observed that the concentration of *L. fermentum* used in the yogurts produced a significant difference in the count of bacteria in the three preparations at time zero at $p<0.05$. This process is also observed at 12 and 24 hours. Seven days after storage, the results showed that in all three samples, the bacterial count decreased slightly. On this day, two Preparations YF-2% and YF-3% had a significant difference compared to YF-1% at $p<0.05$ but no significant difference was observed at $p<0.05$. This trend was observed until the end of the storage period. According to Iranian National Standard No. 11325, it is stated that if the probiotic bacterium is more than 10^6 CFU/g of yogurt at the end of the storage period, this yogurt can be considered as a probiotic. Various factors can affect the survival of probiotic bacteria during yogurt storage. One of the most important factors affecting survival is the environmental conditions of yogurt. The lower the amount of acid present in the product can have a negative effect on the survival of the bacteria. Now, depending on the characteristics of each bacterium, the ability to tolerate acid can vary.

Table 1. Survival count of *Lactobacillus fermentum* in probiotic yogurt.

	Time					
	0	**12 h**	**24 h**	**day 7**	**day 14**	**day 21**
YF-1%	7.15 ± 0.12^{Ca}	7.21 ± 0.24^{Ca}	7.08 ± 0.19^{Ca}	6.89 ± 0.26^{Bb}	6.08 ± 0.16^{Bc}	5.54 ± 0.28^{Bd}
YF-2%	7.53 ± 0.08^{Ba}	7.46 ± 0.23^{Ba}	7.59 ± 0.15^{Ba}	7.24 ± 0.17^{Ab}	6.46 ± 0.12^{Ac}	6.05 ± 0.26^{Ad}
YF-3%	7.89 ± 0.21^{Aa}	7.83 ± 0.15^{Aa}	7.79 ± 0.27^{Aa}	7.32 ± 0.14^{Ab}	6.53 ± 0.22^{Ac}	6.15 ± 0.14^{Ad}

$^{a-e}$ Different lowercase letters within a column indicate significant differences ($p<0.05$).
$^{A-D}$ Different uppercase letters within a row indicate significant differences ($p<0.05$).

Results showed that *L. fermentum* decreased by the end of the storage period. These results indicate that factors in the production process or storage time have reduced the bacterial count. A study by Hekmat et al. (12) on the growth and survival of two probiotic bacteria *Lactobacillus rhamnosus* and *Lactobacillus roteri* in yogurt showed that the type of bacterium could affect its survival. In this study, after one month of storage, *Lactobacillus rutherii* was less than 10 CFU/g of yogurt, but *Lactobacillus rhamnosus* showed no significant change in its initial bacterial count and at the end of the storage period, it was 10^6 CFU/g of yogurt. They suggested that various causes, including bacterial genus and species, could be effective in reducing the survival rate in yogurt. One of the most important reasons for the decreasing survival in yogurt is the acidic condition and oxygen present in the product, both of which can affect the survival of the bacteria. Another study by Antunes et al. (13) examined the survival and acid production of probiotic bacteria. It was shown that the survival of probiotic bacteria is related to factors such as acid content. In another study by Mortazavian

et al. (3), reported that temperature of yogurt can affect the survival of probiotic bacteria. In this study, they stored the yogurt at 2, 5, and 8˚C. based on evaluation during the storage period observed that survival at 2˚C is highest and at 8˚C is lowest (3). The results are the mean of 3 replications ± standard deviation. In each column, capital letters mean there is no significant statistical difference at the p≤0.05 level. Also, lowercase letters in each row indicate there is no significant difference at the p≤0.05 level.

3.2. Changes in E. coli O111 count during storage

The results of changes in *E. coli* bacterial count during storage at Mats are reported in Table 2. The results generally show that by increasing the storage period, *E. coli* count has been decreased. According to the results, it can be said that after 24 hours of storage, the decreasing *E. coli* count was significant compared to the control. There was no significant difference at the p<0.05 level at time *E. coli* between the control and the preparations at storage time. By increasing the storage time to 12 h, bacterial counts decreased in all three preparations. In the YF-1% and YF-2% preparations, the bacterial count did not decrease significantly at p<0.05 compared to the control at this time. But the YF-3% sample was able to significantly reduce the level of *E. coli* at the P <0.05 level compared to the control. This trend was observed until the end of the storage period. Due to new technologies in the pasteurization and sterilization of milk and dairy products, the presence of pathogenic bacteria in dairy products has been minimized. In this study, to evaluate the antimicrobial effect of *L. fermentum* in the storage period, *E. coli* O111 was added to the product. According to various reports, *L. fermentum* was expected to have antagonistic properties against *E. coli*. As reported in various studies, probiotic bacteria can have antimicrobial activity due to the production of metabolites. These metabolites include bacteriocins, carbon dioxide, and organic acids. Bacteria by producing these metabolites can affect the morphological characteristics of pathogenic bacteria and cause cell death. In a study by Kang et al. (14), the antimicrobial effect of *Lactobacillus salivarius* and *L. fermentum* against Staphylococcus bacteria was investigated. In this study, they identified the bacteriocins produced by these bacteria and reported that the cause of the antimicrobial effect of these bacteria is the production of acid and bacteriocins. Another study by Owusu-Kwarteng et al. (15) reported that *L. fermentum* has antimicrobial activity. This bacterium is particularly effective on *E. coli* and *Salmonella* and in some subspecies can be effective on *Listeria monocytogenes* and *Staphylococcus aureus*.

Table 2. Survival results of *Escherichia coli* O111 during storage period.

	Time					
Treatment	**0**	**12 h**	**24 h**	**day 7**	**day 14**	**day 21**
Control	4.53 ± 0.02^{Aa}	4.51 ± 0.02^{Aa}	4.48 ± 0.01^{Aab}	4.46 ± 0.02^{Ab}	4.41 ± 0.02^{Ac}	4.22 ± 0.01^{Ad}
YF-1%	4.51 ± 0.01^{Aa}	4.49 ± 0.02^{Aa}	4.46 ± 0.02^{Ab}	4.28 ± 0.02^{Bc}	4.19 ± 0.01^{Bd}	4.05 ± 0.03^{Be}
YF-2%	4.52 ± 0.02^{Aa}	4.46 ± 0.04^{Aab}	4.45 ± 0.03^{Ab}	4.21 ± 0.03^{Cc}	4.14 ± 0.02^{Cd}	397 ± 0.02^{Ce}
YF-3%	4.51 ± 0.03^{Aa}	4.48 ± 0.03^{Aa}	4.46 ± 0.02^{Aa}	4.17 ± 0.02^{Cb}	4.10 ± 0.03^{Cc}	3.91 ± 0.03^{Dd}

[a–e] Different lowercase letters within a column indicate significant differences (p<0.05).
[A-D] Different uppercase letters within a row indicate significant differences (p<0.05).

According to the results, it can be concluded that the use of simultaneous culture increased the antimicrobial effect compared to a single culture. In this study, we also investigated the effect of different concentrations of bacteria on antimicrobial activity. The results showed that increasing the concentration of bacteria increased their antimicrobial activity. Other results obtained in this study showed that the main cause of the decrease in the growth of pathogenic bacteria in the environment of the studied bacteria was the production of antimicrobial metabolites by these bacteria. The cause of this conclusion is that pH didn't change significantly compared to control samples (16). The results are the mean of 3 replications ± standard deviation. In each column, capital letters mean there is no significant statistical difference at the p≤0.05 level. Also, lowercase letters in each row indicate there is no significant difference at the p≤0.05 level.

3.3. pH variation of yogurt during storage

The results of pH changes of the produced probiotic yogurts are reported in (Table 3). The results show that with increasing storage period the pH changes slightly in order to decrease it. The control sample had a lower reduction than the other samples, as pH variations were about 0.3, whereas in samples containing probiotic YF-2%, YF-1% and YF-3% the pH variation during the storage period was 0.44, 0.55, and 0.60, respectively. At time 0 of storage, no significant pH differences were observed in controls and Preparations at the p<0.05 level. This process continues with increasing storage time up to 24 hours. On day 7 of storage, the control sample showed a significant difference with samples containing probiotics. Also, among the yogurt containing probiotic, the pH decreased with increasing probiotic concentration. This trend was observed until the end of the yogurt storage period. The pH variations depend on the amount of acid produced by the bacteria in the yogurt. Depending on the activity of the bacteria in acid production, the pH variation time may vary. Kailasapathy (17) reported that the pH of probiotic yogurt decreased by 0.3 during the storage period, whereas traditional yogurt pH decreased by 0.6. Depending on the type of probiotic used in yogurt, the pH varies. The results are the mean of 3 replications ± standard deviation. In each column, capital letters mean there is no significant statistical difference at the p≤0.05 level. Also, lowercase letters in each row indicate there is no significant difference at the p≤0.05 level.

Table 3. Results of pH variation during the storage period.

Time	Time					
	0	12 h	24 h	day 7	day 14	day 21
Control	4.53 ± 0.02^{Aa}	4.51 ± 0.02^{Aa}	4.48 ± 0.01^{Aab}	4.46 ± 0.02^{Ab}	4.41 ± 0.02^{Ac}	4.22 ± 0.01^{Ad}
YF-1%	4.51 ± 0.01^{Aa}	4.49 ± 0.02^{Aa}	4.46 ± 0.02^{Ab}	4.28 ± 0.02^{Bc}	4.19 ± 0.01^{Bd}	4.05 ± 0.03^{Be}
YF-2%	4.52 ± 0.02^{Aa}	4.46 ± 0.04^{Aab}	4.45 ± 0.03^{Ab}	4.21 ± 0.03^{Cc}	4.14 ± 0.02^{Cd}	397 ± 0.02^{Ce}
YF-3%	4.51 ± 0.03^{Aa}	4.48 ± 0.03^{Aa}	4.46 ± 0.02^{Aa}	4.17 ± 0.02^{Cb}	4.10 ± 0.03^{Cc}	3.91 ± 0.03^{Dd}

$^{a-e}$ Different lowercase letters within a column indicate significant differences ($p<0.05$).
$^{A-D}$ Different uppercase letters within a row indicate significant differences ($p<0.05$).

3.4. Acidity variation of probiotic yogurt during storage

The results of the acidity variations are reported in (Table 4). The results show that generally, the acidity increased with increasing storage time. In samples containing probiotic, acidity was significantly higher at the end of the storage period at $p<0.05$. At time 0 of storage, no significant differences were observed at all samples in $p<0.05$ level. This trend was also observed up to 24 hours after the storage of yogurt. At day 7 of storage, the results showed that the control sample had significantly lower acidity than the probiotic samples. It was also observed that the YF-1% sample had significantly lower acidity than YF-2% and YF-3%. This trend was observed until the end of the storage period. The acidity variations in yogurt are related to the rate of acid production by the bacteria in the yogurt. The more bacteria present, the more acid they produce. The acidity increases. The results of this study showed that tangible variations in the acidity of the samples did not occur during the storage period. But the samples containing *L. fermentum* changed the acidity of yogurt to a greater extent. Owusu-Kwarteng et al. (15) reported that in increasing acidity or decreasing pH by *L. fermentum*, the behavior of these bacteria is classified into three groups. The first group is bacteria that have a very high acid production rate and produce high acid levels within a few hours. The second group is fermented bacteria that produce acid by mean speed and the last group is bacteria that produce acid very slowly. According to the report of these researchers, it can be stated that

Table 4. Results of acidity variations during the storage period.

Time	Time					
	0	12 h	24 h	day 7	day 14	day 21
Control	68.50 ± 1.12^{Ad}	67.50 ± 1.05^{Ad}	69 ± 1.24^{Acd}	71 ± 1.10^{Cc}	74 ± 0.89^{Cb}	76.50 ± 1.15^{Ca}
YF-1%	67 ± 0.69^{Ad}	69 ± 1.16^{Ad}	67.50 ± 1.52^{Ad}	73 ± 1.06^{Bc}	76.50 ± 1.21^{Bb}	79.50 ± 0.59^{Ba}
YF-2%	69.50 ± 1.21^{Ad}	70 ± 1.26^{Ad}	69 ± 1.08^{Ad}	75.50 ± 0.24^{Ac}	79 ± 1.04^{Ab}	82 ± 1.12^{Aa}
YF-3%	68.50 ± 1.36^{Ad}	68 ± 1.46^{Ad}	70 ± 1.02^{Ad}	76 ± 1.16^{Ac}	81 ± 0.86^{Ab}	84 ± 1.01^{Aa}

$^{a-e}$ Different lowercase letters within a column indicate significant differences ($p<0.05$).
$^{A-D}$ Different uppercase letters within a row indicate significant differences ($p<0.05$).

L. fermentum subspecies can be effective in acid production. Given that the rate of acid production in the yogurt containing fermentum has been slow, it can be concluded that the bacteria used are those of the slow acid-producing group. In another study, Ferdousi et al. (18) examined variations in probiotic yogurt in the cold chain. They reported that pH and acidity do not change significantly after 24 h of storage in the refrigerator. They also reported that probiotic bacteria in general can decrease the pH of the product by acid production and increase the acidity. According to this report, it is possible to find out the similarity of pH and acidity behavior in the present study. In the present study, the rate of changes after 24 hours was not significant. The results are the mean of 3 replications ± standard deviation. In each column, capital letters mean there is no significant statistical difference at the $p\leq0.05$ level. Also, lowercase letters in each row indicate there is no significant difference at the $p\leq0.05$ level.

3.4. Probiotic yogurt syneresis during storage

The results of the syneresis rate in yogurt samples are reported in (Table 5). The results show that syneresis occurred in all samples up to the end of the storage period. According to the results, it can be concluded that the control sample had significantly higher syneresis than the other samples at $p<0.05$ level and YF-3% showed the lowest syneresis. At time 0 of storage, there was no significant difference at the $p<0.05$ level between the samples in the results of syneresis. This trend was observed for the first 24 h of storage. By increasing the storage time up to day 7, the results showed that the control sample had a significant syneresis rate compared to the probiotic samples. By increasing probiotic concentration, it can be concluded that the rate of syneresis decreased compared to control. This trend was observed until the end of the storage period. Syneresis is one of the important factors in evaluating yogurt appearance. This factor is specifically related to the tissue properties of yogurt. One of the characteristics of *Lactobacillus* bacteria is the production of exopolysaccharide. Due to their structural properties, these compounds are capable of bonding and holding water. The higher the production of these compounds in a food system, the lower the rate of syneresis. In another study, Doleyres et al. (19) reported that the presence of exopolysaccharide in yogurt increased the water storage capacity of the samples. In this study, they used the polysaccharides produced by *Lactobacillus rhamnosus*. In a study by Han et al. (20), they investigated the tissue properties of yogurt using different exopolysaccharides of lactic acid bacteria. They explain the cause of the syneresis is weak gel network in the yogurt. The more this structure can be strengthened, the lower the rate of syneresis. The results

Table 5. Results of syneresis variations during the storage period.

Time	Time					
	0	12 h	24 h	day 7	day 14	day 21
Control	18.31±0.58^{Ac}	19.42±1.10^{Ac}	19.14±0.71^{Ac}	21.12±0.45^{Ab}	23.62±0.31^{Aa}	24.14±0.72Aa
YF-1%	17.56±0.82^{Ab}	18.64±0.92^{Ab}	18.45±0.39^{Ab}	19±0.76^{Bb}	22.17±0.51^{Ba}	22.68±0.76B^{Ca}
YF-2%	19±1.12^{Ac}	18.76±0.67^{Ac}	19.15±0.66^{Ac}	20.05±0.51^{Bb}	20.74±0.36^{Cab}	21.72±0.64^{Ca}
YF-3%	18.52±0.82^{Ab}	17.76±0.43^{Ab}	18.94±0.75^{Ab}	19.38±0.61^{Bab}	20.19±0.93^{Ca}	20.79±0.39^{Da}

$^{a–e}$ Different lowercase letters within a column indicate significant differences (p<0.05).
$^{A-D}$ Different uppercase letters within a row indicate significant differences (p<0.05).

that in the samples containing exopolysaccharide, the rate of syneresis was decreased compared to the control sample. They attributed these changes to the existence of exopolysaccharides and modification of the gel network. In a study by Zhang et al. (21), they investigated the production of exopolysaccharide by *L. fermentum* in free fat milk. They reported that *L. fermentum* is capable of producing Viscose exopolysaccharides with a tetra-saccharide structure composed of glucose and galactose units. The contents of the bacterial growth medium as well as the environmental conditions are very influential in the rate of production of this compound. However, pH near 6 and temperature in the range of 37 to 42˚C increase the production of exopolysaccharide by *L. fermentum* (20). The results are the mean of 3 replications ± standard deviation. In each column, capital letters mean there is no significant statistical difference at the p≤0.05 level. Also, lowercase letters in each row indicate there is no significant difference at the p≤0.05 level.

4. Conclusion

Probiotic products are an important category of beneficial foods that are particularly effective in the human digestive system. As mentioned in the previous sections, these products are important in several ways. For this reason, the study of new product having diverse applications can continue to be at the top of the world topics. In this study, *L. fermentum* was used to produce probiotic yogurt. Bacterial survival results showed that *L. fermentum* decreased its survival during storage in the present study but the basic concentration of this bacterium is present, it can be maintained by optimizing the initial concentration of the probiotic product until the end of the storage period, which confirmed the results of this study. Another aim of this study was to investigate the antimicrobial effect of *L. fermentum* on the survival of *E. coli* O111 during our storage period. According to numerous reports on the antimicrobial activity of this bacterium and also the results of this study, it can be concluded that *L. fermentum* has antimicrobial activity against *E. coli* in the storage conditions of this product and the higher the concentration of the primary probiotic bacterium, the greater the effect during storage. Results of yogurt characteristics such as pH and acidity showed no rapid changes during the storage period but significant differences were observed between samples. In addition, it was observed that the products containing probiotic bacteria had lower syneresis rates due to the production of exopolysaccharide. It can be stated that *L. fermentum* has the potential to be used as a probiotic in dairy products, but further steps are still needed ultimate optimizations.

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