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Evaluation of the Growth of some Pathogen Bacteria in Fruit Yoghurts

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SUMMARY

In this study, five groups were established by preparing strawberry, peach, apricot, banana and plain yogurts and, by inoculating *E. coli, S. aureus, and B. cereus* strains during the starter culture addition phase of every group, the effects of pathogen bacteria on the fruit yogurts were investigated. At the end of 30 days of storage of prepared fruit yogurts, the acidity (LA) varied between 0.79 and 1.49; while the lowest initial acidity was found in apricot-added yogurt, the lowest acidity at the end of storage period was found in strawberry-added yogurt as 1.49 and the difference was found to be statistically significant ($p<0.05$). Again, the pH values varied between 3.80 and 4.62; the lowest initial pH was found in peach-added yogurt with 4.16 and the lowest pH at the end of storage period was found in banana-added yogurt with the value of 3.91, while the difference was statistically significant ($p<0.05$). It was observed that the fruit varieties in yogurts played role in growth of pathogens, and they had inhibitory effect on *S. aureus* between 57.2 and 67.8%, *B. cereus* between 16.6 and 45.9%, and *E. coli* between 12.8 and 26.4%. While all of the pathogens were degraded in strawberry-added yogurt by 17.9-67.8%, *E. coli* didn't negatively affect the growth in peach-added yogurt and *B. cereus* was degraded by 23.5% and *S. aureus* by 58.9%. The degradation was 12.8-64.1% effective on all of the pathogens in apricot-added yogurts. In banana-added yogurt, while the *E. coli* growth was not affected negatively, it was determined that *B. cereus* was degraded by 45.9% and *S. aureus* by 63.8%. With high acidity and low pH levels caused due to of fruit in yoghurt, the fruit yogurts have found to have inhibitory effect on *S. aureus* and *B. cereus* and *E. coli* pathogens.

Key Words: *Pathogen Bacteria, Fruit Yoghurt, Growth*

ÖZET

Meyveli Yoğurtlarda Bazı Patojen Bakterilerin Gelişimlerinin İncelenmesi

Bu çalışma da çilekli, şeftalili, kayısılı, muzlu ve sade yoğurtlar hazırlanarak, beş grup oluşturulmuş ve her bir gruba starter kültür ilavesi aşamasında *E.coli, S.aureus, B.cereus* suşları inoküle edilerek, meyveli yoğurtların patojen bakteriler üzerindeki etkisi araştırılmıştır. Hazırlanan meyveli yoğurtlarda 30 günlük muhafaza süresinin sonunda; asitlik (LA) 0.79-1.49 arasında değişmiş en düşük başlangıç asitliği kaysılı yoğurt iken muhafaza sonunda 1.49 ile çilekli yoğurt olmuştur ve aralarındaki farklılık istatistiksel olarak anlamlı bulunmuştur ($p<0.05$). Yine pH değerleri de 3.80 ile 4.62 arasında değişmiş ve 4.16 ile en düşük başlangıç pH'sı şeftalili yoğurt iken depolama süresi sonunda en düşük 3.91 pH ile muzlu yoğurtta olmuş ve yoğurtlar arasında istatistiksel açıdan da fark oluştuğu tespit edilmiştir ($p<0.05$). Yoğurtlardaki meyve çeşitlerinin patojenlerin gelişimi üzerinde etkili olduğu, *S.aureus* üzerine % 57.2-67.8 arasında, *B.cereus* üzerine % 6.6-45.9 arasında ve *E.coli* üzerine % 12.8-26.4 arasında inhibitör etkilerinin olduğu görülmüştür. Çilekli yoğurta tüm pataojenler % 17.9-67.8 arasında yıkımlanırken, şeftalili yoğurtta *E.coli* gelişimi olumsuz etkilenmemiş, *B.cereus* % 23.5 ve *S.aureus* % 58.9 oranında yıkımlanmıştır. Kaysılı yoğurtta yıkımlanma tüm patojenlerde % 12.8-64.1 arasında olmuştur. Muzlu yoğurtta da yine *E.coli* gelişimi olumsuz etkilenmezken *B.cereus* % 45.9 ve *S.aureus* % 63.8 oranında yıkımlandığı tespit edilmiştir. Meyveli yoğurtlarda meyvelerden kaynaklanan yüksek asitlik ve düşük pH değerleriyle yoğurtlar içerisindeki başta *S.aureus* olmak üzere *B.cereus* ve *E.coli* patojenleri üzerine inhibitör etkili oldukları tespit edilmiştir.

Anahtar Kelimeler: *Meyveli yoğurt, Patojen bakteri, Gelişim*

INTRODUCTION

Yogurt, which is a gel-like milk product formed as a result of casein's precipitation by the acid formed by the lactic bacteria, is one of the foods that are important for nutrition and healthy life (Tamine and Robinson 2007). This is because the yogurt is a food containing sufficient and balanced level of nutrient components required for human life. Besides its rich content in terms of organic compounds such as peptides, amino acids, lactic acid, fatty

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acids, antimicrobial matters formed as a result of milk's fermentation and also millions of lactic bacteria, its probiotic characteristics are the properties making it powerful (Özden 2009). Among its lactic bacteria content, especially the *Lb. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* and also *L.casei, L.rhamnosus*, bifidobacteria and some of the yeasts-molds constitute the natural flora of the yogurt (Angelov et al. 2009).

Fermentation technology is one of the oldest food protection methods known. The lactic flora executing the fermentation also plays major role in preventing the pathogen bacteria and their spores. Fruit acids and natural phenolic compounds in fruits' structure have antioxidant and antimicrobial properties (Nizamlıoğlu and Nas 2004) and, thus, it is known that the pathogen bacteria growth in yogurt is limited. Acetic acid, lactic acid and some inhibitory materials secreted by lactic acid bacteria (LAB) are the important compounds preventing the growth of bacteria other than the flora (Meyer et al. 2007). Furthermore, the compounds formed by the lactic bacteria such as the diacetyl, hydrogen peroxide, and CO_2 are the components preventing the growth of other bacteria. Pathogen bacteria are sensitive to either these acids formed or the low pH emerging (Gibbs 1987). Attaie et al. (1987) have reported that the metabolites formed by the bacteria are of inhibitory effect on microorganisms secreting toxins in fermented milk products such as *S. aureus*. Depending on the natural acidity of yogurt, even though the pathogen bacteria may reproduce more difficultly, the yeasts and molds can reproduce very easily. Especially depending on the fruit variety and sugar additive used in fruit yogurts manufactured in order to increase the yogurt consumption and improve the range of product, the number of yeasts increases (Osborne and Pritchard 1974). Again, because of the fruits used in yogurts, there may be molds and bacteria.

In this study, the banana, peach, apricot, and strawberry-added yogurts were prepared and the effects of fruits on pathogen bacteria growth were investigated by inoculating *S. aureus, E. coli and B. cerecus* strains, which can easily contaminate the milk products especially during the culture addition phase and easily cause food intoxication upon the consumption of these products, into the yogurts during adding the starter culture.

MATERYAL ve METOT

Manufacture of Experimental Yogurts

The cow milk used in manufacturing the fruit yogurts during this study were procured from the Veterinary Faculty Farm of Kafkas University. Firstly, the chemical analysis of the milk was performed; the mean fat-free dry matter content was found to be 12.3% and fat rate to be 3.4%. And then, the milks heat-treated for 5 minutes at 90°C were cooled to 45°C and 3% starter culture (Chr. Hansen's Lab. YC-180) was added before 0.01 ml *S. aureus* (RSKK-25923) (6.755 $\log_{10}$ cfu/ml), *E. coli* (ATTC-25922) (7.380 $\log_{10}$ cfu/ml), *B. cerecus (*ATCC-11778) (3.875 $\log_{10}$ cfu/ml), strains procured from Refik Saydam Public Hygiene Center and HEMAKIM were inoculated. And then, in order to manufacture banana, strawberry, peach and apricot-added yogurt, the milk was divided into five and then poured into PET containers containing 15% fruit heat-treated with sugar before, then incubated at 42 °C until pH reaches at, and then stored in refrigerator at +4 °C to use in analyses after pH reached at the desired level (Aksu and Nas 1996).

Analytical Methods

The chemical and microbiological analyses of fruit yogurts were performed at the start of incubation (0^{th} min), mid of the incubation, and 1^{st}, 3^{rd}, 7^{th}, 15^{th}, and 30^{th} day of storage.

Chemical analysis

Acidity; Samples (10 ml) were titrated with 0.1 M NaOH using phenolphthalein as indicator. The amount of NaOH consumed was calculated in LA unit by multiplying with 0.0225 (Anon 1990). *Measurement of pH:* The pH value of samples was determined at room temperature with an pH-meter and combined glass electrode (Hanna HI 8521) standardized with pH 4.01 and 7.01 standard buffer solutions (Merck). The pH of each sample was measured with a digital pH meter equipped with a glass electrode that was inserted directly into the yogurt sample for the measurement (Anon 1990).

Microbiological analysis

In order to observe the microflora growth, 1ml samples were taken from each of the yogurt varieties at the times specified above under aseptic conditions and, after being stirred with 9 ml 0.1% peptone-water within the sterile tube by vortex, the decade dilatations were prepared considering the estimated number of bacteria. For counting *Lb. delbrueckii* subsp. *bulgaricus,* MRS agar (Oxoid CM 361) was used. With pH level set to 5.7, the agar was planted 0.1ml using spread plaque method, then the petri dishes were incubated at 35°C for 48 hours under anaerobic (Anaerobic jar Oxoid HP11) conditions. Typical colonies having 1-3 mm diameter were confirmed with microscopically, and then counted (Anon 1983; Dave and Shah 1996). For counting the *S. salivarius* subsp. *thermophilus*, M17 Agar pH 6.9 (Oxoid CM785) was utilized. After planting by using spread plaque method again, the agar was incubated at 35°C for 48 hours under aerobic conditions. Following the incubation, the typical colonies with 1-2 mm diameter were microscopically confirmed and then counted (Dave and Shah 1996). For mold and yeast counting, the Potato Dextroz Agar pH 5.6 (Oxoid CM 139) was utilized. After the inoculation, following the incubation at 22 °C for 5 days, all of the colonies were counted. Among the pathogen bacteria, Baird Parker Agar pH 6.8 (Oxoid CM275) was used for *S. aureus* counting, Violet Red Bile Agar pH 7.4 (Oxoid CM107) for *E. coli* counting, and Bacillus Cereus Selective Agar pH 7.2 (Oxoid CM617) for *B. cereus* counting. For each of bacteria, after plantation on its own medium by using spread plaque method, the incubation was executed under aerobic conditions at 37 °C for 48 hours (Anon 1983; Elmer and James 2001). After calculation of typical colonies reproduced on mediums, the cfu/ml parameters were calculated.

Statistical Analysis

Statistical analyses were carried out by utilizing SPSS 16 package software (Statistical Software 10.0 for Windows, SPSS Inc., USA). The data obtained from triplicated chemical, microbiological and sensory analyses were analyzed via one-way variance analysis (ANOVA). While investigating the differences between the groups, Tukey test was used ($p < 0.05$).

RESULTS

The results of chemical (acidity and pH) and microbiological (*Lb. delbrueckii* subsp. *bulgaricus*, *S. salivarius* subsp. *thermophilus* and yeast, mold) analyses carried out at the beginning of incubation and during the storage of fruit yogurts are presented in Table 1. According to the chemical analysis results, the acidity (LA) at the beginning of incubation and during the storage are 0.75-0.79 for plain yogurt, 0.68-1.49 for strawberry-added yogurt, 0.59-0.79 for peach-added yogurt, 0.60-1.22 for apricot-added yogurt, and 0.62-1.28 for banana-added yogurt. The pH values were found to be 4.36-4.62 for plain yogurts, 4.31-3.93 for strawberry-added yogurt, 4.31-4.41 for peach-added yogurt, 4.47-4.18 for apricot-added yogurt, and 4.42-3.91 for banana-added yogurt.

In microbiological analyses, where the microflora growth was monitored, the level of *Lb. delbrueckii* subsp. *bulgaricus* ($\log_{10}$ cfu/ml) at the beginning of incubation and at the end of storage period was found to be 4.56-7.31 for plain yogurts, 4.43-6.95 for strawberry-added yogurt, 4.19-7.24, 4.53-7.84 and 4.45-7.29 for banana-added yogurt. That of *S. salivarius* subsp. *thermophilus* ($\log_{10}$ cfu/ml) was found to be 4.30-5.90 for plain yogurts, 4.80-6.19 for strawberry-added yogurt, 5.20-6.46 for peach-added yogurt, 5.04-7.36 for apricot-added yogurt and 4.73-6.21 for banana-added yogurt. Among the pathogen bacteria, the levels of *B. cereus, E. coli and S. aureus* ($\log_{10}$ cfu/ml) were found to be 2.54-2.12/5.71-7.57/5.72-2.44 for plain yogurt, 2.97-2.44/7.19-5.29/6.38-2.05 for strawberry-added yogurt, 2.61-1.99/6.69-7.41/6.54-2.68 for peach-added yogurt, 2.72-2.00/7.01-6.11/6.46-2.31 for apricot-added yogurt and 3.70-2.00/4.48-6.85/5.66-2.07 for banana-added yogurt. The mold growth ($\log_{10}$ cfu/ml) was found to be 3.18-4.97 for plain yogurt, 2.92-3.50 for strawberry-added yogurt, 2.93-4.86 for peach-added yogurt, 2.47-4.21 for apricot-added yogurt and 3.05-3.48 for banana-added yogurt.

DISCUSSION

In this study, where different fruits were used, it was observed that, during 30 days of storage, the fruit yogurts followed a different course in terms of acidity, except for the 1st day (Table 1 and Figure 1-2). This difference was found to be statistically significant between the plan yogurt and banana-added yogurt ($p<0.05$) and others ($p<0.001$). While the lowest level of change in acidity between the beginning and 30th day was seen in plain yogurt with 5.41% change, the highest level of change was observed in strawberry-added yogurt with 116.6% change. From the aspect of pH, different growths were observed between the yogurts other than peach-added yogurt (especially between the plain yogurt and others) during the storage, and the difference was determined to be statistically significant at the level of $p<0.001$. While the lowest level of change in pH between the beginning and 30th day was observed in peach-added yogurt with change of 2.36%, the highest level of change was found in banana-added yogurt with -11.6% change.

The differences between the fruit yogurts throughout the storage period in terms of *L.bulgaricus, S. salivarius* subsp. *thermophilus, B. cereus, E. coli, S. aureus* and yeast-mold values and changes are presented in Table 2 and Figure 3-8. The number of *Lb. delbrueckii* subsp. *bulgaricus*, among the bacteria constituting the microflora of yogurt, showed difference at 1st day between the yogurt varieties ($p<0.05$). In next days of storage, the differences between plain, banana ($p<0.05$), and peach ($p<0.001$) yogurts were found to be statistically significant. At the end of storage period, the number of *S. salivarius* subsp. *thermophilus* was found to be at lowest level in plain yogurt (5.903) and at highest level in apricot-added yogurt (7.368). Although the difference from plain yogurt was found to be statistically significant ($p<0.05$), the differences among the fruit yogurts were found to be statistically non-significant. The lowest number of *Lb. delbrueckii* subsp. *bulgaricus* was determined in strawberry-added yogurt (6.952), while the highest value was found in apricot-added yogurt (7.842). Although the difference from plain yogurt was found to be statistically significant ($p<0.05$), the differences among the fruit yogurts were found to be statistically significant only between banana and peach-added yogurts. From the aspect of microflora's percentages of change, the lowest level of change of *L.bulgaricus* was found to be 56.7% in strawberry-added yogurt and the highest level of change was found to be 73.1% in apricot-added yogurt. The lowest level of *S. salivarius* subsp. *thermophilus* change was determined to be 24.1% in strawberry-added yogurt, and the highest level of change was observed to be 45.9% in apricot-added yogurt.

From the aspect of pathogen bacteria growth, the highest number of *B. cereus* during the storage period was found in strawberry-added yogurt (2.442), while the lowest one was found in peach-added yogurt (1.998). On the other hand, the highest number of *E. coli* was found in plain yogurt (7.571) and the lowest one was found in strawberry-added yogurt (5.298), while the lowest and highest numbers of *S. aureus* were found in strawberry-added yogurt (2.058) and peach-added yogurt (2.688). The differences were found to be statistically significant ($p<0.05$). The differences between the days in terms of decreases in number of *E. coli* during the storage period were found to be statistically significant ($p<0.05$ and $p<0.001$). From the aspect of effects of pathogens, it can be seen that the lowest level of decrease in *B. cereus* was found to be in plain yogurt (57.2%) and the highest number of decrease was found in banana-added yogurt with 67.89% decrease, while the lowest and highest levels of decrease in *S. aureus* were observed in plain (16.6%) and strawberry (45.9%) yogurts. In *E. coli*, different changes were observed and, while its level decreased in strawberry and apricot-added yogurt, the level of *E. coli* increased in other varieties. In total, it can be seen that the highest level of decreasing effect among the fruit yogurts was observed on *S. aureus*.

From the aspect of yeast-mold number, the highest value was found in plain yogurt (4.975), while the lowest value was observed in strawberry-added yogurt (3.507). Even though the highest yeast-mold values were observed in plain yogurt, the highest level of statistical difference was found in banana-added yogurt ($p<0.001$). Statistically significant differences were observed between 1st, 7th and 15th days, when the analyses were performed, in terms of yeast-mold numbers ($p<0.05$). Considering the effect of fruit varieties on yeast-mold, the lowest level of increase was determined in banana-added yogurt with 13.8% increase, while the highest level of increase was observed in apricot-added yogurt with 70.1% increase.

Özden (2009), who reported that yoghurt has *in vitro* inhibitor effects on many microorganisms such as *Salmonella, Shigella, Staphylococcus, E. coli, Bacillus, Clostridium, Pseudomonas, Brucella, Vibrio, Klebsiella,* and *Mycobacterium*, has also reported in same article that the most resistant bacteria in yogurt were *B. subtilis* and *B. mesentericus*, while the most sensitive one was *E. coli*.

Airaii et al. (2011) have found 10 coliform bacteria in fruit yogurt, which they have prepared by using banana and stored for 6 days, on 1st day of storage, and determined no *E. coli* and yeast-mold. Tirloni et al. (2015), have inoculated *E. coli and L. monocytogenes* strains into strawberry and plain yogurts and stored for 68 days.

Table 1. Acidity level (LA) and pH values of fruit yogurts and the difference percentages

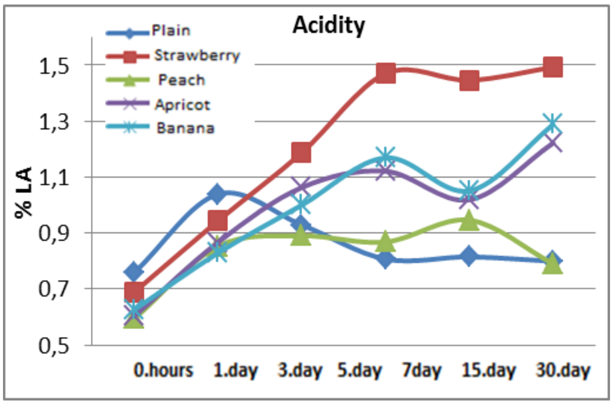
	Yogurt kind	0. hours	1.day	3.day	7.day	15.day	30.day	p	Variation	%
Acidity	Plain	0.758±0.02^{c1}	1.039±0.03^{c1}	0.929±0.02^{ab1}	0.807±0.07^{a1}	0.815±0.04^{a1}	0.799±0.10^{a1}	*	0.041	5.4
	Strawberrry	0.689±0.01^{b1}	0.944±0.03^{ab12}	1.187±0.13^{b12}	1.470±0.23^{b2}	1.446±0.21^{b2}	1.493±0.24^{b2}	**	0.804	116.7
	Peach	0.594±0.00^{a1}	0.853±0.02^{a2}	0.890±0.02^{a2}	0.868±0.03^{a2}	0.946±0.02^{a2}	0.790±0.06^{a2}	**	0.196	33.0
	Apricot	0.603±0.01^{a1}	0.867±0.01^{a12}	1.063±0.04^{ab23}	1.121±0.08^{ab23}	1.019±0.01^{a23}	1.222±0.05^{ab3}	**	0.619	102.6
	Banana	0.626±0.01^{ab1}	0.829±0.01^{a2}	1.000±0.02^{ab3}	1.169±0.02^{ab4}	1.051±0.01^{ab3}	1.288±0.04^{ab5}	*	0.662	105.7
	p	*		**	**	**	**			
pH	Plain	4.362±0.03^{ab12}	4.202±0.08^{b12}	4.122±0.13^{c12}	4.352±0.12^{b12}	4.062±0.02^{c1}	4.622±0.20^{b2}	**	0.26	5.9
	Strawberrry	4.316±0.00^{a3}	3.872±0.05^{a12}	4.040±0.06^{ab2}	3.850±0.03^{a12}	3.798±0.00^{a1}	3.938±0.06^{a12}	*	- 0.378	-8.8
	Peach	4.316±0.01^{a23}	4.380±0.07^{b3}	3.920±0.04^{ab1}	4.100±0.05^{ab12}	3.878±0.05^{ab1}	4.418±0.08^{b3}		0.102	2.4
	Apricot	4.470±0.00^{c5}	4.400±0.00^{b4}	3.800±0.00^{a1}	3.980±0.01^{a2}	3.802±0.00^{a1}	4.182±0.02^{ab3}	*	-0.288	-6.4
	Banana	4.428±0.00^{bc2}	4.326±0.03^{b2}	4.406±0.00^{c2}	3.896±0.03^{a1}	4.002±0.04^{bc1}	3.912±0.04^{a1}	*	-0.516	-11.6
	p	*	**	*	**	**	**			

The values indicated with a, b, c … represent the statistical difference in same column, while those indicated with 1, 2, 3… represent the statistically difference in same row.
*p<0.05, **p<0.001

Table 2. Microbiological analysis results and difference percentages of fruit yogurts

	Yogurt kind	0. hours	1.day	3.day	7.day	15.day	30.day	p	Variation	%
L.bulgaricus	Plain	4.569±0.21^{a1}	7.241±0.15^{ab3}	7.443±0.03^{a3}	7.201±0.10^{ab3}	7.250±0.10^{ab3}	7.311±0.10^{ab3}	*	2.742	60.0
	Strawberry	4.435±0.17^{a1}	6.707±0.16^{a34}	7.081±0.13^{a34}	6.738±0.22^{a34}	6.793±0.22^{a34}	6.952±0.14^{a34}		2.517	56.7
	Peach	4.196±0.06^{a1}	7.482±0.09^{ab3}	7.308±0.11^{a3}	7.145±0.06^{ab3}	7.165±0.06^{ab3}	7.246±0.05^{ab3}	**	3.05	72.7
	Apricot	4.530±0.18^{a1}	7.768±0.19^{b3}	7.455±0.08^{a3}	7.809±0.21^{b3}	7.798±0.22^{b3}	7.842±0.20^{b3}		3.312	73.1
	Banana	4.459±0.32^{a1}	6.748±0.36^{b2}	7.129±0.08^{a3}	7.181±0.18^{ab3}	7.212±0.18^{ab3}	7.291±0.15^{ab3}	*	2.832	63.5
	p		*							
S.thermophilus	Plain	4.305±0.26^{a1}	5.691±0.45^{a12}	5.379±0.16^{a12}	5.783±0.44^{a12}	5.853±0.42$^{a1.2}$	5.903±0.41^{a12}	*	1.598	37.1
	Strawberry	4.804±0.20^{a1}	6.408±0.20^{ab2}	6.448±0.08^{bc2}	6.023±0.36^{a2}	6.101±0.36^{a2}	6.192±0.36^{a2}		1.388	28.9
	Peach	5.204±0.14^{a1}	6.213±0.21^{ab3}	6.496±0.14^{c3}	6.321±0.15^{a3}	6.480±0.17^{a3}	6.460±0.17^{a3}		1.256	24.1
	Apricot	5.048±0.21^{a1}	7.204±0.32^{b2}	7.064±0.16^{c2}	7.348±0.35^{a2}	7.340±0.34^{a2}	7.368±0.34^{a2}		2.32	45.9
	Banana	4.731±0.37^{a1}	6.112±0.29^{ab1}	5.645±0.32^{ab1}	5.965±0.48^{a1}	6.192±0.51^{a1}	6.218±0.52^{a1}		1.487	31.4
	p									
B. cereus	Plain	2.545±0.14^{a1}	2.097±0.09^{a1}	2.182±0.18^{ab1}	2.132±0.12^{a1}	2.140±0.13^{a1}	2.122±0.11^{ab1}		-0.423	-16.6
	Strawberry	2.975±0.06^{a1}	2.559±0.13^{b1}	2.854±0.30^{b1}	2.682±0.06^{b1}	2.889±0.50^{a1}	2.442±0.09^{b1}	*	-0.533	-17.9
	Peach	2.614±0.10^{a2}	2.058±0.06^{a1}	2.058±0.06^{a1}	1.998±0.00^{a1}	1.998±0.00^{a1}	1.998±0.00^{a1}	*	-0.616	-23.5
	Apricot	2.725±0.07^{a2}	2.002±0.00^{a1}	2.002±0.00^{a1}	2.002±0.00^{a1}	2.002±0.00^{a1}	2.002±0.00^{ab1}	**	-0.723	-26.5
	Banana	3.707±0.30^{b3}	2.813±0.01^{b2}	2.463±0.06^{ab12}	2.802±0.04^{b2}	2.643±0.03^{a2}	2.002±0.00^{ab1}	**	-1.705	-45.9
	p	*	*	**	*	*	**			
E. coli	Plain	5.716±0.43^{ab1}	5.978±0.32^{b12}	6.189±0.29^{b12}	6.123±0.17^{a12}	7.213±0.26^{ab23}	7.571±0.14^{b3}		1.855	32.4
	Strawberry	7.198±0.33^{b1}	6.569±0.40^{b1}	6.230±0.51^{b1}	5.364±0.14^{a1}	5.654±0.67^{a1}	5.298±0.60^{a1}	*	-1.9	-26.4
	Peach	6.699±0.16^{ab23}	6.028±0.37^{b12}	5.522±0.01^{b1}	5.886±0.09^{a1}	7.428±0.06^{b3}	7.417±0.10^{b3}	*	0.718	10.7
	Apricot	7.019±0.04^{b2}	6.784±0.13^{b2}	6.568±0.11^{b12}	5.356±0.19^{a1}	6.096±0.37^{ab12}	6.118±0.54^{ab12}	*	-0.901	-12.8
	Banana	4.487±1.10^{a12}	3.871±0.83^{a12}	3.437±0.63^{a1}	5.447±0.59^{a12}	5.893±0.29^{ab12}	6.853±0.23^{ab2}	*	2.366	52.7
	p	**	*	*	**	**	*			
S. aureus	Plain	5.724±0.12^{ab2}	5.180±0.81^{a2}	4.779±0.79^{ab12}	5.150±0.80^{a2}	3.831±0.46^{abc12}	2.449±0.16^{a1}		-3.275	-57.2
	Strawberry	6.387±0.23^{bc3}	5.821±0.13^{a3}	6.207±0.13^{b3}	6.079±0.34^{b3}	4.858±0.08^{c2}	2.058±0.06^{a1}		-4.329	-67.8
	Peach	6.544±0.11^{c4}	5.588±0.27^{a3}	6.212±0.17^{b34}	5.950±0.10^{b34}	4.308±0.04^{bc2}	2.688±022^{a1}		-3.856	-58.9
	Apricot	6.465±0.05^{c4}	5.900±0.16^{a34}	5.996±0.12^{b34}	5.316±0.08^{ab3}	3.477±0.38^{ab2}	2.319±0.31^{a1}		-4.146	-64.1
	Banana	5.669±0.21^{a4}	5.551±0.01^{a34}	4.042±0.48^{a23}	3.555±0.63^{a12}	2.828±0.29^{a12}	2.077±0.03^{a1}	**	-3.592	-63.4
	p		*	*			*			
Mould-Yeast	Plain	3.184±0.07^{b2}	2.620±0.03^{b1}	4.220±0.05^{b3}	4.306±0.08^{a3}	4.768±0.03^{b4}	4.975±0.08^{b4}		1.791	56.2
	Strawberry	2.929±0.15^{ab12}	2.407±0.01^{a1}	3.413±0.14^{ab2}	4.356±0.08^{a4}	4.208±0.15^{a34}	3.507±0.29^{a23}	*	0.578	19.7
	Peach	2.934±0.03^{ab2}	2.616±0.03^{b1}	3.599±0.09^{ab3}	4.483±0.03^{a4}	4.579±0.11^{ab45}	4.862±0.06^{b5}		1.928	65.7
	Apricot	2.476±0.14^{a1}	2.633±0.00^{b1}	3.149±0.28^{a1}	4.178±0.21^{a2}	4.283±0.16^{ab2}	4.213±0.23^{b2}		1.737	70.1
	Banana	3.057±0.09^{b12}	2.577±0.02^{b1}	3.197±0.26^{a12}	3.852±0.28^{a23}	4.691±0.02^{ab3}	3.481±0.34^{a12}	**	0.424	13.8
	p		*		*	*				%

The values indicated with a, b, c … represent the statistical difference in same column, while those indicated with 1, 2, 3… represent the statistically difference in same row.
*p<0.05, **p<0.001



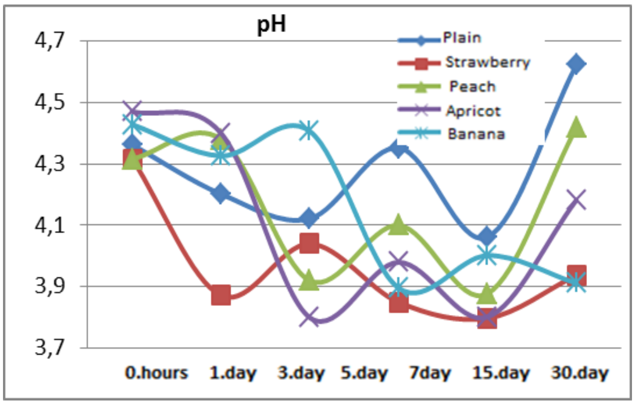
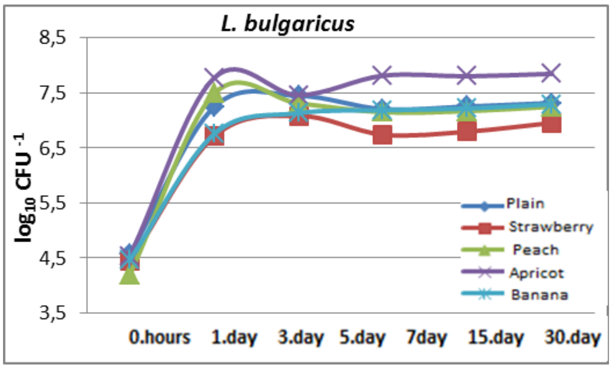


Figure 1, 2. Acidity and pH levels of yogurts during the storage period



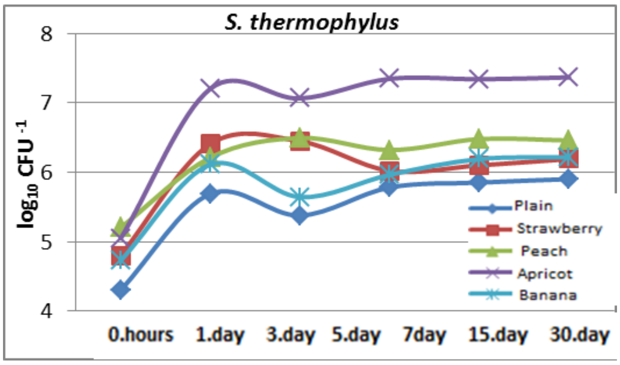
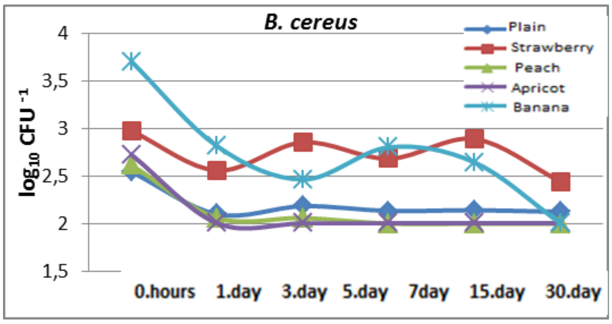


Figure 3, 4. *Lb. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* levels of yogurts during the storage period



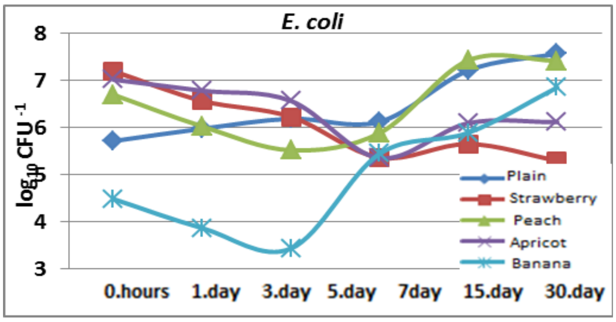
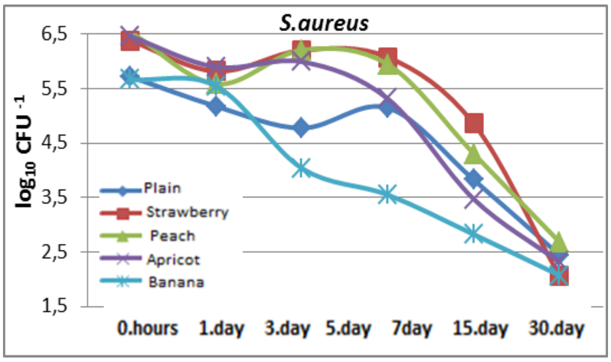


Figure 5, 6. *B. cereus and E. coli* levels of yogurts during the storage period



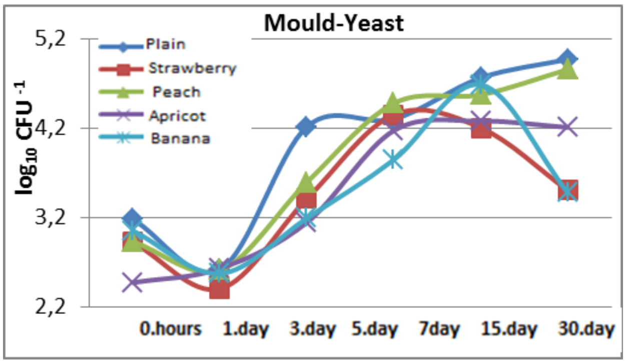


Figure 7, 8. *S. aureus* and yeast-mold levels of yogurts during the storage period

They have reported the *E. coli* concentration to be 2 log_{10} cfu/g, while it totally disappeared on 26th day of storage in strawberry-added yogurts under favor of increasing acidity, and on 33rd day of storage in plain yogurts. In this study, similarly, it was observed at the end of 30 days of storage that the number of *E. coli* was lower than the initial values in strawberry and plain yogurts. For peach and banana-added yogurts, Canganella et al. (1998) have determined in their study that *E. coli* bacteria have shown resistance to the flora bacteria and the acidity they caused during the further days of storage. Dong et al. (2012) have reported that strawberry fruit contain phenolic compounds having natural antimicrobial effects. The strawberry extract has been reported to have antimicrobial effects on gram-negative bacteria such as *E. coli*, *S. thypimurium, Candida albicans, and C. jejuni* and to be useful as a natural antifungal. Nohynek et al. (2006), in their study, have reported that the yogurt variety exhibiting highest level of inhibitory effect on bacteria other than *B. cerecus* was strawberry-added yogurt, and our results corroborates this result. In this study, it was observed that all of the yogurt varieties showed high-level of antibacterial effect on *S. aureus*. Although there is no same study in literature, it has been determined in an *in-vitro* study that *Lb. delbrueckii* subsp. *bulgaricus* insulated from village and town yogurts had inhibitory effect on *S. aureus* coagulase (+) and (-) strains (Aslım and Beyatlı 1997). In another study, where the antimicrobial effects of yogurt and its water on steak tartar a la turca, it has been determined that the number of *S. aureus* in samples prepared by adding yogurt and its water on 0th hour was lower than the control group (Dogan et al. 2014). In another study, it has been emphasized that, among the yogurt bacteria, *Lb. delbrueckii* subsp. *bulgaricus* had antimicrobial effect on *S. aureus* because of a compound with hydrogen peroxide character that *Lb. delbrueckii* subsp. *bulgaricus* produces (Dahiya and Speck 1968). Belickova et al. (2001), in their study, have found the number of *S. aureus* (0.70-2.0 log_{10} cfu/ml) to be lower in strawberry-added yogurts when compared to other varieties. In this study, similarly, it was seen that the yogurt species that are effective on *S. aureus* at highest level were strawberry and banana-added yogurts.

Ahmed et al. (2014), in their study investigating the effects of yogurt starter cultures on *E. coli*, have been emphasized that the addition of $6.5x10^7$ *E. coli* disappeared on 9th day and the numbers of starter culture *(S.thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) were $11.4x10^{10}$, while pH and acidity were 4.35 and 0.82%. Osali et al. (2013), in their study, have reported that the level of *E. coli* O157:H7 at the end of 7th day of storage at 4 °C was unmeasurable. Tsiraki and Savvaidis (2016) have reported that 2 ml*kg addition of Citrox component obtained from citrus fruits into the yogurts decreased the levels of *B. cereus* and *S. enterica* by 2.8 log_{10} cfu/g in 12-28 days. Even though the researchers such as Coghill and Juffs, Ahmed et al., Wong et al., Griffiths and Phillips, Larsen and Jorgensen, Zhou et al., and Wong et al. have reported that they have determined various levels of *B. cereus* vegetative and spore forms in various milk products, authors have reported that they have found none of them in yogurts (Miller 2008).

Kılıç (1990), have emphasized that the effect of *Lb. delbrueckii* subsp. *bulgaricus* on *B. cereus* was weak, Akpınar et al. (2011) in their study where they have investigated the antimicrobial effectiveness of *S. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* insulated from yogurts, have reported that 11% of *S.termophilus* insulated from yogurts was effective on *B. cereus,* while *Lb. delbrueckii* subsp. *bulgaricus* had higher suppressor effect (44%). In this study, it was observed that all of the yogurt varieties, especially the banana-added yogurt, had high-level of inhibitory effect on *B. cereus*. This conclusion corroborates the findings of Kılıç (1990), who has reported that the antimicrobial effect of *S. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* as starter culture is higher when combined.

In a study, where the microbiological characteristics of the fresh home-made yogurts have been investigated in Konya region, it has been reported that the mean number of yeast-mold was $30x10^4$ cfu/g (Durak et al. 2008). Tarakçı and Küçüköner (2003), on contrary with this study, have determined that, in fruit yogurts that they have prepared by using various fruits, the number of yeast-mold was lower than control group. They have emphasized that this result might be caused from faster yeast growth and slower mold growth in fruit yogurts due to the sugar content in medium (Osborne and Pritchard 1974). In another study, where 5%, 7% and 10% concentrations of Grewia Tenax fruit have been utilized, the number of yeast-mold in yogurts has been found to be between 3.69 and 4.12 log_{10} cfu/ml (Mohamed et al. 2015). Okoye and Animalu (2009) have reported no fungal growth in yogurts they have prepared by adding sweet potatoes, Tirloni et al. (2015) have emphasized that, depending on the storage temperature, the yeast could reproduce much enough to cause spoiling of fruit yogurt. In this study, even though they showed small fluctuations during the period of storage, the numbers of yeast-mold increased in proportion to the initial values.

Karagözlü (1997) has reported that, in strawberry and peach-added yogurts stored for 28 days, the number of *S. salivarius* subsp. *thermophilus* in strawberry and peach-added yogurts to be $1.1\text{-}27.0x10^7$ cfu/ml and $0.5\text{-}23.6\ x10^7$ cfu/ml, respectively, and that there was a decrease in parallel with time, while the number of *Lb. delbrueckii* subsp. *bulgaricus* has been shown in same study to decrease throughout the period of storage. Their number has been reported to be $4.1\text{-}19.5x10^7$ cfu/ml in strawberry-added yogurt and $3.2\text{-}16.3\ x10^7$ cfu/ml in peach-added yogurt. In this study, in parallel with the study of Boycheva et al. (2011), the number of *S. salivarius* subsp. *thermophilus* in fruit yogurts showed higher level of increase during the storage period in proportion to plain yogurts. Michael et al. (2010), in yogurts they have prepared by adding herb extracts, have emphasized that the number of *Lb. delbrueckii* subsp. *bulgaricus* were higher than 6 log cfu mL^{-1}. In this study, similarly, the number of *Lb. delbrueckii* subsp. *bulgaricus* during the storage period was found to be higher when compared to plain yogurts.

Uraltaş and Nazlı (1998) have determined the acidity level of fruit yogurts to vary between 0.9% and 1.1%. In another study, where the physiochemical, microbiological and sensory properties of fruit yogurts have been investigated, the titration acidity has been reported to vary between 0.65% and 1.11% (Şahan et al. 1999). Similarly, in our study, the acidity varied between 0.59% and 1.49% throughout the storage, and increased in course of time. In another similar study, the increase of acidity in course of time has been emphasized to be a result of the transformation of lactose by yogurt bacteria and the release of free fatty acids as a result of hydrolization of fats (Çakmakçı et al. 1997). In study of Şahan et al. (1999), pH have fluctuated throughout the storage period, and varied between 4.08 and 4.36. In this study, the similar fluctuations were observed and pH varied between 3.80

and 4.62. The minimum initial pH was found in peach-added yogurt as 4.16, while the minimum pH at the end of storage period was found to be in banana-added yogurt as 3.91. It was determined that there was statistically significant difference between the yogurts. These decreases in pH of yogurts during the storage were understood to be caused from the fact that the acidity of fruits used decreases the pH of fruit yogurts.

In this study, it was observed that the addition of various fruits into the yogurts had effects on growth of pathogen bacteria, and the levels of their destructive effects were found to vary between 57.2% and 67.8% for *S. aureus*, 16.6% and 45.9% for *B. cereus* and 12.8% and 26.4% for *E. coli*. While all the pathogens were destructed by 17.9-67.8% in strawberry-added yogurt, while the growth of *E. coli* in peach-added yogurt was not affected negatively and *B. cereus* was destructed by 23.5% and *S. aureus* by 58.9%. The destruction of all the pathogens in apricot-added yogurts varied between 12.8% and 64.1%. Furthermore, while growth of *E. coli* was not affected negatively in banana-added yogurt, the *B. cereus* and *S. aureus* were found to be destructed by 45.9% and 63.8%, respectively.

With high acidity and low pH levels because of phenolic compound contents of fruits, the fruit yogurts have inhibitory effect on especially the *S. aureus and B. cereus* and *E. coli* pathogens. Besides their positive effects from sensory aspect by improving the range of product in yogurt production, they have also been shown to prevent the pathogen growth at various levels.

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