

Effect of Potassium Sorbate on Some Microbiological Properties of Cokelek Stored at Different Temperatures

Gülsüm ÖKSÜZTEPE * O. İrfan İLHAK *  Abdullah DİKİCİ **
Mehmet ÇALICIOĞLU * Bahri PATİR *

* Fırat Üniversitesi Veteriner Fakültesi, Besin Hijyeni ve Teknolojisi Bölümü, TR-23119 Elazığ - TÜRKİYE

** Tunceli Üniversitesi Mühendislik Fakültesi, Gıda Mühendisliği, TR-62000 Tunceli - TÜRKİYE

Makale Kodu (Article Code): KVFD-2010-1840

Summary

In this study, the effect of potassium sorbate at 0.01%, 0.05% and 0.1% on the some microbiological (total aerobic mesophilic bacteria-TAMB, coliform bacteria, *Lactobacillus-Leuconostoc-Pediococcus*, *Stapylococcus-Micrococcus*, enterococcus and yeast-mold) and chemical (pH, acidity) properties of cokelek stored at $4\pm 1^\circ\text{C}$ or $22\pm 1^\circ\text{C}$ was investigated. It was seen that adding potassium sorbate at 0.01%, 0.05% and 0.1% to cokelek did not have an effect on numbers of TAMB, coliform bacteria, *lactobacillus-leuconostoc-pediococcus*, *staphylococcus-micrococcus* in cokelek stored at both $4\pm 1^\circ\text{C}$ and $22\pm 1^\circ\text{C}$ ($P>0.05$). However, adding potassium sorbate at 0.05% and 0.1% to cokelek had an effect on numbers of enterococci and yeast-mold in cokelek stored at $4\pm 1^\circ\text{C}$ ($P<0.05$). It was also not observed significant difference in pH and acidity levels between control group and treatment groups containing potassium sorbate during storage period ($P>0.05$).

Keywords: *Cokelek, Microbiological quality, Storage temperature, Potassium sorbate*

Potasyum Sorbatın Farklı Sıcaklıklarda Muhafaza Edilen Çökeleğin Bazı Mikrobiyolojik Özellikleri Üzerine Etkisi

Özet

Bu çalışmada, %0.01, 0.05 ve %0.1 oranında potasyum sorbatın $4\pm 1^\circ\text{C}$ ve $22\pm 1^\circ\text{C}$ 'de muhafaza edilen çökeleğin bazı mikrobiyolojik (toplam aerobic mezofil bakteri, koliform bakteri, *Lactobacillus-Leuconostoc-Pediococcus*, *Stapylococcus-Micrococcus*, enterococcus ve maya-küf) parametreleri ile pH ve toplam asidite (laktik asit cinsinden) üzerine etkileri araştırıldı. Hem $4\pm 1^\circ\text{C}$ 'de hem de $22\pm 1^\circ\text{C}$ 'de muhafaza edilen çökelek örneklerine %0.01, 0.05 ve %0.1 oranında potasyum sorbat ilavesinin toplam aerobic mezofil bakteri, koliform bakteri, *Lactobacillus-Leuconostoc-Pediococcus*, *Stapylococcus-Micrococcus* sayıları üzerine herhangi bir etkisinin olmadığını görüldü ($P>0.05$). Bununla birlikte, $4\pm 1^\circ\text{C}$ 'de muhafaza edilen çökelek örneklerinde %0.05 ve %0.1 oranında potasyum sorbat ilavesinin enterococcus ve maya-küf sayıları üzerine etkili olduğu bulundu ($P<0.05$). Çökelek örneklerinin muhafaza süreleri boyunca, kontrol grubu ve potasyum sorbat ihtiva eden gruplar arasında pH ve asidite bakımından bir fark görülmedi ($P>0.05$).


Anahtar sözcükler: *Çökelek, Mikrobiyolojik kalite, Muhafaza sıcaklığı, Potasyum sorbat*


INTRODUCTION

Cokelek is a popular traditional dairy product in Turkey. Cokelek is produced by diluting yoghurt at 1:1 ratios with potable water and churned for separation of milk fat, and then fat is removed. The remaining portion is boiled until precipitation. The arising precipitate is placed into cloth bags and hanged for overnight for

removal of excess fluid. Then, it is kept under pressure for a short time (ca. 1 h) to reduce water content. It is then removed into a large pot and kneaded by hand adding salt at 1 or 2% (w/w). The average values of the chemical parameters of cokelek are 3.8 for pH, 1.25% for acidity (lactic acid), 0.95 for water activity, 1.38% for fat,

 İletişim (Correspondence)

 +90 424 237000/3962

 oiilhak@firat.edu.tr

21.43% for dry substance, and 17.91% for protein ¹. Cokelek is sold without packing under refrigerated or ambient temperature in markets or bazaar. The major problem regarding cokelek is that the shelf life of the product is very short due to traditional production method is used, and the main cause of spoilage is the growth of yeast and molds ².

Various preservatives have been used against spoilage of dairy products, especially against mold growth. The most frequently used preservative for this purpose is the sorbic acid and its salts ^{3,5}. Antimicrobial effects of sorbates may vary depending on multiple factors in foods including pH, water activity (a_w), initial microbial flora and other additives used ⁶. Potassium sorbate is permitted to be used in dairy products up to 1000 mg/kg concentration according to Turkish Food Codex ⁷. There are some studies investigating the effects of potassium sorbate in some dairy products which is popular in Turkey including white cheese, kashar cheese and yoghurt ^{8,9}. At present, no published study was found on use of sorbates for preservation of cokelek. There is also no standard for cokelek in Turkey.

The aim of this study was to investigate (i) the minimum concentration of potassium sorbate that is needed to limit the growth of yeast and mold in cokelek (ii) the effect of potassium sorbate on microbiological quality, pH and acidity levels of cokelek stored at 4 ± 1 or $22\pm 1^\circ\text{C}$.

MATERIAL and METHODS

Preparation of Cokelek

Experimental cokelek samples were produced from yoghurt at our laboratory. Briefly, yoghurt was diluted 1/1 ratio with tap water and heated to 90°C . After it was cooled to room temperature, it was transferred to cloth bags and hanged for overnight for removal of excess fluid. Then, it was kept under pressure for a short time (ca. 1 h) to reduce water content. The resulting curd was called as crude cokelek.

Adding Potassium Sorbate

Crude cokelek was divided into 4 equal portions for treatment groups. Treatment groups were called as group A with 2% salt (control), group B with 2% salt + 10 mg/kg potassium sorbate, group C with 2% salt + 100 mg/kg potassium sorbate, and group D with 2% salt + 1000 mg/kg potassium sorbate. A 100 g portion of cokelek samples in each group were placed into styrofoam plates and wrapped with saran film. The resulting packages in each group were further divided

into two groups, and then, they were stored at $4\pm 1^\circ\text{C}$ or $22\pm 1^\circ\text{C}$.

Microbiological Analyses

The samples were taken from crude cokelek, and on days 0, 5, 15, 25, 35, 45, 55, 65 and 75 during storage period of cokelek for microbiological and chemical analyses. A 10 g sample from each package was aseptically transferred to sterile stomacher bag and 90 ml $\frac{1}{4}$ Ringer solution was added before homogenizing for 2 min using a stomacher (BagMixer[®] 400, Interscience). Serial dilutions were prepared from the 10^{-1} dilution. Bacterial populations were enumerated using Plate Count Agar (Oxoid) for total aerobic mesophilic bacteria after incubation at $30\pm 1^\circ\text{C}$ for 72 h, Violet Red Bile Agar (Oxoid) for coliforms after incubation at $30\pm 1^\circ\text{C}$ for 24 h, Ragosa Acetate Agar for *Lactobacillus- Leuconostoc- Pediococcus* after incubation at $30\pm 1^\circ\text{C}$ for 5 days, Thallous Acetate Tetrazolium Glucose Agar (TITA) of Barnes for *Enterococcus spp.* after incubation at $45\pm 1^\circ\text{C}$ for 48 h ¹⁰, Potato Dextrose Agar (Oxoid) with pH 3.5 (acidified with 10% tartaric acid) for yeast and mold after incubation at $21\pm 1^\circ\text{C}$ for 5 days ¹¹, Mannitol Salt Agar (Oxoid) for *Staphylococcus- Micrococcus* after incubation at $37\pm 1^\circ\text{C}$ for 36-48 h ¹².

Chemical Analyses

The pH values of samples were recorded by using a pH meter (pH 2001, Selecta). Titratable acidity as %lactic acid was determined in accordance using method described for yoghurt by Turkish Standards Institute (TS 1330) ¹³.

Statistical Analyses

The study was composed of three independent replicates. The numbers of bacteria were converted to \log_{10} cfu/g before calculating means and performing statistical analyses. The data were analyzed by analysis of variance (ANOVA) for main (fixed) effects (treatment, storage temperature, and sampling days) and three way interactions between treatments, storage temperature and sampling days by using Statistical Analysis System ¹⁴. The means were separated by using Fisher's Least Significant Differences (LSD) test according to General Linear Model (GLM) procedures. Statistical significant level was accepted as 5% ($P < 0.05$).

RESULTS

The data for microbiological analyses of cokelek stored at $4\pm 1^\circ\text{C}$ or $22\pm 1^\circ\text{C}$ were presented in [Table 1](#) and [2](#), respectively. The data for pH level and titratable acidity of cokelek samples during storage at $4\pm 1^\circ\text{C}$ or $22\pm 1^\circ\text{C}$ were showed in [Fig. 1, 2, 3](#) and [4](#), respectively.

Table 1. Effects of various potassium sorbate levels on viability of some microorganisms in cokelek during storage at 4±1°C (log₁₀ cfu/g)**Tablo 1.** Çeşitli potasyum sorbat düzeylerinin 4±1°C'de muhafaza edilen çökeleklere bazı mikroorganizmaların yaşamı üzerine etkisi (log₁₀ cfu/g)

Microorganisms	Crude Cokelek	Groups	Storage Days							
			0	5	15	25	35	45	55	65
Total Aerobic Mesophilic Bacteria	6.90	A	7.89 ^{az}	6.98 ^{az}	7.94 ^{az}	7.98 ^{az}	8.05 ^{az}	-	-	-
		B	7.27 ^{az}	6.50 ^{az}	6.86 ^{az}	7.14 ^{az}	7.49 ^{az}	8.91 ^{az}	-	-
		C	7.72 ^{az}	6.50 ^{az}	6.64 ^{az}	6.66 ^{az}	7.88 ^{az}	8.02 ^{az}	8.65 ^{az}	-
		D	7.77 ^{az}	6.11 ^{az}	6.54 ^{az}	6.57 ^{az}	7.48 ^{az}	7.71 ^{az}	7.76 ^{az}	8.02 ^a
Coliform	1.00	A	1.67 ^{az}	1.49 ^{az}	1.67 ^{az}	1.33 ^{az}	1.00 ^{az}	-	-	-
		B	2.09 ^{az}	1.69 ^{az}	1.63 ^{az}	1.56 ^{az}	1.12 ^{az}	<1.00 ^{az}	-	-
		C	1.00 ^{az}	1.72 ^{az}	1.45 ^{az}	1.30 ^{az}	<1.00 ^{az}	<1.00 ^{az}	<1.00 ^{az}	-
		D	1.00 ^{az}	1.51 ^{az}	1.33 ^{az}	1.20 ^{az}	<1.00 ^{az}	<1.00 ^{az}	<1.00 ^{az}	<1.00 ^a
Lactobacillus-Leuconostoc-Pediococcus	5.99	A	7.98 ^{az}	7.28 ^{az}	6.86 ^{az}	7.03 ^{az}	7.04 ^{az}	-	-	-
		B	6.06 ^{az}	7.54 ^{az}	6.43 ^{az}	6.15 ^{az}	6.60 ^{az}	6.15 ^{az}	-	-
		C	6.89 ^{az}	7.18 ^{az}	5.48 ^{az}	5.28 ^{az}	6.04 ^{az}	5.91 ^{az}	6.85 ^{az}	-
		D	7.06 ^{az}	7.04 ^{az}	6.30 ^{az}	5.61 ^{az}	5.64 ^{az}	5.90 ^{az}	6.33 ^{az}	7.03 ^a
Staphylococcus-Micrococcus	1.83	A	2.83 ^{az}	2.33 ^{az}	2.02 ^{az}	1.92 ^{az}	2.38 ^{az}	-	-	-
		B	2.10 ^{az}	2.03 ^{az}	1.59 ^{az}	2.10 ^{az}	2.32 ^{az}	2.13 ^{az}	-	-
		C	3.06 ^{az}	1.99 ^{az}	1.62 ^{az}	1.69 ^{az}	2.30 ^{az}	2.02 ^{az}	1.82 ^{az}	-
		D	2.66 ^{az}	2.02 ^{az}	1.65 ^{az}	1.64 ^{az}	2.01 ^{az}	1.49 ^{az}	1.68 ^{az}	1.10 ^a
Enterococcus	1.85	A	1.85 ^{azy}	1.51 ^{az}	2.02 ^{az}	2.03 ^{az}	1.32 ^{az}	-	-	-
		B	2.19 ^{az}	1.00 ^{bz}	1.59 ^{az}	1.36 ^{abz}	1.01 ^{bz}	<1.00 ^{bz}	-	-
		C	1.77 ^{azy}	1.00 ^{az}	1.62 ^{az}	1.70 ^{az}	1.43 ^{az}	<1.00 ^{az}	<1.00 ^{az}	-
		D	<1.00 ^{ay}	<1.00 ^{az}	1.65 ^{az}	<1.00 ^{az}	<1.00 ^{az}	<1.00 ^{az}	<1.00 ^{az}	<1.00 ^a
Yeast and Mold	5.07	A	5.77 ^{az}	6.86 ^{az}	6.61 ^{az}	7.01 ^{az}	6.89 ^{az}	-	-	-
		B	6.24 ^{az}	6.25 ^{az}	6.25 ^{az}	6.07 ^{azy}	5.92 ^{azy}	5.73 ^{az}	-	-
		C	5.79 ^{az}	5.97 ^{az}	5.68 ^{az}	5.39 ^{azy}	5.20 ^{azy}	4.96 ^{az}	4.81 ^{az}	-
		D	5.38 ^{az}	5.49 ^{az}	5.10 ^{az}	4.83 ^{ay}	4.74 ^{ay}	4.64 ^{az}	4.35 ^{az}	4.19 ^a

- : Not analyzed due to spoilage of the product, **a,b**: Means with the different superscripts within the same row are significantly different (P<0.05), **z,y**: Means with the different superscripts within the same column are significantly different (P<0.05)

Table 2. Effects of various potassium sorbate levels on viability of some microorganisms in cokelek during storage at 22±1°C (log₁₀ cfu/g)**Tablo 2.** Çeşitli potasyum sorbat düzeylerinin 22±1°C'de muhafaza edilen çökeleklere bazı mikroorganizmaların yaşamı üzerine etkisi (log₁₀ cfu/g)

Microorganisms	Crude Cokelek	Groups	Storage Days			
			0	5	15	25
Total Aerobic Mesophilic Bacteria	6.90	A	7.89 ^{az}	8.62 ^{az}	-	-
		B	7.27 ^{az}	8.04 ^{az}	-	-
		C	7.72 ^{az}	8.26 ^{az}	-	-
		D	7.77 ^{az}	8.66 ^{az}	7.67 ^a	8.11 ^a
Coliform	1.00	A	1.67 ^{az}	2.32 ^{az}	-	-
		B	2.09 ^{az}	2.24 ^{az}	-	-
		C	1.00 ^{az}	2.41 ^{bz}	-	-
		D	1.00 ^{az}	2.40 ^{bz}	1.62 ^{ab}	1.42 ^a
Lactobacillus-Leuconostoc-Pediococcus	5.99	A	7.98 ^{az}	7.52 ^{az}	-	-
		B	6.06 ^{az}	7.00 ^{az}	-	-
		C	6.89 ^{az}	6.91 ^{az}	-	4.94 ^b
		D	7.06 ^{az}	6.34 ^{az}	5.25 ^{ab}	-
Staphylococcus-Micrococcus	1.83	A	2.83 ^{az}	2.34 ^{az}	-	-
		B	2.10 ^{az}	2.01 ^{az}	-	-
		C	3.06 ^{az}	1.83 ^{az}	-	-
		D	2.66 ^{az}	2.40 ^{az}	1.87 ^a	1.47 ^a
Enterococcus	1.85	A	1.85 ^{azy}	1.73 ^{az}	-	-
		B	2.19 ^{az}	1.62 ^{az}	-	-
		C	1.77 ^{azy}	1.48 ^{az}	-	-
		D	<1.00 ^{ay}	<1.00 ^{ay}	<1.00 ^a	<1.00 ^a
Yeast and Mold	5.07	A	5.77 ^{az}	7.46 ^{az}	-	-
		B	6.24 ^{az}	6.78 ^{az}	-	-
		C	5.79 ^{az}	5.67 ^{az}	-	-
		D	5.38 ^{az}	6.50 ^{az}	4.68 ^a	5.36 ^a

- : Not analyzed due to spoilage of the product, **a,b**: Means with the different superscripts within the same row are significantly different (P<0.05), **z,y**: Means with the different superscripts within the same column are significantly different (P<0.05)

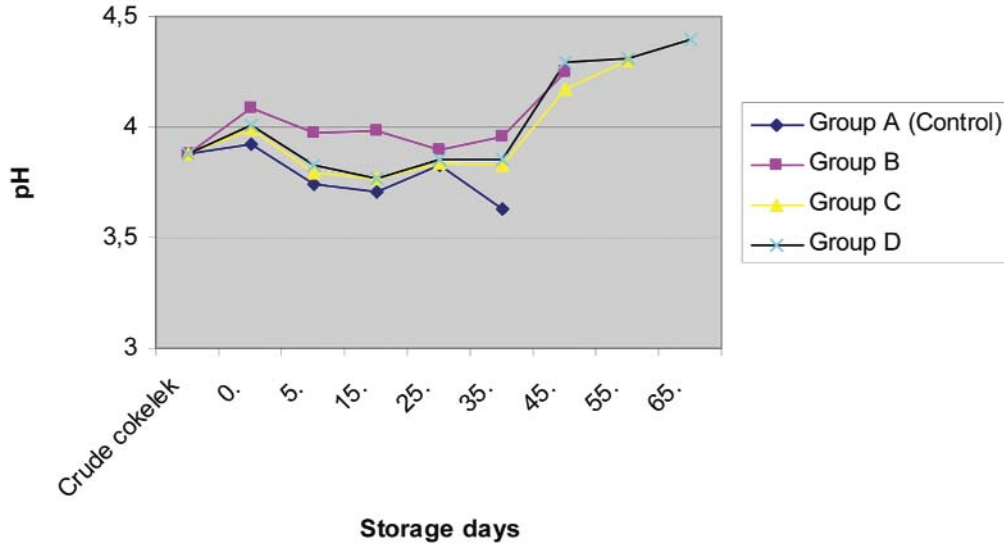


Fig 1. Effects of various potassium sorbate levels on pH levels in cokelek during storage at $4\pm 1^\circ\text{C}$

Şekil 1. Çeşitli potasyum sorbat düzeylerinin $4\pm 1^\circ\text{C}$ 'de muhafaza edilen çökeleklerin pH düzeyleri üzerine etkisi

Fig 2. Effects of various potassium sorbate levels on % acidity levels in cokelek during storage at $4\pm 1^\circ\text{C}$

Şekil 2. Çeşitli potasyum sorbat düzeylerinin $4\pm 1^\circ\text{C}$ 'de muhafaza edilen çökeleklerin % asitlik düzeyleri üzerine etkisi (laktik asit cinsinden)

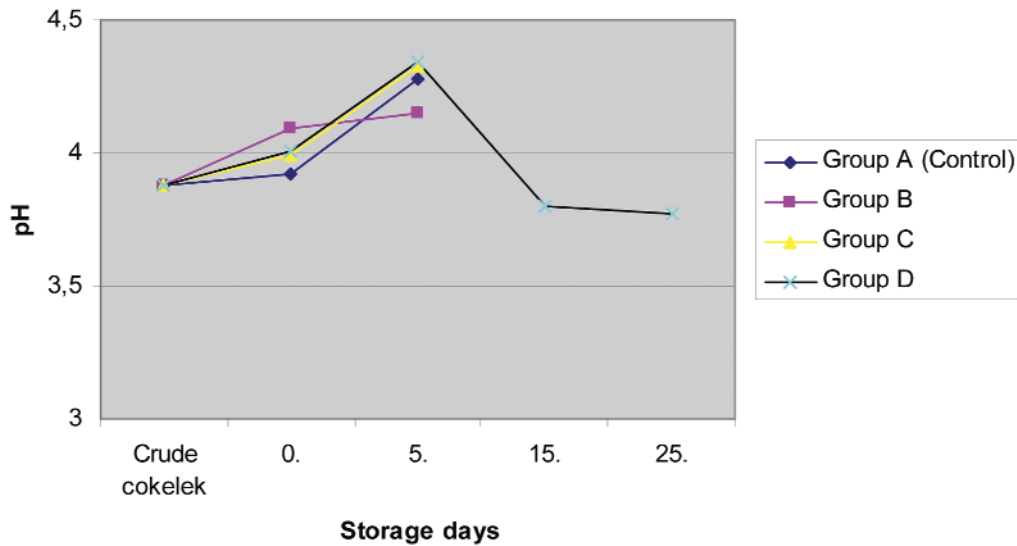
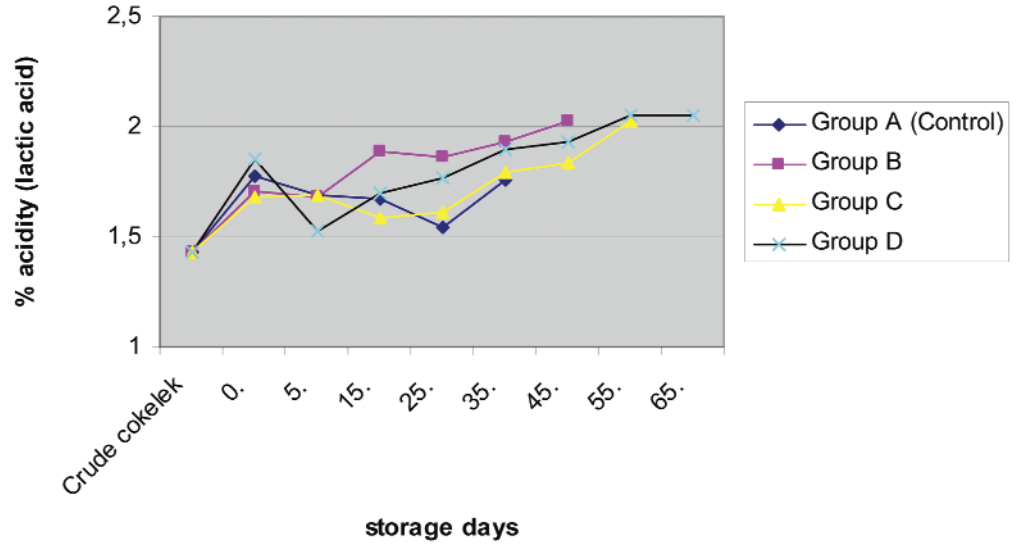
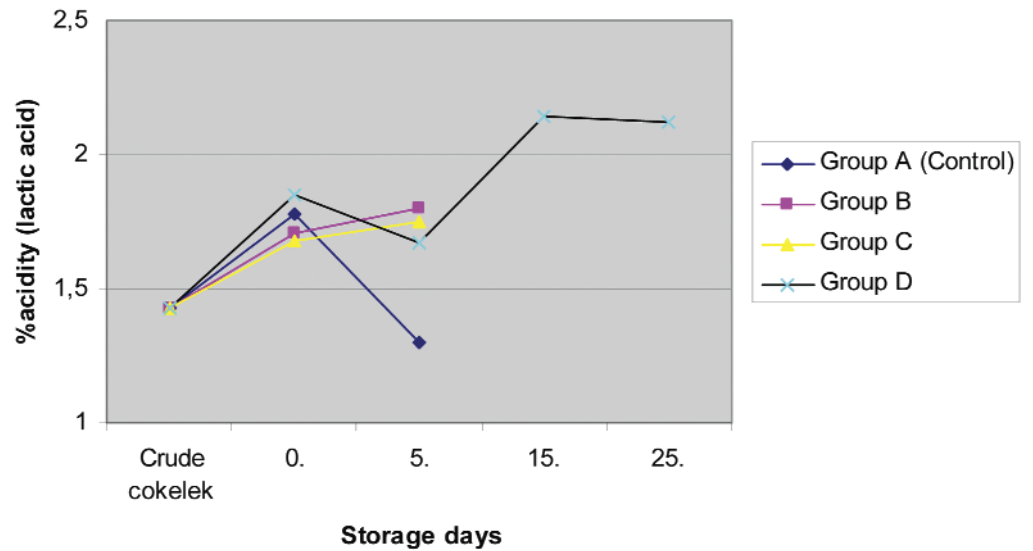


Fig 3. Effects of various potassium sorbate levels on pH levels in cokelek during storage at $22\pm 1^\circ\text{C}$

Şekil 3. Çeşitli potasyum sorbat düzeylerinin $22\pm 1^\circ\text{C}$ 'de muhafaza edilen çökeleklerin pH düzeyleri üzerine etkisi

Fig 4. Effects of various potassium sorbate levels on % acidity levels in cokelek during storage at $22\pm 1^\circ\text{C}$

Şekil 4. Çeşitli potasyum sorbat düzeylerinin $22\pm 1^\circ\text{C}$ 'de muhafaza edilen çökeleklerin % asitlik düzeyleri üzerine etkisi (laktik asit cinsinden)



DISCUSSION

When it was inspected the effects of potassium sorbate with different concentration on counts of total aerobic mesophilic bacteria (TAMB) of cokelek samples within the same storage group (Table 1 and 2), it was not seen significant differences during their storage period ($P>0.05$). After treatment groups were prepared, TAMB increased in all groups on day 0 of storage period. And then, the numbers showed regular increase during the storage period and were found to be $8.05 \log_{10}$ for control group on day 35, $8.91 \log_{10}$ for group B on day 45, $8.65 \log_{10}$ for group C on day 55 and $8.02 \log_{10}$ for group D on day 65 of storage period at $4\pm 1^\circ\text{C}$ (Table 1). Numbers of TAMB, in all samples stored at $22\pm 1^\circ\text{C}$, were found to be above $8 \log_{10}$ on day 5 of storage (Table 2). Lack of significant effect of potassium sorbate on TAMB has been supported by the results of previous studies carried out in white cheese and kashar cheese ^{8,9,15}. However, it should be emphasized that there are other studies reporting that potassium sorbate had been found to be significant effect on TAMB ¹⁶⁻¹⁸. This discrepancy may be due to the different material and the composition of flora.

Level of coliform bacteria insignificantly increased in group A (control) and group B on day 0, and group C and group D on day 5 of storage period at $4\pm 1^\circ\text{C}$ (Table 1). After day 15 of storage period, coliform numbers continuously decreased in all groups stored at $4\pm 1^\circ\text{C}$ during the remaining period of storage. Their numbers dropped below detection limit on day 35 in group C and D, on day 45 in group B. Numbers of coliforms in samples stored at $22\pm 1^\circ\text{C}$ increased on day 5 of storage. After day 5, coliform numbers in group D continuously

decreased and was found to be $1.42 \log_{10}$ on day 25 of storage (Table 2). In a study ¹⁵ carried out on kashar cheese noted that the potassium sorbate at 500 mg/kg concentration in the cheese samples decreased coliform bacteria counts. However, it was not seen statistical analyses on effect of potassium sorbate on coliform bacteria in the study mentioned. In the present study, when the results related to coliform bacteria counts were inspected, it was seen a decrease their counts in all groups during the storage period, not excepting control group. However, no significant differences were observed between treatment groups within the same storage group or the different storage group ($P>0.05$). There have not been sufficient literatures about the antimicrobial effect of potassium sorbate on growth of coliform bacteria.

The numbers of *Lactobacillus - Leuconostoc - Pediococcus*, in all groups stored at $4\pm 1^\circ\text{C}$, decreased until on day 25 of storage period, and then continuously increased at the remaining period of storage (Table 1). No significant differences were observed between treatment groups during storage period ($P>0.05$). These results are in agreement with findings of other researchers ^{8,15,17} who reported that inhibitory effect of potassium sorbate on this group of microorganisms was very limited ($P>0.05$). When it was inspected this group of bacteria in samples stored at $22\pm 1^\circ\text{C}$, however, their numbers continually decreased in group D (1000 mg/kg potassium sorbate) during storage time of 25 days, and it was seen significant difference between days in this group of cokelek (Table 2). This result may be due to more decrease in pH level (pH 3.8) or increase in titratable acidity (2.14% as lactic acid) in this group on day 15 of storage (Fig. 3 and 4).

The numbers of *Stapylococcus-Micrococcus* was lower in products treated with higher concentration of potassium sorbate and stored at $4\pm 1^\circ\text{C}$ (Table 1). Statistical analyses showed that the numbers of these bacteria were not significantly different between the treatment groups ($P>0.05$). Moreover, changes in the numbers of these organisms within individual groups during storage indicates that potassium sorbate was not effective on *Stapylococcus-Micrococcus* ($P>0.05$) (Table 1 and 2). Our results are contradictory to the results reported by Nizamlioglu et al ⁹. This difference can possibly be explained by the use of higher concentration potassium sorbate (1%, 2%, 3%) in their study.

The numbers of enterococci decreased in all treatment groups until day 5 of the storage. In those products stored at $4\pm 1^\circ\text{C}$, enterococci numbers increased until day 10, and then showed a decrease during the remaining period of the storage. Moreover, their number dropped below detection limit in groups C and B on day 45. In group D in which potassium sorbate was used at high level, no *enterococcus spp.* was recovered during the storage, even on day 0 (Table 1). In products stored at $22\pm 1^\circ\text{C}$, numbers of enterococci decreased on day 5 of storage and as expected, no enterococci were recovered in group D during storage period. Numbers of enterococci were lower in cokelek containing potassium sorbate at 0.05% or 0.1% than control groups ($P<0.05$) (Table 1 and 2). This may indicate that potassium sorbate might be effective on survival of this group of bacteria, as previously reported by Dogruer et al ⁸.

The numbers of yeast and mold was initially 5.07 log cfu/g in crude cokelek, and it was generally lower in groups containing potassium sorbate compared to control group (Table 1 and 2). The numbers of yeast and mold was lower in product treated with higher concentration of potassium sorbate (group D) during storage period at $4\pm 1^\circ\text{C}$. It was also observed that the numbers of yeast and mold continuously decreased in groups containing potassium sorbate as storage periods increased (Table 1). No significant differences were observed between days ($P>0.05$) during storage period of products at $4\pm 1^\circ\text{C}$, and there were no significant differences between groups until day 25 of storage. On day 25 and 35 of storage, significant differences were observed between group D and control group depending on continuously decrease of the numbers of yeast and mold in the group D treated with higher level of potassium sorbate during storage period ($P<0.05$) (Table 1). Our findings are in agreement with findings of other researchers who studied on the effect of potassium sorbate on yeast and mold in different products ^{5,18,19}. Statistical analyses, however, showed that potassium

sorbate was not effective on yeast and mold in cokelek stored at $22\pm 1^\circ\text{C}$ ($P>0.05$), even though in higher concentration such as 1000 ppm (Table 2). It has been noted that antimicrobial effect of potassium sorbate increases as the storage temperature decrease ^{17,20}. This information may explain, in the present study, why potassium sorbate had no effect on yeast and mold in cokelek stored at $22\pm 1^\circ\text{C}$.

The data for pH and acidity were presented in Fig. 1-4. pH levels in crude cokelek was 3.9, this level insignificantly decreased to 3.7 until on day 15 of storage, and then increased to 4.2 for group B on day 45, 4.3 for group C on day 55 and 4.4 for group D on day 65 of storage at $4\pm 1^\circ\text{C}$ (Fig. 1). pH level of control group was detected 3.6 on day 35 of storage. Acidity level as lactic acid was 1.43 in crude cokelek. This level increased to 1.76 for control group on day 35, 2.03 for group B and C on day 45 and 55, 2.05 for group D on day 65 of storage at $4\pm 1^\circ\text{C}$ (Fig. 2). As for samples stored at $22\pm 1^\circ\text{C}$, pH levels of samples increased to 4.2-4.3 for all groups on day 5. After day 5, pH level of group D decreased to 3.7 on day 25 of storage (Fig. 3). Acidity levels of these samples were found to be 1.3% for control, 1.8% for group B and C, 1.7% for group D on day 5. After day 5, acidity level of group D increased to 2.1 on day 25 of storage (Fig. 4). Statistical analyses showed that data obtained from samples were not significantly different ($P>0.05$) between control and treatment groups depending on temperature and period of storage.

Determining the right concentration of a preservative to be used in a food is not easy. The type of the food, the initial microbiological load, and conditions of processing and storage are important factors that effect the concentration of the preservative to be used ⁵. In the present study, all findings showed that application of potassium sorbate at concentration of 100, 500 and 1000 ppm did not have an effect on numbers of total mesophilic aerobic bacteria, coliform bacteria, *lactobacillus- leuconostoc- pediococcus* and *staphylococcus-micrococcus* in cokelek stored at both $4\pm 1^\circ\text{C}$ and $22\pm 1^\circ\text{C}$. However, potassium sorbate of 500 and 1000 ppm had an effect on numbers of enterococci and yeast-mold in cokelek stored at $4\pm 1^\circ\text{C}$, not $22\pm 1^\circ\text{C}$. It was also observed that shelf life of cokelek was depending on the level of potassium sorbate added and storage temperature. However, due to cokelek is produced under unmechanized or artisanal conditions in small dairy plants or on farms; it is difficult to find a standard product with respect to composition and microbiological quality of cokelek. Therefore, it is needed further research on suitable preservatives on cokelek.

REFERENCES

1. **Öksüztepe GA, Patir B, Dikici A, Bozkurt Ö, Çalicioğlu M:** Microbiological and chemical quality of cokelek marketed in Elazığ. *Fırat Univ Sađ Bil Derg*, 21 (1): 27-31, 2007.
2. **Kıvanç M:** Gıda koruyucusu olarak sorbik asit ve tuzları: I. Genel özellikleri. *Gıda*, 14 (5): 315-320, 1989.
3. **Marth EH, Constance MC, Hasenzahl L, Jackson HW, Hussong RV:** Degradation of potassium sorbate by *Penicillium* species. *J Dairy Sci*, 49 (10): 1197- 1205, 1966.
4. **Han JH, Floros JD:** Potassium sorbate diffusivity in American processed and Mozzarella cheeses. *J Food Sci*, 63 (3): 435-437, 1998.
5. **Mihyar GF, Yamani MI, Al-Sa'ed AK:** Resistance of yeast flora of Labaneh to potassium sorbate and sodium benzoate. *J Dairy Sci*, 80 (10): 2304-2309, 1997.
6. **Campos CA, Gerchenson LN:** Effect of certain additives on sorbate stability. *Food Res Int*, 29 (2): 147-154, 1996.
7. **Turkish Food Codex:** Gıda katkı maddeleri sıralı EC numaraları kullanım dozu ve özel şartları, Ek 1-13. Ministry of Agriculture and Rural Affairs, Ankara, 2000.
8. **Doğruer Y, Gürbüz Ü, Nizamlioğlu M:** Potasyum sorbatın beyaz peynirin kimyasal ve mikrobiyolojik kalitesine etkisi. *Selçuk Univ Vet Bil Derg*, 12 (1): 109-116, 1996.
9. **Nizamlioğlu M, Gürbüz Ü, Doğruer Y:** Potasyum sorbatın kaşar peynirin kimyasal ve mikrobiyolojik kalitesine etkisi. *Selçuk Univ Vet Bil Derg*, 12 (2): 23-29, 1996.
10. **Harrigan WF:** Laboratory methods in food microbiology. 3rd ed. Academic Press, London, 1998.
11. **Oxoid:** The Oxoid Manuel. 50th ed. Published by Oxoid Ltd, Hampshire, 1982.
12. **British Standards Institution:** Methods of microbiological examination for dairy purposes. BS 4285. British Standards Institution, London, 1968.
13. **Turkish Standards Institute:** Yoğurt. TS1330. Ankara, 2006.
14. **Statistical Analyses System:** SAS, Inst. Inc. Cary. 8. Version, North Caroline, 1999.
15. **Ozdemir C, Demirci M:** Selected microbiological properties of kashar cheese samples preserved with potassium sorbate. *Int J Food Prop*, 9, 515-521, 2006.
16. **Bradley RL, Harmon LG, Stine CM:** Effect of potassium sorbate on some organisms associated with cottage cheese spoilage. *J Milk and Food Techn*, 25, 318-323, 1962.
17. **Sofos JN, Busta FF:** Antimicrobial activity of sorbate. *J Food Protect*, 44 (8): 614-622, 1981.
18. **Yılmaz L, Kurdal E:** Effect of sorbic acid and potassium sorbate addition to the brine on microbiological and chemical properties of Turkish white cheese during ripening. *Food Sci Technol Res*, 14 (5): 437-444, 2008.
19. **Aly ME:** Prolongation of the keeping quality of mozzarella cheese by treatment with sorbate. *Nahrung*, 4, 194-200, 1996.
20. **Gram L:** Inhibition of mesophilic spoilage *Aeromonas spp.* on fish by salt, potassium sorbate, liquid smoke and chilling. *J Food Protect*, 54 (6): 436-442, 1991.