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# Some Microbiological Properties of Kefir Yogurts Produced by Using Milk Powder and Whey Powder

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## ABSTRACT

The purpose of this research is to evaluate the recycling of whey and gaining cost using demineralized whey powder as a dry additive instead of or in combination with milk powder in the food sector and to emphasize the importance of kefir in terms of health by increasing interest in kefir yogurt. Therefore, kefir yogurts produced by using different proportions of skim milk powder and demineralized whey powder as three replicates (A (control): kefir yogurt produced using 0% demineralized whey powder and 3% milk powder, B: kefir yogurt produced using %1 demineralized whey powder and 2% milk powder, C: kefir yogurt produced using 2% demineralized whey powder and 1% milk powder, D: kefir yogurt produced using 3% demineralized whey powder and 0% milk powder). Some chemical properties of milk powder and whey powder used in the production were determined, pH and titration acidity analyses of samples were carried out during incubation and microbiological properties were determined during storage. According to the results obtained, during the incubation, the kefir yogurt; pH and titration acidity values of kefir yogurt were significantly influenced by the incubation time ($p<0.05$) while the effects of milk powder and demineralized whey powder were not significant ($p>0.05$) on titration acidity and pH values. Storage time affected statistically significant effects ($p<0.05$) on total aerobic mesophilic bacteria and total yeast count while the effects of milk powder and demineralized whey powder were not significantly ($p>0.05$) on microbiological properties of kefir yogurt.

**Keywords:** Kefir yogurt, microbiological properties, milk powder, whey powder

## 1. INTRODUCTION

According to the Turkish Food Codex Fermented Dairy Products Report, kefir is stated as fermented milk product that starter cultures contain specifically strains of *Lactobacillus* kefir, *Leuconostoc, Lactococcus* and *Acetobacter* species and yeasts which are fermented (*Kluyveromyces marxianus*) and non-fermented (*Saccharomyces unisporus, Saccharomyces cerevisiae* and *Saccharomyces exiguus*) lactose or in which kefir grains are used (Anon, 2009).

Kefir, a Caucasian origin fermented milk product, the most distinctive feature from other fermented milk product is due to symbiotic activity of bacteria and yeast in kefir grains, combination with lactic acid and alcoholic fermentation is carried out together (Dinç, 2008; Assadi *et al.,* 2000; Yilmaz *et al*., 2006).

The lactic acid bacteria, yeast and polysaccharide (kefiran), which form the kefir grains, give kefir unique properties in a symbiotic relationship (Beshkova *et al.*, 2002). Furthermore, thanks to the lactic acid, acetaldehyde, acetoin, ethanol and other fermentation by-products obtained from the diversity of this microorganism, kefir is a relaxing fermented product with unique sensory properties (Ertekin and Güzel-Seydim, 2010; Esmek and Güzeler, 2015). After fermentation, kefir grains are used again for the next milk by healing itself (Karatepe and Yalçın, 2014).

It has been reported that the most important feature of the kefir grains is the filtration and re-use at the end of fermentation (Yüksekdağ and Beyatlı, 2003). Besides the kefir produced from kefir grains, industrial kefirs produced as starter culture are sold as ready for consumption (Dinç, 2008).

**Table 1.** Microbiological properties of kefir yogurts (Farnworth, 2006)

| **Product** | **Lactococcus (log cfu/g)** | **Laktobacillus (log cfu/g)** | **Yeast (log cfu/g)** |
|---|---|---|---|
| Kefir grain | 7.37 | 8.94 | 8.30 |
| Kefir mother culture | 8.43 | 7.65 | 5.58 |
| Kefir drink | 8.54 | 7.45 | 5.24 |

Kefir grains have a gelatinous and irregular structure with a diameter ranging from 1 to 6 mm. When looking at the structure of these grains, they contain lactic acid bacteria (*lactobacilli, Lactococci, leuconostocs*), acetic acid bacteria, and yeast mixture, which is important due to the production of ethanol and carbon dioxide during fermentation, combined with casein and complex sugars by a matrix of polyssacharide (Iriyogen *et al*., 2005).

According to Turkish Food Codex the Report on Fermented Dairy Products, yogurt is expressed as a fermented milk product, which is specifically used in fermentation, using symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Anon, 2009).

Compared to kefir and yogurt, yogurt-containing bacteria keep the digestive system clean and provide nutrients for other beneficial organisms that are host there, while kefir also has the property of colonizing the digestive system, which yogurt does not have (Anon, 2012).

In order to make yogurt more attractive, kefir culture, which is added to the milk in certain proportions, is left to incubate at 41-43 ºC and kefir yogurt is produced (Esmek and Güzeler, 2015).

The critical step in the process of yogurt production is to increase the total fat-free dry matter content of the milk and the main purpose of this process is to obtain the desired physical and sensory properties in the final product and to provide a great deal of consumer appreciation. Many methods (boiling, milk powder additive, buttermilk powder additive, whey powder or concentrate, caseinate additive, evaporation and membrane techniques) are used in the fat-free dry matter content of the milk (Özer, 2006).

Because of the problems caused by the minerals contained in the raw material and because these substances have a negative effect on the drying process and the end product, demineralization of the whey must be separated from its minerals by special filtration techniques. Demineralized whey powder can also be used partially in place of skimmed milk powder and therefore the cost of some products can be reduced (Üçüncü, 2004).

The aim of this research is to use demineralized whey powder in order to eliminate salty taste, to provide cost saving and utilizing whey by using demineralized whey powder instead of milk powder or collocation of both powder in food sector and to emphasize the importance of kefir in terms of health by increasing interest kefir yogurt. For this purpose, compositions of demineralized whey powder and skimmed milk powder were determined, pH and titration aciditiy of samples were analysed during the incubation period and microbiological analyses of kefir yogurt produced by using different proportions of demineralized whey powder and skimmed milk powder were performed.

## 2. MATERIAL AND METHODS

### *2.1 Material*

In the production of kefir yogurt using demineralized whey powder and skimmed milk powder, cow milk provided from Cukurova University Faculty of Agriculture Research Application Farm Livestock Branch was used. Kefir grains which were used in pre-production and stored frozen were used in culture production after being activated. In this study the 70% demineralized whey powder was provided from Ekso Milk and Food Product Industry and Trade Incorporated Company and skimmed milk powder from Bakkalbaşıoğlu Dairy Products Industry and Trade Incorporated Company. For packaging of kefir yogurt produced by using demineralized whey powderand skimmed milk powder, 200 g capped polypropylene boxes were obtained from Cukurova University Faculty of Agriculture Research Application Farm Livestock Branch.

### *2.4 Method*

This research was carried out in the Milk Technology Research Laboratory of the Food Engineering Department of the Faculty of Agriculture at Cukurova University. As a result of preliminary trials, kefir was found to be 9% as starter culture which was used in the production of kefir yogurt.

In the production of kefir yogurts produced using different proportions of demineralized whey powder; milk is standardized after necessary controls are made. Cow milk is divided into four parts: 0% demineralized whey powder, 3% milk powder to sample A, 1% demineralized whey powder, 2% milk powder to sample B, 2% demineralized whey powder, 1% milk powder to C sample, 3% demineralized whey powder,% 0 milk powder to sample D was added and each was heat treated at 90ºC for 5 minutes. The milk cooled to 42 ± 1ºC was fermented at 9% with kefir culture. Milk, that is filled containers, was incubated at 41 ± 1ºC until the pH reached 4.7, and it was cooled at 4 ± 1ºC at the end of the incubation.

Protein, titratable acidity (in terms of lactic acid), dry matter (by gravimetric method), fat, ash (by gravimetric method) determination (Oysun, 1991) and lactose ratio calculation method were performed in skimmed milk powder and 70% demineralized whey powder.

In incubation samples, according to Cemeroğlu (1992) pH determination was performed by using Testo 230 brand pH meter and titration acidity (in terms of lactic acid) (TSE, 2002; TSE, 2006) was determined during incubation at certain time intervals.

The total mesophilic aerobic bacteria count of the yogurt samples was incubated in aerobic conditions at 30 ºC for 2 days using Plate Count Agar (PCA, Merck) and all of the colonies grown in petri plates were evaluated by counting. In the yeast and mold count, the yogurt samples were incubated at 25 ºC for 5 days at aerobic conditions using Potato Dextrose Agar (PDA) and the colonies grown in petri plates were calculated by counting. In lactic acid bacteria count, yogurt samples were incubated in anaerobic conditions at 37 ºC for 2 days using de Man Ragosa Sharpe Agar (MRS, Merck) and all of the colonies grown in petri plates were evaluated by counting (Keleş, 2003).

Statistical analysis were performed using SPSS package program according to paket "Randomized Parcel Trial Plan". Duncan multiple comparison test was used to compare the means (Düzgüneş *et al*., 1987).

## 3. RESULTS AND DISCUSSION

In this section, the compositions of the demineralized whey powder and skimmed milk powder, which were used in production of kefir yogurts, were investigated. Some changes of kefir yogurts were analysed during the incubation period. The microbiological properties of kefir yogurts were investigated during storage. The effects of different proportions of demineralized whey powder on the properties of kefir yogurt were discussed and the results were statistically evaluated and the results were compared with the similar studies.

**Table 2.** The compositions of the 70% demineralized whey powder and skimmed milk powder (n=3)

| Properties | Skimmed Milk Powder | 70% Demineralized Whey Powder |
|---|---|---|
| **Titration Acidity (% lactic acid)** | 0.17±0.01 | 0.14±0.01 |
| **Dry matter (%)** | 95.12±0.16 | 96.23±0.25 |
| **Fat (%)** | 0.93±0.11 | 0.97±0.06 |
| **Protein (%)** | 35.33±0.58 | 7.00±0.30 |
| **Ash (%)** | 7.03±0.03 | 4.50±0.30 |
| **Lactose (%)** | 51.82±0.28 | 83.77±0.25 |

As shown in Table 2, skimmed milk powder used in the production of kefir yogurt; Considering the Turkish Food Codex Report on Condensed Milk and Milk Powder, the fat content and moisture content of milk powder were found to be appropriate according to the values in this report (Anon, 2005). In addition, it is stated that the protein content of the milk powder is between 34.0% and 37.0%, the lactose ratio is between 49.5% and 52.0% and the ash rate is between 8.20% and 8.60% (Özer, 2006). According to the Turkish Food Codex Cheese Report, the moisture content of the whey powder must be at most 5%. The moisture content according to the dry matter value corresponds to the report (Anon, 2015). According to some studies, the protein value of whey powder was low (Hafıza, 2000; Güven and Karaca, 2003). The reason for low protein value is thought to be related to production of raw materials and powder products.

The incubation was completed in all samples in an average of 330 minutes. One-way analysis of variance of pH and titration acidity results of incubation samples and changes in incubation period are given in Table 3, Table 4 and the graphs formed by these values are given in Figure 1, Figure 2.

**Table 3.** pH values of kefir yogurts during the incubation period (n=3)

| Incubation Time (min.) | Kefir Yogurts | | | |
|---|---|---|---|---|
| | **A** | **B** | **C** | **D** |
| 0 | 6.11±0.02$^{aK}$ | 6.12±0.04$^{aK}$ | 6.15±0.03$^{aK}$ | 6.17±0.02$^{aK}$ |
| 45 | 6,04±0.06$^{aK}$ | 6.05±0.06$^{aK}$ | 6.05±0.08$^{aK}$ | 6.10±0.10$^{aK}$ |
| 150 | 5.06±0.11$^{aL}$ | 5.06±0.11$^{aL}$ | 5.10±0.09$^{aL}$ | 5.12±0.08$^{aL}$ |
| 225 | 4.85±0.05$^{aM}$ | 4.86±0.06$^{aM}$ | 4.89±0.06$^{aM}$ | 4.93±0.06$^{aM}$ |
| 290 | 4.74±0.04$^{aN}$ | 4.74±0.04$^{aN}$ | 4.80±0.05$^{aMN}$ | 4.82±0.05$^{aN}$ |
| 330 | 4.68±0.00$^{aN}$ | 4.68±0.01$^{aN}$ | 4.69±0.04$^{aN}$ | 4.69±0.01$^{aO}$ |

a: The values shown in different letters in the same row are different from each other at p <0.05 level.
K, L, M, N, O: The values shown with different exponential letters in the same column are different from each other at p<0.05 level.

As shown in the table, the pH values of kefir yogurts ranged from 4.68 to 6.17 during incubation. In general, the highest pH values were taken D sample during incubation, followed by C, B and A (control) samples. During the incubation, the samples were not significantly affected by their pH values (p>0.05). Since the water holding and hydration capacity of the yogurt is most favourable, which the pH is in the range of 4.2-4.6, the incubation is terminated at about 4.5-4.6 (Özer, 2006).

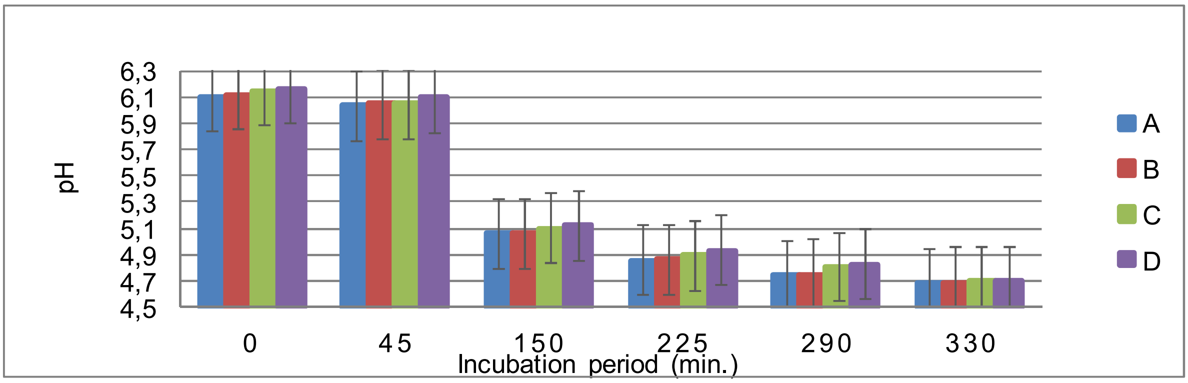


**Figure 1.** pH values of kefir yogurts during the incubation period

As shown in the figure, the pH values of the samples were generally decreased throughout the incubation. The pH values of the samples were significantly affected by incubation time ($p<0.05$). When the status was evaluated in terms of incubation times, all samples decreased to the same pH at the same time. Öztürk (2013) observed that the yogurts produced by using different proportions of powdered milk and buttermilk decreased the pH values throughout the incubation. Güzel-Seydim *et al.* (2000) observed a decrease in pH values during fermentation in production of kefirs.

**Table 4.** Titration acidity values of kefir yogurts during the incubation period (n=3)

| Incubation Period (min.) | Kefir Yogurts | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 0 | $0.31\pm0.01^{aO}$ | $0.27\pm0.01^{bP}$ | $0.27\pm0.01^{bP}$ | $0.27\pm0.01^{bP}$ |
| 45 | $0.37\pm0.01^{aN}$ | $0.33\pm0.01^{bO}$ | $0.32\pm0.01^{cO}$ | $0.31\pm0.06^{dO}$ |
| 150 | $0.69\pm0.01^{aM}$ | $0.63\pm0.01^{bN}$ | $0.59\pm0.01^{cN}$ | $0.55\pm0.03^{dN}$ |
| 225 | $0.70\pm0.01^{aM}$ | $0.69\pm0.02^{abM}$ | $0.66\pm0.06^{bcM}$ | $0.66\pm0.01^{cM}$ |
| 290 | $0.81\pm0.02^{aL}$ | $0.74\pm0.01^{bL}$ | $0.74\pm0.01^{bL}$ | $0.73\pm0.03^{bL}$ |
| 330 | $0.93\pm0.02^{aK}$ | $0.86\pm0.01^{bK}$ | $0.85\pm0.02^{bK}$ | $0.84\pm0.01^{bK}$ |

a, b, c, d: The values shown in different letters in the same row are different from each other at $p<0.05$ level.
K, L, M, N, O, P: The values shown with different exponential letters in the same column are different from each other at $p<0.05$ level.

As shown in the table, titratable acidity values of kefir yogurts ranged from 0.27 to 0.93 during incubation. Generally, the highest titratable acidity value was taken A sample during incubation, followed by samples B, C and D respectively. The samples were significantly influenced by incubation for their titratable acidity values ($p<0.05$). As the ratio of demineralized whey powder increased, the titratable acidity values of the samples decreased. It is thought that the demineralized whey powder has a low titratable acidity value from milk powder. Arslaner (2002), in yogurts produced using whey powder and milk powder, the titration acidity of the sample of whey powder used is low levels, because, after a certain level, serum proteins was caused lactic acid bacterial growth by slowing.

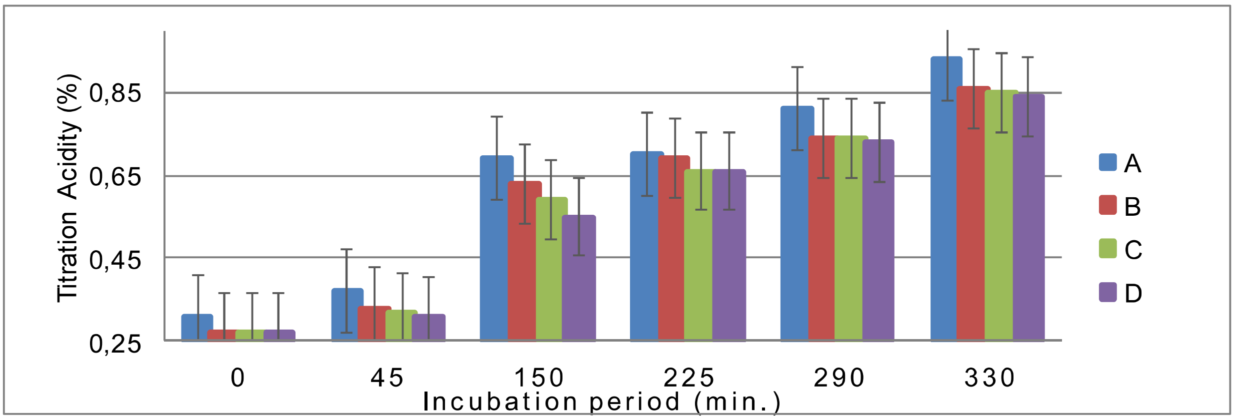

**Figure 1.** Titration acidity values of kefir yogurts during the incubation period

As shown in the figure, titratable acidity values of the samples were generally increased during the incubation. The titratable acidity values of samples were significantly affected by incubation time ($p<0.05$). The decrease in pH values and the increase of titratable acidity during the incubation are caused by the bacteria in the culture to produce lactic acid by metabolizing lactose (Özer, 2006). Şekerli (2013) reported that different temperature norms and yogurt cultures applied samples of the pH values decreased, titratable acidity of the value increased during the incubation. It is thought that the incubation time is longer because kefir culture is used instead of yogurt culture and the incubation temperature is at low temperature (41 °C).

Kefir grains have a complex microflora containing acetic acid bacteria, yeasts and lactic acid bacteria which is included mesophilic lactococci with homologous and heterofermentative thermophilic lactobacilli. The fermentation takes place by addition of kefir grains to the milk and kefir is obtained by the metabolic activity of lactic acid bacteria and the metabolic activity products of the microorganism group consisting of various bacteria and yeasts in this fermentation (Özkaya *et al.,* 2013). Therefore, total aerobic mesophilic bacteria, total lactic acid bacteria and total yeast count were determined in order to evaluate microbiological characteristics of kefir yogurts produced by using different amounts of demineralized whey powder.

**Table 5.** Microbiological properties of kefir yogurts (log cfu/g)

| Kefir Yogurts | 1st day | 7th day | 21st day |
|---|---|---|---|
| | **Total Aerobic Mesophilic Bacteria Count** | | |
| **A** | 7.47±0.81$^{aK}$ | 7.75±0.57$^{aK}$ | 7.29±1.14$^{aK}$ |
| **B** | 7.65±0.87$^{aK}$ | 7.33±0.03$^{aK}$ | 7.27±0.24$^{aK}$ |
| **C** | 8.47±0.80$^{aK}$ | 7.32±0.13$^{aL}$ | 7.10±0.14$^{aL}$ |
| **D** | 8.19±1.27$^{aK}$ | 7.16±0.77$^{aK}$ | 7.43±0.43$^{aK}$ |
| | **Total Lactic Acid Bacteria Count** | | |
| **A** | 7.56±0.72$^{aK}$ | 7.15±0.97$^{aK}$ | 7.07±0.10$^{aK}$ |
| **B** | 7.42±1.06$^{aK}$ | 7.11±0.21$^{aK}$ | 7.08±0.08$^{aK}$ |
| **C** | 7.86±1.00$^{aK}$ | 7.41±0.18$^{aK}$ | 7.21±0.23$^{aK}$ |
| **D** | 7.90±0.72$^{aK}$ | 7.51±0.78$^{aK}$ | 7.21±0.24$^{aK}$ |
| | **Total Yeast Count** | | |
| **A** | 6.60±0.17$^{aK}$ | 6.81±0.13$^{aK}$ | 6.85±0.10$^{aK}$ |
| **B** | 6.36±0.20$^{aL}$ | 6.50±0.17$^{aKL}$ | 6.79±0.16$^{aK}$ |
| **C** | 5.99±0.92$^{aK}$ | 6.84±0.38$^{aK}$ | 6.95±0.27$^{aK}$ |
| **D** | 5.96±1.22$^{aK}$ | 6.37±0.58$^{aK}$ | 6.97±0.30$^{aK}$ |

a: The values shown in different letters in the same row are different from each other at $p < 0.05$ level.
K,L: The values shown with different exponential letters in the same column are different from each other at $p<0.05$ level.

As shown in the table, total aerobic mesophilic bacteria count of the kefir yogurts ranged from 7.10 to 8.47 log cfu/g, the total lactic acid bacteria count was between 7.07 and 7.90 log cfu/g, and the total number of yeasts varied between 5.96 and 6.97 log cfu/g in the storage. The use of different amounts of demineralized whey powder did not significantly affect the total number of aerobic mesophilic bacteria of the kefir yogurts, the total number of lactic acid bacteria and the total number of yeasts ($p>0.05$).

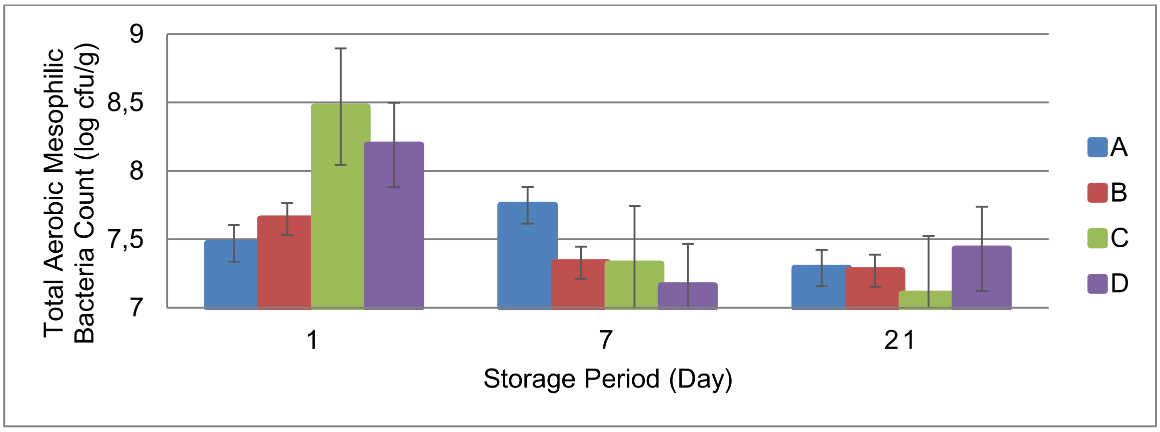


**Figure 3.** Total aerobic mesophilic bacteria count of the kefir yogurts during storage

As shown in the figure; total aerobic mesophilic bacterial counts of kefir yogurts decreased regularly in samples B and C, while they changed irregularly in samples A and D during storage. The storage period was statistically significant ($p<0.05$) in C sample on these differences and it was found to be insignificant in other samples ($p>0.05$). According to the Turkish Food Codex Fermented Milk Products Report, it is stated that the total number of specific microorganisms in yogurt, kefir and fermented milk products should be at least $10^7$ cfu/g (7 log cfu/g) (Anon, 2009). According to this report, kefir yogurts were found to be in proper values during storage. Ünal (2013) reported that the total bacterial count of the set kefir produced from different dry matter of milks with starter culture and grain, ranged from 8.80 log cfu/ml to 10.05 log cfu/ml. In addition, the total bacterial count of the Kefir samples increased up to the 8th day of storage and decreased on the other days of storage and decreased the resistance of the microorganisms due to increased acidity was reported to cause this situation.

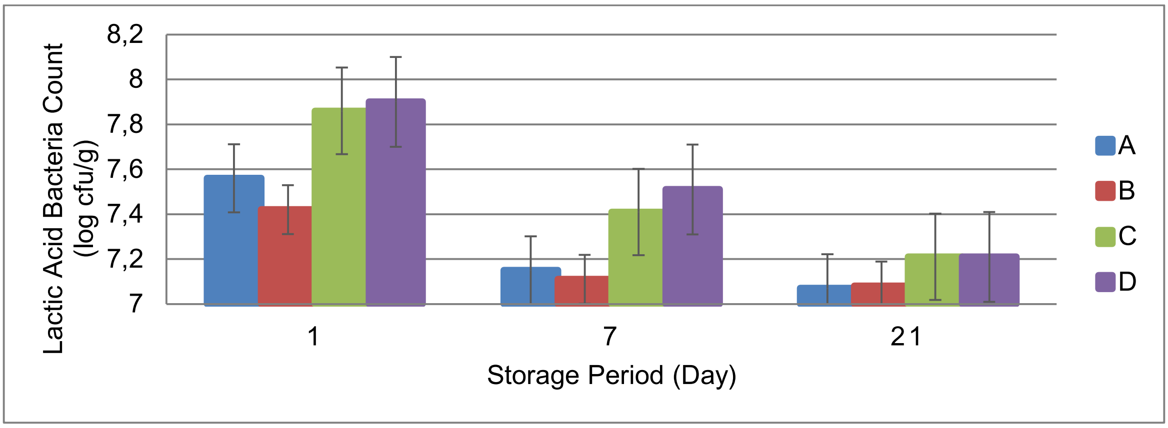


**Figure 4.** Total lactic acid bacteria count of the kefir yogurts during storage

As shown in the figure, the count of lactic acid bacteria was regularly decreased in all samples. The effect of storage time on lactic acid bacterial counts of kefir yogurts was not statistically significant ($p>0.05$). The reduction in lactic acid bacteria during storage is thought to be unable to resist the increased acidity of lactic acid bacteria. Güzeler *et al.* (2017) found the average number of lactic acid bacteria is 7.85 log cfu/g and 6.35 log cfu/g respectively on kefir and kefir yogurt produced. Nalchi (2014) reported that the effect of storage of yogurts on lactic acid bacterial count was not statistically significant ($p>0.05$) in a study done. In a study done by Gölge (2002) reported that the effect of storage on the number of lactic acid bacteria of yogurts is significant.

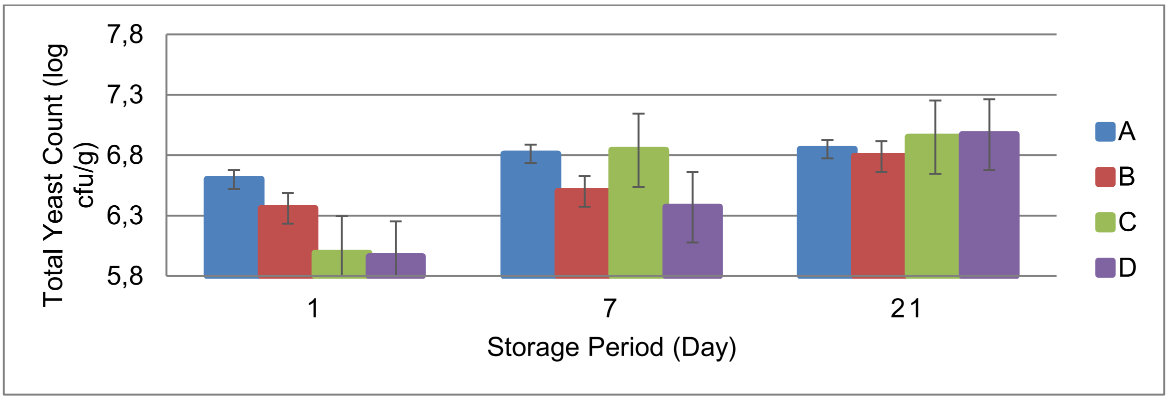


**Figure 5.** Total yeast count of kefir yogurts during storage

As shown in the figure; during the storage period, yeast count of kefir yogurts increased regularly. The storage period was found to be statistically significant ($p<0.05$) in sample B on these differences and it was found that there was no effect on other samples ($p>0.05$). According to the Report on Fermented Milk of Turkish Food Codex it is stated that kefir should have at least $10^4$ cfu/g (4 log cfu/g) yeast. Kefir yogurts have taken appropriate values for this report during the storage (Anon, 2009). Okur *et al.* (2008), in a study, yeast of yogurt obtained with kefir support culture was found to be significantly higher ($p<0.05$) than yogurt obtained by the only culture of yogurt and they reported that kefir culture contained in the yeast microflora reflected on all of the yogurt is used in the culture of kefir. Unal (2013) produced set type kefir by starter culture and grain in different dry matter proportion of milk. It is reported that although the average yeast count of this set type kefir on the first and 8 days of storage wasn't found to be statistically important, the yeast count of the kefirs was found to be important statistically kefir on the 15 days of storage from other two values and this change was directly related to increased acidity. Güzel-Seydim *et al.* (2005) reported that between 7 and 14 days of storage, and between the $14^{th}$ and the $21^{st}$ days, the amount of yeast in kefir increased (5.72-6.52-6.56 log cfu/ml, respectively) and this increase was due to the increase in ethanol concentration.

## 4. CONCLUSION

According to the results of the analysis made in the skimmed milk powder and demineralized whey powder, the results were observed to comply generally with the conditions stated in the Turkish Food Codex Report on Condensed Milk and Milk Powder and the Turkish Food Codex Cheese Report. During the incubation period, pH and titration acidity values of samples were examined at specific intervals. The use of demineralized whey powder at different rates, the pH values of the samples were not significant during the incubation period ($p>0.05$), and the titratable acidity values were significantly affected ($p<0.05$). Incubation time significantly affected the pH and titratable acidity values of the samples ($p<0.05$). It was thought that the decrease in titratable acidity values in the samples as the ratio of demineralized whey powder increased and that the value of titration acidity of demineralized whey powder was lower than that of milk powder. It is thought that the reason for the long incubation period was due to the use of kefir culture instead of yogurt culture and the low temperature of the incubation temperature. While the use of demineralized whey powder at different proportions on total aerobic mesophilic bacteria count of kefir yogurts was not significantly affected ($p>0.05$), storage time was significantly affected in C sample ($p<0.05$). According to the Turkish Food Codex Fermented Milk Report, total bacterial counts of kefir yogurts were found to be suitable during storage. The use of different amounts demineralized whey powder and storage period of kefir yogurts were not significantly affected by the total lactic acid bacteria numbers ($p>0.05$). While the use of demineralized whey powder at different rates on the total yeast numbers of kefir yogurts was not statistically significant ($p>0.05$), the storage period was significantly affected in B sample ($p<0.05$). It is stated that the amount of yeast contained in the kefir culture is reflected to the samples.

According to the results of the research, it was found appropriate to use certain proportions of demineralized whey powder in kefir yogurt production as an increasing dry matter. In addition demineralized whey powder can use in order to utilize whey and gain cost.

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