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RESEARCH ARTICLE

Observation of Lactic Acid Bacteria and Yeast Populations During Fermentation and Cold Storage in Cow's, Ewe's and Goat's Milk Kefirs

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Summary

Changes in the population numbers of *Lactobacillus* spp, *Lactococcus* + *Leuconostoc* spp. and yeasts in the kefirs made of cows, ewes and goats milk were determined in this study. The highest count of *Lactobacillus* spp. was in ewe's milk. There was a slight or no difference in the goats and cow's milk. At 24 h, one $\log_{10}$ cfu/ml decrease was observed for *Lactobacillus* spp. in cows and ewes milk. *Lactobacillus* spp. were more steady in goat milk with a half $\log_{10}$ cfu/ml decrease. *Lactococcus* + *Leuconostoc* spp. similarly reached their maximum populations in cow, ewe and goat milk at the time period of 15-24 h. *Lactococcus* + *Leuconostoc* spp. in all milks were in parallel with the maximum populations of *Lactobacillus* spp. in ewe milk at the time period of 12-15 h. Yeasts had similar numbers in cow, ewe and goat milk at the time period of 15-21 h. Some decreases were observed in the numbers of *Lactobacillus* spp, *Lactococcus* + *Leuconostoc* spp, and yeasts of the kefirs kept at 4ºC for 7 days. The decreases of *Lactobacillus* spp. in cows' and goats' milk kefirs were slight and similar, while one and half $\log_{10}$ cfu/ml decrease was counted in ewes' milk kefir. The decrease in the numbers of *Lactococcus* + *Leuconostoc* spp. was more pronounced than those of *Lactobacillus* spp. and yeasts. pH decreased during the fermentation and all remained constant over the storage period of 7 days. The type of milk had an influence on the population development of kefirs. Ewe's milk supported the growth of *Lactobacillus* spp. and *Lactococcus* + *Leuconostoc* spp. better. This indicates that different milks may influence the population development of kefir microflora which may affect the quality of kefir.

Keywords: *Kefir, Milk, Lactic acid bacteria*

İnek, Koyun ve Keçi Sütünden Yapılan Kefirlerde Fermentasyon Süresince ve Soğukta Muhafazada Laktik Asit Bakteri ve Maya Populasyonunun Gözlemlenmesi

Özet

Bu çalışmada, inek, koyun ve keçi sütünden yapılan kefirlerde *Lactobacillus* spp, *Lactococcus* + *Leuconostoc* spp. ve mayaların popülasyon düzeyindeki değişim belirlendi. En yüksek *Lactobacillus* spp. düzeyi koyun sütünde saptandı. Keçi ve inek sütünde ise çok az veya hiç fark yoktu. Yirmi dördüncü saatte inek ve koyun sütünde *Lactobacillus* spp. için bir $\log_{10}$ cfu/ml azalma gözlemlendi. *Lactobacillus* spp. keçi sütünde yarım $\log_{10}$ cfu/ml azalma ile daha istikrarlıydı. *Lactococcus* + *Leuconostoc* spp. benzer olarak maksimum popülasyonlarına inek, koyun ve keçi sütünde 15-24 saatlik zaman periyodunda ulaştı. *Lactococcus* + *Leuconostoc* spp. bütün sütlerde, *Lactobacillus* spp.'nin koyun sütünde 12-15 saatlik zaman periyoundaki maksimum popülasyonu ile parelellik içersindeydi. Mayalar inek, koyun ve keçi sütünde 15-21 saatlik zaman periyodunda benzer düzeylere sahipti. 4ºC'de 7 gün muhafaza edilen kefirlerde *Lactobacillus* spp., *Lactococcus* + *Leuconostoc* spp. ve maya düzeylerinde azalmalar gözlemlendi. *Lactobacillus* spp.'de koyun sütü kefirinde bir buçuk $\log_{10}$ cfu/ml azalma saptanırken, inek ve keçi sütü kefirlerindeki azalmalar az veya paraleldi. *Lactococcus* + *Leuconostoc* spp. düzeyindeki azalma, *Lactobacillus* spp. ve maya'nın düzeyine göre daha belirgindi. pH fermentasyon sürecinde düşerken, 7 günlük depo süresi boyunca değişmeden kaldı. Sütün çeşidinin kefirdeki popülasyon gelişmesine etkisi oldu. Koyun sütünün *Lactobacillus* spp. ve *Lactococcus* + *Leuconostoc* spp.'nin üremesi için daha uygun olduğu görüldü. Bu durum farklı sütlerin kefir mikroflorasının popülasyon gelişimini etkileyebileceğine diğer bir ifadeyle kefirin kalitesini etkileyebileceğine işaret etmektedir.

Anahtar sözcükler: *Kefir, Süt, Laktik asit bakterisi*

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INTRODUCTION

Milk has an essential role in most diets world-wide. Consumption of milk and fermented milk products is widespread and yet increasing. In developed countries where there is an established dairy industry, new products are being developed to sustain consumer interest.

In recent years, there has been more interest in different fermented milk products known only to particular countries with a view to adapting them for commercial large-scale production in other parts of world. Among the best known are yoghurt, acidophilus milk, kefir, koumiss and yakult. In Turkey, kefir is made traditionally at home but interest is growing and there has been a promotion for its large-scale production due to its health benefits [1]. Recently, kefir has been produced on a commercial scale by a few dairy companies in Turkey and available in big supermarkets. Few people, however, recognize that these products are prepared by bacterial and/or yeast action, and the characteristic flavours and textures of these products are results of these fermentations. In natural fermentations lactic acid bacteria implement competitive characteristics which allow them to produce good quality milk fermentations.

For the manufacture of kefir, the traditional starter cultures are in the form of grains of variable sizes, which resembles cauliflower florets in shape and colour [2,3] but also sheet-like structures [4] and a globular, sagy structure resembling small pouches are exist [5]. These grains contain a wide and varying microflora, such as lactic acid bacteria, yeasts, acetic acid bacteria and moulds [6-13], but the culture is dominated by a *Lactobacillus*/yeast population. The evidence suggest that population development in kefir fermentation appears to be controlled and there is a pattern of microbial succession [14] and compared to other milk fermentations (e.g. yoghurt and cheese) the microbiology of kefir grain is less well understood [15].

The majority of fermented milk products are made from cow milk, but sheep, goat, buffalo, camel, and horse milk can be also used [16]. Kefir is mainly produced from cow milk and little information is available on kefir made from different mammalian milk [17]. Therefore, this paper deals with the changes in the *Lactobacillus*/yeast flora during the kefir fermentations in cow's, ewe's and goat's milk.

MATERIAL and METHODS

This study was performed at Kafkas University Animal Research Center (HAUM) between May and June in 2006. Milk samples of cow, ewe, and goat were obtained from local farms in Kars city, Turkey. Kefir grains were purchased from the Department of Dairy Technology of Agriculture Faculty, Ege University in Izmir, Turkey, in the sterile 0.9% NaCl solution, and were propagated at the Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Kafkas University in Kars, Turkey, by daily transfer into UHT milk at room temperature.

The milk samples were heated at 85°C for 30 min and cooled to 25°C in a water bath before inoculation. A liter of each sample was inoculated with 30 g active kefir grains. Fermentation was carried out at 25°C for 21 h. Subsequently all the fermentate was transferred to the cold store at 4°C. Microbiological analysis for total *Lactobacillus* spp., *Lactococcus + Leuconostoc* spp., and yeasts were performed. *Lactobacillus* spp. was grown on MRS agar at 30°C for 48 h in an anaerobic jar (Gas-pack anaerobic system, BBL). Total number of *Lactococcus + Leuconostoc* spp. was enumerated on M17 agar medium (Oxoid). Yeasts were grown on Potato Dextrose Agar (PDA, Oxoid).

The pH was determined electrometrically (Orion Model 420A) and acidity by titration with N/10 NaOH in the presence of phenolphthalein. Acidity was expressed as per cent lactic acid (LA).

Chemical analyses were performed in accordance with the inditacions by Oysun [18].

RESULTS

The chemical composition of each type of milk is shown in *Table 1*. The pH and acidity values of cows', ewes' and goats' milk kefirs during 21 h (fermentation process) at 25°C, and 7 days storage-ripening at 4°C are given in *Table 2* and *Table 3*, respectively. The population numbers of *Lactobacillus* spp., *Lactococcus + Leuconostoc* spp. and yeasts in the kefirs made from ewe, cow and goat milk during 21 h at 25°C fermentation, and 7 days storage-ripening at 4°C are given in *Tables 4-6* and *Tables 7-9*, respectively.

Table 1. *The chemical composition of cow, ewe, and goat milk*
Tablo 1. *İnek, koyun ve keçi sütünün kimyasal kompozisyonu*

Parameter	Cow Milk	Ewe Milk	Goat Milk
Total solid (%)	12.0	19.3	13.0
Protein (%)	3.3	6.0	3.6
Carbohydrates (%)	4.7	5.4	4.5
Ash (%)	0.7	0.9	0.8

Table 2. *Changes in pH and acidity of each milk sample during kefir fermentations at 25ºC for 21 h*
Tablo 2. *Yirmi bir saat süreyle 25ºC'de kefir fermentasyonu süresince her bir süt örneğindeki asit ve pH değişimi*

Time (h)	pH Cows' Milk	Acidity Cows' Milk	pH Ewes' Milk	Acidity Ewes' Milk	pH Goats' Milk	Acidity Goats' Milk
0	7.02	0.17	7.03	0.24	7.01	0.17
3	6.62	0.2	6.82	0.27	6.72	0.22
6	6.36	0.24	6.63	0.31	6.52	0.25
9	6.04	0.28	6.29	0.38	6.17	0.35
12	5.70	0.38	5.26	0.56	5.8	0.43
15	4.81	0.55	5.26	0.72	5.3	0.64
18	4.56	0.68	5.06	0.94	5	0.89
21	4.54	1.2	4.52	1.21	4.6	0.96

Table 3. *Changes in pH and acidity of each kefir sample during storage at 4ºC for 7 days*
Tablo 3. *Yedi gün 4ºC'de depolama süresince her bir kefir örneğindeki asit ve pH değişimi*

Day	pH of Kefir Made from Cows' Milk	Acidity of Kefir Made from Cows' Milk	pH of Kefir Made from Ewes' Milk	Acidity of Kefir Made from Ewes' Milk	pH of Kefir Made from Goats' Milk	Acidity of Kefir Made from Goats' Milk
1	4.41	1.34	4.49	1.66	4.51	1.21
2	4.28	0.78	4.42	1.17	4.33	1.04
3	4.24	0.72	4.37	1.19	4.27	1
4	4.22	0.85	4.34	1.13	4.22	1.01
5	4.12	0.83	4.31	1.12	4.2	0.99
6	4.08	0.82	4.3	1.12	4.15	0.98
7	4.02	0.81	4.3	1.12	4.03	0.97

Table 4. *The counts (cfu/ml) of Lactobacillus spp. in cow's, goat's and ewe's milk during kefir fermentation at 25ºC for 21 h*
Tablo 4. *Yirmi bir saat süreyle 25ºC'de kefir fermentasyonu sürecinde inek, keçi ve koyun sütünde Lactobacillus spp. miktarı (cfu/ml)*

Time (h)	Cow's Milk	Ewe's Milk	Goat's Milk
0	4.11	4.30	4.04
3	5.04	5.47	5.48
6	6.08	6.41	5.84
9	6.90	7.38	6.38
12	7.25	9.54	7.69
15	8.69	9.68	8.50
18	8.78	8.69	8.00
21	7.57	8.79	8.00

Table 5. *The counts (cfu/ml) of Lactococcus+Leuconostoc spp. populations in cow's, goat's and ewe's milk during kefir fermentation at 25ºC for 21 h*
Tablo 5. *Yirmi bir saat süreyle 25ºC'de kefir fermentasyonu sürecinde inek, keçi ve koyun sütünde Lactococcus + Leuconostoc spp. miktarı (cfu/ml)*

Time (h)	Cow's Milk	Ewe's Milk	Goat's Milk
0	4.36	4.14	4.62
3	4.78	5.77	4.95
6	6.08	5.95	5.95
9	6.60	7.28	6.69
12	7.28	8.61	8.25
15	8.69	9.78	9.17
18	8.95	9.45	9.36
21	9.28	9.32	8.11

Table 6. *The counts (cfu/ml) of yeast populations in cow's, goat's and ewe's milk during kefir fermentation at 25ºC for 21 h*
Tablo 6. *Yirmi bir saat süreyle 25ºC'de kefir fermentasyonu sürecinde inek, keçi ve koyun sütünde maya miktarı*

Time (h)	Cow's Milk	Ewe's Milk	Goat's Milk
0	3.60	3.30	3.36
3	4.39	5.17	4.30
6	4.90	5.34	4.60
9	4.60	5.15	4.84
12	5.20	5.39	5.00
15	5.30	5.38	5.41
18	5.67	5.47	5.58
21	5.72	5.86	5.20

Table 7. *The counts (cfu/ml) of Lactobacillus spp. populations in kefir samples made from cow's, goat's and ewe's milk during 7 days storage at 4ºC*
Tablo 7. *Dört santigrat derecede 7 gün süreyle depolamada inek, keçi ve koyun sütünden yapılan kefir örneklerinde Lactobacillus spp. miktarı (cfu/ml)*

Day	Cow's Milk	Ewe's Milk	Goat's Milk
1	7.48	8.84	8.00
2	7.41	8.15	7.95
3	7.30	7.76	7.47
4	7.47	7.47	7.69
5	7.17	7.23	7.60
6	7.04	7.28	7.54
7	7.00	7.23	7.51

Table 8. *The counts (cfu/ml) of Lactococcus + Leuconostoc spp. populations in kefir samples made from cow's, goat's and ewe's milk during 7 days storage at 4°C*

Tablo 8. *Dört santigrat derecede 7 gün süreyle depolamada inek, keçi ve koyun sütünden yapılan kefir örneklerinde Lactococcus + Leuconostoc spp. miktarı (cfu/ml)*

Day	Cow's Milk	Ewe's Milk	Goat's Milk
1	9.68	9.00	8.95
2	9.55	9.11	8.20
3	9.56	8.95	8.30
4	8.60	8.30	8.38
5	7.60	8.20	8.00
6	7.30	8.00	7.90
7	7.25	8.00	7.72

Table 9. *The counts (cfu/ml) of Yeast populations in kefir samples made from cow's, goat's and ewe's milk during 7 days storage at 4°C*

Tablo 9. *Dört santigrat derecede 7 gün süreyle depolamada inek, keçi ve koyun sütünden yapılan kefir örneklerinde maya miktarı (cfu/ml)*

Day	Cow's Milk	Ewe's Milk	Goat's Milk
1	5.81	5.84	5.69
2	5.90	5.60	5.47
3	5.50	5.58	5.44
4	5.48	5.47	5.30
5	5.60	5.47	5.30
6	5.47	5.47	5.25
7	5.47	5.44	5.00

DISCUSSION

During the fermentation a sharpe decrease of around 2 pH units was observed but pH did not vary during storage. This may be due to the presence of yeasts since Collar [19] reported that lactic acid bacteria multiply and produce lactic and acetic acids more slowly in mixture with yeasts than in pure culture. pH values at 21 h fermentation were in agreement with the average samples pH at 22 h fermentation as reported by Guzel-Seydim et al.[20] and Cais-Sokolinska et al.[21]. Maximum acidity was observed between the time period of 21 h and the first day of storage. This showed similarity with the results of Fontan et al.[22] but was slightly higher than the results of Beshkova et al.[23].

The lactic acid bacteria and yeasts are the pre-dominating microflora in kefir grains [24]. Considering the population counts of viable *Lactococcus + Leuconostoc* spp., our results are in agreement with those of Wszolek et al.[17], Simova et al.[24] and also Ninane et al.[25] who reported that *Lactococcus* showed by far the greatest variability comparing to *Lactobacillus* spp. and yeasts.

During the fermentation for 21 h, increases were observed in the numbers of *Lactobacillus* spp., *Lactococcus + Leuconostoc* spp. and yeasts. Maximum populations of *Lactobacillus* spp., *Lactococcus + Leuconostoc* spp. and yeasts were reached at different time periods, and showed variations in different milks *(Table 4-6)*. The highest counts of *Lactobacillus* spp. were observed in ewe's milk between 12 h and 15 h time period *(Table 4)*. It was 2 $\log_{10}$ cfu/ml higher than the numbers observed for *Lactobacillus* spp. in cow's and goat's milk at 12 h. This may be due to variations in composition between different types of milks (cow, ewe and goat) as ewe milk has a higher content of protein and fat than cow and goat milk [26]. Furthermore, it contains higher levels of vitamins, such as folic acid, pantothenic acid, riboflavin and niacin which are considered to be essential for the growth of some *Lactobacillus* spp. strains [27], providing perhaps the best substrate for the manufacture of a fermented milk product [28]. As far as the goat and cow milk were concerned, it was interesting to mention that there was only a slight or no difference in the population numbers of *Lactobacillus* spp. After 21 h fermentation, there was almost one $\log_{10}$ cfu/ml decrease in the population numbers of *Lactobacillus* spp. in cow and ewe milk, as compared to the maximum population numbers of *Lactobacillus* spp. at 15 h in the same milks. The numbers of *Lactobacillus* spp. were steadier in goat milk with a half $\log_{10}$ cfu/ml decrease comparing to the maximum population number at 15 h in goat milk. The maximum numbers of *Lactobacillus* spp. at 21 h and in following 2 days storage at 4°C were in parallel with the results of Irigoyen et al.[16], Wszolek et al.[17], Guzel-Seydim et al.[20], Fontan et al.[22], Witthuhn et al.[29], and Witthuhn et al.[30].

Unlike *Lactobacillus* spp., *Lactococcus + Leuconostoc* spp. reached their maximum populations which were similar in numbers in cow, ewe and goat milk but at the different time periods of 18-21 h, 15-21 h and 15-18 h, respectively *(Table 5)*. The maximum population numbers of *Lactococcus + Leuconostoc* spp. in cow, ewe and goat milk were in parallel with the maximum populations of *Lactobacillus* spp. in ewe milk at the time period of 12-15 h. Likewise, yeasts had similar maximum population numbers in cow, ewe and goat milk at the time periods of 18-21 h, 18-21 h and 15-18 h, respectively *(Table 6)* and these values were slightly lower than the values of Guzel-Seydim et al.[20] at 22 h fermentation time but our 7^{th} day storage values showed similarity with the 7^{th} day values of Guzel-Seydim et al.[20].

The storage and ripening of cows', ewes' and goats' milk kefirs at 4°C for 7 days resulted in decreases of the population numbers of *Lactobacillus* spp., *Lactococcus +*

Leuconostoc spp. and yeasts *(Table 7-9).* The decrease of *Lactobacillus* spp. numbers in cows' and goats' milk kefirs were slight and similar, while one and half $\log_{10}$ cfu/ml decrease was counted in ewes' milk *(Table 7).* Likewise, the decrease in the population numbers of *Lactococcus + Leuconostoc* spp. was more pronounced rather than in the populations of *Lactobacillus* spp. and yeasts, and there were 2.43, 1.23 and 1 $\log_{10}$ cfu/ml difference in cows', goats' and ewes' milk kefirs, respectively *(Table 8).* For the yeasts, a slight decrease was also observed in goats' cows' and ewes' milk kefirs *(Table 9).* These results were in parallel with the study of Irigoyen et al.[16] and Oner et al.[31]. As expected, lactic acid bacteria and yeasts were the predominant flora in fresh (21 h) and stored (7 days) kefirs but some strains decreased by 1 to 2.43 $\log_{10}$ cfu/ml during the storage period. *Lactobacillus* spp. reached the highest population numbers in ewe's milk while *Lactococcus + Leuconostoc* spp. reached the highest population numbers in ewe's and cow's milk. Yeast population numbers were not affected largely by the type of milk used. Since these population numbers may indicate that ewe's milk supports the growth of the *Lactobacillus* spp. and *Lactococcus+ Leuconostoc* spp. better, which may lead to the thought of ewe's milk being better choice, as compared to cow's and goat's milk. Likewise, Wszolek et al.[17] reported that the firmness of the product was also influenced by the type of milk used stating the order of ovine>bovine >caprine. Furthermore Wojtowski et al.[32] claimed that kefir produced from sheep milk can have a considerably more advantageous effect on the health of the consumers than kefirs produced from goat or cow milk, however the ratings for acceptability of kefirs were ranked in the order of kefir made from cow milk>ewe milk>goat milk. On the other hand, Sahan [33] concluded that organoleptic qualities of kefirs made from cow's, ewe's and goat's milk indicated that the best kefir could also be produced from goat's milk as well as the cow's milk. Likewise, Kaptan and Gursel [34] also stated that the best quality kefir can be made from goat milk.

In conclusion, this study showed that the type of milk has an influence on the population development of the kefir flora. Although the differences in the population development may influence the quality of kefir, considering the previous studies [12,21] it can be presumed that the type of milk has a greater influence than the starter cultures and their population development on the sensory profile.

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