Comparison of microbial population of household and commercial kefirs in Ankara, Turkey

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Abstract: Kefir is a fermented milk product produced by a complex symbiotic metabolic activity of lactic acid bacteria, acetic acid bacteria and yeast species in kefir grains. Although it was discovered by Caucasian people centuries ago, kefir is still manufactured and consumed by many nations because of its health benefits. Kefir has an acidic and mildly alcoholic, unique flavor due to the multiple actions of bacteria and yeasts. The population composition differs primarily from their source or country of origin. It's also affected from the ratio of kefir grains to milk, the ratio of species to each other, method of cultivation of the grains, incubation time and temperature, sanitation during separation of kefir grains, washing of grains, cold storage and the properties of substrates. The aim of this study was to compare the microbial population of household and commercial kefirs in Ankara, Turkey. The samples were analyzed for lactic acid bacteria, lactoccocci, yeast, enterococci, *Enterobacteriaceae* and coliform bacteria. Commercial kefir samples contained higher amounts of lactococci than lactic acid bacteria and yeast compared to household kefir samples. Besides, in one of the commercial kefir sample enterococci counts were observed and one of the household kefir samples contained coliform bacteria.

Keywords: Commercial, household, kefir, microbial population

Ankara ilinde tüketilen ticari ve ev yapımı kefirlerin mikrobiyal popülasyonlarının kıyaslanması

Öz: Kefir tanelerinde bulunan laktik asit bakterilerinin, asetik asit bakterilerinin ve mayaların kompleks simbiyotik metabolic aktiviteleri sonucu oluşan fermente bir süt ürünü olan kefir, Kafkaslar'da asırlar öncesinde keşfedilmiş olmasına rağmen, sağlığa olan katkılarından ötürü günümüzde de hala pek çok ulus tarafından tüketilmektedir. Kefirin kendine özgü asidik ve hafif alkolik lezzeti bakteri ve mayalardan ileri gelen bir takım reaksiyonlardan şekillenmektedir. Bu popülasyonun kompozisyonu kefirin kaynağına ya da geldiği ülke kökenine göre değişmektedir. Ayrıca kefir tanelerinin süte oranı, mikroorganizmaların birbirlerine oranları, kefir tanelerinin yetiştirilme metodu, inkübasyon periyodu ve sıcaklığı, kefir tanelerinin küçültülmesi esnasında hijyen kurallarına uyulup uyulmadığı, tanelerin yıkanması, soğuk muhafaza gibi pek çok etken de mikrobiyal popülasyonu etkileyebilmektedir. Bu çalışmada, Ankara'da tüketilen ticari ve ev yapımı kefirlerin mikrobiyal popülasyonlarını kıyaslamak amaçlanmıştır. Örnekler laktik asit bakterileri, laktokok, maya, enterokok, Enterobacteriaceae ve koliform bakteriler bakımından analiz

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edilmiştir. Laktik asit bakterileri ve mayalara oranla, ticari kefirlerin ev yapımı kefirlere göre daha fazla laktokok içerdiği tespit edilmiştir. Ayrıca ticari kefir örneklerinden birinde enterokok, bir ev yapımı kefir örneğinde ise koliform bakteri varlığı belirlenmiştir.

Anahtar sözcükler: Ev yapımı, kefir, mikrobiyal popülasyon, ticari

Introduction

Kefir is a viscous, acidic, and mildly alcoholic milk beverage produced by the fermentation of milk using kefir grains as a starter culture (12).

The Caucasian people discovered this unique mildly alcoholic beverage while they were carrying the fresh milk in leather bags or oak vats. Although it was discovered centuries ago, kefir is still manufactured and consumed by many nations because of its health benefits. Kefir has been assigned a variety of health claims in addition to its nutritional value. Many studies regarding kefir's biological activities have established that kefir possess the anti-inflammatory, immune-modulating, antimicrobial, anti-tumoral effects and it has the potential to become a type of functional food (5, 10, 13, 19, 21, 22).

The distinct microorganism groups identified in this beverage performed three different kinds of fermentations; lactic, alcoholic and acetic fermentations. The increase in lactic acid bacteria population causes an increase in the lactic acid concentration in the beverage, whereas the increase in yeast population favored the ethanol formation. Alcohol fermentation is the result of the addition of yeasts in the form of kefir grains. Because of the multiple fermentation process, the resulting product possesses flavor that is characterized by a balance of lactic acid, diacetyl, acetaldehyde, acetoin, ethanol and CO_2. Moreover during the fermentation, vitamin B_1, vitamin B_{12}, calcium, amino acids, folic acid and vitamin K increase in the kefir (8, 10, 15, 16).

Kefir is an excellent example of the co-occurrence of yeasts and bacteria. The lactic acid bacteria, lactococci, yeast and acetic acid bacteria contents of kefir grains differ due to variety of effects (1, 16, 24). Different reports indicate that microbial content of kefir grains include lactic acid bacteria, lactococci, yeast and acetic acid bacteria and product quality strongly depend on the origin of the grains (7, 9). Thereof, the objective of this study was to compare the microbial population of household and commercial kefirs in Ankara, Turkey.

Material and Methods

Samples: Three commercial kefir samples from different retail markets and three household kefir samples were collected in Ankara province (Turkey) from local householders. The commercial kefir samples were transported to the laboratory under refrigerated conditions and analyzed on the same day. On the other hand, the household kefir grains were transported to the laboratory in sterile water, immediately prepared for fermentation in hygienic conditions and incubated at room temperature for 24 hours in sterile milk.

Manufacturing of household kefir samples: Kefir grains (2%) were added to sterile milk and incubated for 24 h at 25 °C. After the incubation period, pH of the kefir samples were measured at the time of sampling using a pH meter (Hanna Instruments, HI-2221 pH Bench Meter, Romania).

Microbiological analysis: 10 ml of the kefir samples were homogenized with 90 ml of a sterile 0.1% peptone water solution using a

stomacher (Aes Easy Mix, Combourg, France) for 2 min. Serial dilutions were made with sterile peptone water and plated in duplicates on specific media. Lactic acid bacteria counts were performed on de Man, Rogosa and Sharpe (MRS, Merck, 110660) medium at 30ºC under anaerobic conditions for 48 h (7). Lactococci counts were carried out on M17 medium (Oxoid CM0785) with 50 ml of sterile lactose solution (10% w/v) in pH 6.9 at 30ºC for 48 h (7). Dilutions were also plated on Slanetz-Bartley (SB, Oxoid CM377), Violet Red Bile Glucose (VRBG, Oxoid CM485) and Violet Red Bile Lactose (VRBL, CM0968) agars for enterococci, *Enterobacteriaceae*, and coliform bacteria, respectively. These were incubated aerobically at 37ºC for 24-48 h. Yeasts were enumerated in Sabouraud Dextrose Agar (SDA, Oxoid CM0041) supplemented with chloramphenicol (0.05 mg/mL) after the incubation period at 25ºC for 5 days (2).

Results

According to the results, bacterial counts of the kefir samples and household kefir samples generally contained similar amount of lactic acid bacteria, lactococci and yeast, while commercial kefir samples contained higher amounts of lactococci than lactic acid bacteria and yeast.

In one of the commercial kefir samples enterococci counts were observed as 2.3 log cfu/mL while no enterococci counts were determined from any other kefir samples.

Yeast counts were variable among the kefir samples as the minimum counts of yeast from commercial kefir samples was observed as 2.0 log cfu/mL. On the other hand, one of the household samples contained 7.0 log cfu/mL as the maximum yeast count. However, household kefir samples contained much more yeast content than the commercial samples (Table 1).

On the other hand, one of the household kefir samples contained coliform bacteria with the count of 3.3 log cfu/mL.

After 24 h incubation, pH of the kefir samples were measured as 5.35, 5.80, 5.55 in household kefir samples (kefir 4, 5, 6), respectively.

Table 1: Bacterial counts of the commercial and household kefir samples (cfu/g)

Tablo 1: *Ticari ve ev yapımı kefir örneklerinin bakteri sayımları (kob/mL)*

	Commercial			Household		
	Kefir 1	**Kefir 2**	**Kefir 3**	**Kefir 4**	**Kefir 5**	**Kefir 6**
Lactococci	9.2 log	8.1 log	7.9 log	7.2 log	5.3 log	6.0 log
Lactic acid bacteria	5.0 log	7.2 log	4.3 log	7.3 log	5.6 log	6.3 log
Yeast	5.0 log	2.0 log	2.0 log	6.9 log	5.6 log	7.0 log
Enterococci	2.3 log	*	*	*	*	*
Coliform	*	*	*	3.3 log	*	*
Enterobacteriaceae	2.6 log	*	*	3.6 log	*	*

**: <1.3 log*

Discussion and Conclusion

Kefir is produced by a diverse spectrum of microbial species. The composition of microbial population differs primarily from their source or country of origin (17, 26). It is also affected from the ratio of kefir grains to milk, the ratio of species to each other, method of cultivation of the grains, incubation time and temperature, sanitation during separation of kefir grains, washing of grains, cold storage and the properties of substrates. On the other hand, different types of milk as the substrate affect the microbiota depending on their carbohydrate, fat and protein content (3, 9, 16, 24, 25).

Lactic acid bacteria are presented as the largest portion (65–80 %) of the microbial population, while lactococci, yeasts and sometimes acetic acid bacteria constituting the remaining portion of the microorganisms in the kefir grains (24, 25). According to the microbial counts of the Turkish household kefir samples in this study, it was observed that they contained similar amounts of lactic acid bacteria, lactococci and yeast; while commercial kefir samples contained higher amounts of lactococci than lactic acid bacteria and yeast. Our study's results are similar with Kök-Tas et al. (11) in terms of the *Lactococcus* spp. and yeast contents of the kefir grains from Antalya, Turkey.

A wide variety of *Lactobacillus* species have been isolated from both kefir beverages and grains, including *L. kefiri, L. kefiranofaciens, L. kefirgranum* and *L. parakefiri* which constitute dominant populations (7, 12, 18, 20). However, some studies show that *Lactococcus lactis* were identified as dominant in the fermented product by both culturing and culture-independent techniques (4, 6, 20). Similarly, in our study we found that commercial Turkish kefir samples contained lactococci as dominant species. *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, which are thought to be closely associated with kefir and responsible for acidification, are commonly reported in kefirs (15). Similar to our results, in a study that was conducted in Bursa, Turkey, Cetinkaya et al. (3) determined lactobacilli, lactococci, enterococci, *Enterobacteriaceae* and yeast counts as $3.6x10^7$cfu/ml, $1.8x10^8$ cfu/ml, $4.8x10^4$ cfu/ml, $7.3x10^3$ cfu/ml, and $7.7x10^4$ cfu/ml, respectively. Also, it was reported that 22% (11/50) and 16% (8/50) of the samples were contaminated with *Escherichia coli* and *Staphylococcus aureus*, respectively.

Yeast is important in kefir fermentation because of the production of ethanol and carbon dioxide. Kefir usually contains lactose fermenting yeast such as *Kluyveromyces lactis, K. marxianus, Torula kefir*, also non-lactose-fermenting yeasts such as *Candida kefir* and *Saccharomyces cerevisae* were dominantly isolated (10, 15, 20). Besides these yeasts, *K. marxianus*, *Torulaspora delbrueckii*, *Saccharomyces unisporus*, *Pichia fermentans*, *Kazachastania aerobia*, *Lachancea meyersii*, *Yarrowia lipolytica* and *Kazachstania unispora* were also detected in various kefir studies from different countries (14, 15, 20, 23). In our study, we also found variable yeast counts in the kefir samples and household kefir samples contained much more contents of yeast than the commercial samples.

The origin of the kefir grains plays a significant role of constituting the microbial flora. Many studies from different countries have focused on identifying the content of kefirs grains. Simova et al. (20) detected that the kefir from Bulgaria contain *L. subsp. lactis, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, L. helveticus, L. casei subsp. pseudoplantarum* and *L. brevis*. In the study, identified yeasts (10–17

%) were *Kluyveromyces marxianus var. lactis, S. cerevisiae, Candida inconspicua* and *C. maris*. In Irish kefir, Rea et al. (18) determined the counts of lactococci, leuconostocs, lactic acid bacteria, acetic acid bacteria and yeasts at the end of the fermentation as 10^9, 10^8, 5×10^6, 10^5 and 10^6 cfu/ml respectively. In another study, Motaghi et al. (17) determined that *L. brevis, L. kefiri, Leuconostoc mesenteroides,* and *Acetobacter aceti* were predominant species in Iranian kefir. Among the yeasts, *S. cerevisiae, S. fragilis, S. lactis* and *Candida kefir* were isolated. *C. kefir* and *S. cerevisiae* were the most commonly isolated species when compared to the rest of the microflora.

As a conclusion, the results of this study show that commercial kefir samples contain higher amounts of lactococci than lactic acid bacteria and yeast compared to household kefir samples. The microbial content of kefir is primarily dependent on its source which can differ from region to region. Therefore, it is very important to make species-based identification of the microorganisms in the kefir to demonstrate the health benefits of the products. On the other hand, in one of the commercial kefir samples enterococci were observed and one of the household kefir samples was found to be contaminated with coliform bacteria. This can be considered as a fecal contamination during production where the kefir grain had been collected previously. However, it is hard to say how many passages have been made after the contamination occurred. At the same time, it is utmost important to decide whether to decontaminate or eliminate such contaminated kefir grains from production. For this reason, initial microbial population of kefir grains should be known and attention is needed to be paid to hygiene during the preparation of the product.

References

1. Altay F, Karbancıoglu-Güler F, Daskaya-Dikmen C, Heperkan D (2013): *A review on traditional Turkish fermented non-alcoholic beverages: Microbiota, fermentation process and quality characteristics.* Int J Food Microbiol, **167**, 44–56.

2. Bergmann RSO, Pereira MA, Veiga SMOM, Schneedorf JM, Oliveira NMS, Fiorini JE (2010): *Microbial profile of a kefir sample preparations: grains in natura and lyophilized and fermented suspension*. Ciênc Tecnol Aliment Campinas, **30**(**4**): 1022-1026.

3. Cetinkaya F, Elal Mus T (2012): *Determination of microbiological and chemical characteristics of kefir consumed in Bursa.* Ankara Üniv Vet Fak Derg, **59**, 217-221.

4. Chen HC, Wang SY, Chen MJ (2008): *Microbiological study of lactic acid bacteria in kefir grains by culture-dependent and culture-independent methods.* Food Microbiol, **25**(**3**): 492-501.

5. Diniz RO, Garla LK, Schneedorf JM, Carvalho JCT (2003): *Study of antiinflammatory activity of Tibetan mushroom, a symbiotic culture of bacteria and fungi encapsulated into a polysaccharide matrix.* Pharmacol Res, **471**, 49–52.

6. Dobson A, O'sullivan O, Cotter PD, Ross P, Hill C (2011): *High-throughput sequence-based analysis of the bacterial composition of kefir and an associated kefir grain.* FEMS Microbiol Lett, **320**(**1**): 56-62.

7. Garrote GL, Abraham AG, Antoni GL (2001): *Chemical and microbiological characterisation of kefir grains.* J Dairy Res, **68**, 639-652.

8. Guzel-Seydim ZB, Seydim AC, Grenee AK, Bodine AB (2000): *Determination of organic acids and volatile flavor substances in kefir during fermentation*. J Food Compos Anal, **13**(**1**): 35–43.

9. Guzel-Seydim Z, Wyffels JT, Seydim AC, Greene AK (2005): *Turkish kefir and kefir grains: microbial enumeration and electron microscobic observation*. Int J Dairy Technol, **58**(**1**): 25-29.

10. Irigoyen A, Arana I, Castiella M, Torre P, Ibanez FC (2005): *Microbiological, physicochemical, and sensory characteristics of kefir during storage*. Food Chem, **90,** 613–620.

11. Kok-Tas T, Ekinci FY, Guzel-Seydim ZB (2012): *Identification of microbial flora in kefir grains produced in Turkey using PCR*. Int J Dairy Technol, **65**(**1**): 126–131.

12. Leite AMO, Mayo B, Rachid CTCC, Peixoto RS, Silva JT, Paschoalin VMF, Delgado S (2012): *Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis*. Food Microbiol, **31**, 215-221.

13. Liu JR, Wang SY, Chen MJ, Chen HL, Yueh PY, Lin CW (2006): *Hypocholesterolaemic effects of milk-kefir and soya milk kefir in cholesterol-fed hamsters*. Br J Nutr, **95**(**5**): 939–946.

14. Magalhães KT, Pereira MA, Nicolau A, Dragone G, Domingues L, Teixeira JA, Silva JBA, Schwan RF (2010): *Production of fermented cheese whey-based beverage using kefir grains as starter culture: evaluation of morphological and microbial variations*. Bioresour Technol, **101**(**22**): 8843-8850.

15. Magalhães KT, Pereira GVM, Campos CR, Dragone G, Schwan RF (2011): *Brazilian Kefir: Structure, Microbial Communities and Chemical Composition*. Braz J Microbiol, **42**, 693-702.

16. Mistry VV (2004): *Fermented liquid milk products*. 939–957. In: YH Hui, Meunier-Goddik, L, Hansen OS, Josephsen J, Nip W, Stanfield PS, Toldra F (Eds.), Handbook of Food and Beverage Fermentation Technology. Marcel Dekker, New York.

17. Motaghi M, Mazaheri M, Moazami N, Farkhondeh A, Fooladi MH, Goltapeh EM (1997): *Short communication: Kefir production in Iran.* World J Microbiol Biotechnol, **13**, 579-581.

18. Rea MC, Lennartsson T, Dillon P, Drinan FD, Reville WJ, Heapes M, Cogan TM (1996): *Irish kefir-like grains: their structure, microbial composition and fermentation kinetics*. J Appl Bacteriol, **81**(**1**): 83–94.

19. Silva KR, Rodrigues SA, Xavier L, Lima AS (2009): *Antimicrobial activity of broth fermented with kefir grains*. Appl Microbiol Biotechnol, **152**(**2**): 316–325.

20. Simova E, Beshkova D, Angelov A, Hristozova T, Frengova G, Spasov Z (2002): *Lactic acid bacteria and yeasts in kefir grains and kefir made from them*. J Ind Microbiol Biotechnol, **28**, 1–6.

21. Urdaneta E, Barrenetxe J, Aranguren P, Irigoyen A, Marzo F, Ibanez FC. (2007): *Intestinal beneficial effects of kefir-supplemented diet in rats.* Nutr Res, **27** (**10**): 653–658.

22. Vinderola CG, Duarte J, Thangavel D, Perdigon G, Farnworth E, Matar C (2005): *Immunomodulating capacity of kefir*. J Dairy Res, **72**(**2**): 195–202.

23. Wang SY, Chen HC, Liu JR, Lin YC, Chen MJ (2008): *Identification of yeasts and evaluation of their distribution in Taiwanese kefir and viili starters*. J Dairy Sci, **91(10)**: 3798–3805.

24. Witthuhn RC, Schoeman T, Britz TJ (2005): *Characterisation of the microbial population at different stages of kefir production and kefir grain mass cultivation.* Int Dairy J, **15**, 383–389.

25. Wouters JTM, Ayad EHE, Hugenholtz J, Smit G (2002): *Microbes from raw milk for fermented dairy products.* Int Dairy J, **12(2-3)**: 91–109.

26. Wszolek M, Tamime AY, Muirs DD, Barclay MNI (2001): *Properties of kefir made in Scotland and Poland using bovine, caprine and ovine milk with different starter cultures*. Lebensm Wiss Technol, **34**, 251-261.

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