GIDA (2010) 35 (3): 177-182 *Research* /Araştırma

EFFECTS OF DIFFERENT MILK TYPES AND STARTER CULTURES ON KEFIR

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Received / Geliş tarihi: 01.06.2009
Received in revised form / Düzeltilerek geliş tarihi: 13.08.2009
Accepted / Kabul tarihi: 18.08.2009

Abstract
In this study, physicochemical and microbiological properties of kefir produced from cow, ewe and goat milk with grain and DVI cultures were investigated throughout 15 days storage period. Samples were taken after 1, 7 and 15 d of storage and acidity, pH, total solid as well as lactococci, lactobacilli and yeast counts were determined. Analyses of ethanol were carried out by using headspace gas chromatography. In all kefir samples, acidity increased during the storage period. While lactococci counts increased one logarithmic unit in the all DVI kefir samples at d 7, lactococci of grain kefir samples showed a decrease. Lactobacilli and yeast counts were similar at the first day in all samples. Coliforms and *E. coli* were absent. Concentration of ethanol was influenced by the type of starter culture and storage time.

Keywords*:* Kefir, lactic acid bacteria, yeast, ethanol

FARKLI SÜT ÇEŞİTLERİ VE STARTER KÜLTÜRLERİN KEFİR ÜZERİNE ETKİLERİ

Özet
Bu çalışmada, inek, koyun ve keçi sütü ile tane ve ticari kefir mayası kullanılarak üretilmiş kefirlerin 15 günlük depolama sürecinde kimyasal ve mikrobiyolojik özellikleri incelenmiştir. 1., 7. ve 15. günlerde alınan örneklerde asitlik, pH ve kurumadde tayinleri yapılmış, laktokok, laktobasil ve maya sayıları tespit edilmiştir. Etanol üretimleri head space gaz kromatografi ile belirlenmiştir. Kefir örneklerinin tümünde depolama süresince asitlik yükselmiştir. DVI kültürü kullanılarak üretilen kefir örneklerinde laktokok sayısı bir logaritmik birim artarken, tane ile üretilen kefir örneklerinde laktokok sayısı azalmıştır. Laktobasil ve maya sayısı bütün örneklerde ilk günkü değerlere benzer bulunmuştur. Kefir örneklerinde koliform ve *E. coli* tespit edilmemiştir. Etanol miktarı kullanılan starter kültürün çeşidine ve depolama süresine bağlı olarak değişmiştir.

Anahtar kelimeler: Kefir, laktik asit bakterileri, maya, etil alkol

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INTRODUCTION

Kefir is a fermented milk product which has acidic properties and has a slightly alcoholic flavor (1, 2). It is believed that kefir originated from the regions of Caucasian mountains (3). It is traditionally produced from kefir grains which are small, gelatinous, yellowish in colour, and look like small clamps of irregularly shaped cauliflower (4). These grains contain a mixture of the complex microflora such as lactic acid bacteria, yeasts and sometimes acetic acid bacteria as well as a polysaccharide matrix "kefiran". These microorganisms, being peculiar to kefir, inhibit the pathogen growth by the production of lactic acid, antibiotics and bactericide substances (5). Kefiran is also reported to show anti-tumor activity. Furthermore, kefir grains contain vitamins, minerals, amino acids and easily digestible complete proteins which are essential for a healthy body (6). Because of these characteristics, kefir is described as the yogurt of 21st Century (7). As the nutritional value and positive effects of kefir on health are better understood, commercial production of kefir is encouraged. However, by way of using kefir grains, the commercial kefir production is difficult. Although natural microflora of kefir grains has a symbiotic stability, the species and the amount of various microorganisms show significant variation in the production pathway, *kefir grains® kefir® secondary batch kefir* (8). The composition of the population may differ either on its source or the method and the substrates used (9).

Therefore, the usage of the cultures prepared to standardize kefir production is becoming increasingly widespread. Presently, in order to produce these cultures, which are special to kefir, microflora of kefir grains is studied. The microbial structure of the grains is extremely suitable to isolate pure cultures. Lactobacilli make up the largest portion (65-80%) of the microbial population and the remaining portion is lactococci and yeasts (10). Lactic acid bacteria and yeasts identified by isolating from kefir grains include: *Lactobacillus brevis*, *L. helveticus*, *L. kefir*, *Leuconostoc mesenteroides*, *Kluyveromyces lactis*, *K. marxianus* and *Pichia fermentas* (5). Defined and freeze-dried kefir cultures are commercially available for the production of bulk starter or for direct-to-vat inoculation (DVI) of the milk base (11). In parallel with this, due to the widespread availability of kefir grains commercially in place of kefir only being sold as beverage, a method for mass cultivation of kefir grains was recently developed (4).

Although kefir can be manufactured from any type of milk such as cow, ewe, goat, coconut, rice and soy, in general, cows' milk is used. After fermentation of milk with grain or commercial starter culture, lactic acid, acetic acid, CO_2, ethanol and aromatic compounds are formed. The unique aroma and flavor of kefir is the result of yeast and lactic acid bacteria coexisting in a symbiotic association (6, 12). Although kefir is manufactured by using various types of milk and kefir grains or by commercial starter cultures, little is known as to how these methods affect the quality of kefir.

In this study, the chemical and microbiological properties of kefir, which was manufactured either with grain or starter culture using cow, goat, ewe milk, were determined and the variations resulting after during a 15 day storage period were evaluated.

MATERIALS AND METHODS

Materials

In this study, cow, ewe and goat milk were provided from the region of Isparta, Turkey. For kefir production, two types of starter cultures were used:

a) Traditional kefir grains preserved in sterile saline solution,

b) DVI freeze-dried culture. (Kefir C1 Wiesby).

Manufacturing of kefir

Milks were heated to 95 °C for 15 min and cooled to 25 °C. Then each heat treated milk base was divided into two equal batches and used for manufacturing of kefir.

Kefir 1. Kefir grains were re-activated three times in heat treated milk. After each growth cycle at 25 °C for 18 hours, the grains were separated by using a sieve. Active kefir grains were inoculated into milk cooled to 25 °C with a 3% inoculation rate, and incubated at 25 °C for 22 hours until pH fell to ~4.5. After incubation, the grains were separated from kefir and washed with sterile water, then maintained at 4 °C until the next production.

Kefir 2. Commercial DVI starter culture composes of *Lactococcus lactis*, *Lc. cremoris*, *Lc. diacetylactis*, *Leuconostoc sp.*, *Lactobacillus kefyr*, *Candida kefyr*

and *Saccharomyces unisporus*. DVI kefir starter culture was inoculated (2%) into the heat treated and cooled milk and then incubated at 25 °C for 22 hours until pH fell to ~4.5 (11).

After incubation of kefir 1 and kefir 2, fermented products were cooled to 10 °C and stored at 4 °C. The samples which were taken from all groups immediately after incubation and at the 7th and 15th days of storage and chemical and microbiological analyses were carried out. The trials were performed in two replicates.

Chemical analyses

Total solid (TS), fat and titratable acidity (lactic acid %) were determined according to the Turkish Standards (13). The pH values of the kefir were measured with a pH meter (HANNA).

Antibiotic test was made by the production of acid in milk. The test could be completed in under 30 min, it provides a simple, routine guide to the acceptability of the milk for production.

Ethanol was analyzed using gas chromatograph (Perkin Elmer Autosystem xL). The system was equipped with a flame ionization detector (Perkin Elmer) with a 50 m CP WAX 52 capillary column (inner diameter, 0.32 mm; film thickness, 1.2 mm; Carbowax 20M, Varian). The chromatograph was connected to an automatic head-space sampler (Model: Turbo Matrix 16, Perkin Elmer). Operating parameters of the chromatograph were as follows: 25 psi of head pressure, 95 °C injector temperature and 250 °C detector temperature. The oven temperature was held 70 °C for 1 min; the temperature was the increased in increments of 4 °C/min up to 150 °C with a total cycle time of 5.5 min. The parameters of headspace sampler were as follows: 70 °C sample temperature, 30 min thermostat time, 80 °C needle temperature, 90 °C transfer line temperature, 3 min. pressurization time, and 0.04 min of injection time.

All chemical analyses were carried out in duplicate.

Microbiological analyses

Kefir samples (10 g) were weighed aseptically and homogenized in sterile Ringer's solution (Oxoid BR52; ¼ strength). In the same solution, decimal dilutions were prepared. By using a standard culture method, lactobacilli counts were determined in MRS Agar, lactococci in M17 Agar (Difco, USA) and yeast in PDA Agar (Difco, USA). Lactic acid bacteria were incubated at 30 °C for 24-48 hours, yeast and molds at 25 °C for 5 days. In the kefir samples Most Probable Number methods were used to determine coliforms and *E. coli* whether or not any contamination occurred during kefir making process (14-16). All microbiological analyses were conducted in duplicate.

Statistical analysis

Statistical analysis was performed by using the SAS System for Windows V7. Duncan's multiple range test was used to compare milk type with kefir quality (SAS Institute Inc., Cary, N.C., U.S.A).

RESULTS AND DISCUSSION

Quality of raw milk

Chemical properties of raw milk are shown in Table 1. The differences in total solids, fat and acidity (SH) arise from species of mammals. Total solid and fat differences among milk types were important statistically ($P<0.05$), while differences of acidity were not significant ($P>0.05$). Although the ewes' milk contained about twice more total solid and fat than cows' milk, goats' milk displayed similarities cows' milk. These results agreed with the average values of milk types (17) however differed from Wszolek and coworkers' findings (11). On the other hand the fat ratio of cows' milk does not conform to national legal specifications. Fat content of cows' milk should be minimum 3% in whole milk (18). There was not any antibiotic residue in milk samples.

Table 1. Chemical properties of raw milks

Milk	Total solid (g/100 g)	Fat (g/100 g)	Acidity (SH)	pH
Cow	10.84±0.41[a]	2.8±0	9.15±0.49	6.32±0.49
Ewe	18.18±1.90	7.0±1.13	10.1±0.70	6.54±0.02
Goat	13.04±0.94	4.5±1.13	8.05±0.63	5.98±0.58

[a]Data represent the mean values from two independent experiments and their standard deviations.

Enumeration of microorganisms

Although kefir grains contain various microorganisms (19), in this study 3 different microbiological media were used to make comparison for microbial counts between kefirs produced with two different starter cultures. The data obtained from microbial counts are given in Table 2. There is no correlation among the starter culture counts, storage period, and milk species (P>0.05). Lactococci counts in kefir samples manufactured from DVI starter culture increased to about 1 log unit during a 7 day storage period, while showing a slight decrease at 15th day except for kefir produced from goat's milk. On the other hand, lactococci in kefir samples from kefir grains declined throughout storage period, except for cows' milk kefir at 15th day. Lactobacilli remained almost stable during storage period in all types of kefir. Many studies have investigated the composition of the microorganisms present in kefir grains and reported that *Lactobacillus* is the most frequently found microbe (2, 20). Fontan et al (21) have shown, in the manufacture of kefir, by fermentation of heat-treated cows' milk using a commercial starter culture, *Lactococcus* spp. predominated during the first 24h, but *Lactobacillus* spp. became the most abundant microbial group on increasing fermentation time to 168h. No *Leuconostoc* strains were isolated during the fermentation process. Yeasts developed during late fermentation (>48 h) and reached a final mean count that was lower than that reported by other authors for kefir (21).

Yeast counts changed slightly in kefir samples with DVI starter culture; however a variation was determined in other kefir samples. Lactic acid bacteria counts were higher than yeast counts. Although microbial population of kefir grains is changed by the miscellaneous factors, similar results were obtained for the counts of lactic acid bacteria and yeasts, in the various studies (4, 11, 12, 22). However in this study, yeast counts of DVI kefir were higher than grain kefir. Wszolek et al. (11) were found 6.66 log cfu/g yeast in a commercial kefir culture. According to kefir flavor as sour or mild, yeast contents of commercial starter cultures could be changed (23). Kefir produced with grains is reported to contain yeasts in a wide range such as 10^4-10^7 cfu/ml (11, 23, 24). The yeasts can be irregularly distributed in kefir grains. As electron microscopy has shown, lactose negative yeasts are more prevalent in the center of grain, whereas lactose-positive yeasts settle more on the surface. Too much washing leads to an impoverished yeast population on the grains' surface (23). For this reason, it can be suggested that the excessive washing of grains may cause the reduction of yeast counts.

Molds and Coliforms were not detected in kefir samples. This was accepted as an indication of good sanitary conditions during manufacture of kefir.

Chemical properties of kefir samples

Chemical properties of kefir samples are given in Table 3. The data showed that pH values, total solid, ethanol amounts were affected by the milk and culture types and storage period (P<0.05). Although pH values of all groups decreased with the increase of storage period, more rapid reduction occurred in kefir samples with grain. pH of kefir samples with DVI starter culture reduced slightly at the first week, while a rapid acidity increase was

Table 2. Microbial counts of kefir samples made from different starter cultures (log cfu/g)

Microorganisms	Days	Cow		Ewe		Goat	
		Grain	DVI	Grain	DVI	Grain	DVI
Lactococcus spp.	0	8.176	7.267	8.148	7.491	8.193	7.176
	7	7.255	8.059	7.001	8.154	6.698	8.180
	15	8.230	7.560	6.906	7.707	6.039	8.265
Lactobacillus spp.	0	8.146	7.622	8.822	7.897	8.087	7.750
	7	8.568	7.406	8.309	7.771	8.585	7.742
	15	8.505	7.531	8.404	7.631	7.477	7.788
Yeasts	0	4.698	6.658	4.207	6.150	4.847	6.403
	7	5.301	6.430	4.419	6.658	4.265	6.637
	15	5.342	6.372	5.671	6.007	5.056	6.176

Table 3. Chemical properties of kefir samples

Properties	Days	Cow		Ewe		Goat	
		Grain	DVI	Grain	DVI	Grain	DVI
pH	0	4.50	4.47	4.29	4.63	4.50	4.44
	7	4.10	4.48	3.85	4.51	3.60	4.42
	15	4.10	4.30	4.10	4.37	3.60	4.23
Total solid (g/100g)	0	11.15±1.13	11.07±2.27	21.20±1.27	22.21±3.24	13.02±2.92	13.99±1.63
	7	11.09±2.32	10.98±4.59	20.49±2.65	21.20±2.92	13.58±1.01	13.65±0.60
	15	10.85±3.01	11.00±2.97	20.67±3.82	21.41±4.02	13.54±0.52	13.28±1.81
Ethanol (mg/ml)	0	0.062±0.03[a]	1.210±0.06	0.286±0.06	0.630±0.06	0.039±0.03	0.696±0.06
	7	0.282±0.06	1.845±0.53	0.142±0.02	0.703±0.06	0.106±0.04	1.335±0.01
	15	0.506±0.06	2.080±0.19	0.167±0.03	1.390±0.05	0.128±0.06	1.455±0.47

[a]Data represent the mean values from two independent experiments and their standard deviations.

determined at the second week of storage. The highest acidity developed at grain goat kefir samples. pH decreased to 3.6 at 7 days of storage and remained stable after 15 days of storage. Similar tendency was observed at DVI goat kefir samples. Although the initial pH values of all milk types were similar, decrease of pH in kefir samples produced from goat's milk was relatively more. Starter culture type, storage period ($P<0.05$) and mammals species ($P<0.05$) had significant effects on these pH changes. Oktar and Karagözlü (25) and Koroleva (26) reported similar results for different type of kefir samples.

The total solid content of kefir samples with grain decreased slightly except for goat milk kefir during storage, while a significant drop of total solid in kefir samples with commercial starter culture was not determined ($P>0.05$). Ethanol which is an aroma component in kefir is formed in all of milk types by mainly yeasts. During the storage, ethanol regularly increased except for grain in kefir samples produced from ewe's milk. The highest ethanol amounts were determined in DVI in kefir samples produced from cows' milk after 15 days of storage. However, milk's type was not important for ethanol production ($P>0.05$). DVI cultures produced ethanol higher amount than grain cultures. These differences are originated from the microflora of the products. So, it is concluded that the changes of ethanol quantity is important according to the storage time and culture type ($P<0.05$). The reported ethanol contents of kefir samples had a wide range between 0.01 and 1% (27).

CONCLUSIONS

Kefir could be produced by using various milk and culture types. Chemical and microbiological properties of these products were similar. Lactobacilli, lactococci and yeasts composed of the dominant microflora of all kefir types. However kefir made by using commercial starter culture had differences in ethanol content compare with grain. Ethanol contents were influenced by the storage time. As considered the concentration of ethanol contents, commercial starter culture was found to be suitable to kefir production. The effects of seasonal variations and milk type on the kefir properties were not searched in Turkey, mainly the chemical and microbiological characteristics of various kefir samples were determined in this study. At the second step of the project the effects of seasonal variations and milk type on the production of aroma compounds such as; acetaldehyde, acetoin and diacetyl in kefir will be determined.

Acknowledgement

The authors are grateful to Prof. Dr. Ender Olcayto for editing the manuscript.

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