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Effect of different fermentation conditions on composition of kefir microbiota

Hecer, C., *Ulusoy, B. and Kaynarca, D.

Near East University Faculty of Veterinary Medicine Food Hygiene and Technology Department, Nicosia, Cyprus

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Abstract

In the present work, experimental groups were incubated at 20°C, 25°C and 30°C for 18, 24 and 48 h, and at 4°C for 3, 6 and 9 d. The aim was to investigate the microbiota changes under different incubation conditions. The results demonstrate that the total dry matter (%) values of the samples were very close to each other and were not significantly different ($p > 0.005$) by the different fermentation conditions. The microorganisms were enumerated and identified by VITEK® 2 Compact automated ID/AST instrument and API System. *Kocuria* spp. was obtained in the groups incubated at 4°C, but was not isolated in the groups incubated at 20°C, 25°C and 30°C. *Micrococcus* spp. and *Candida kefyr* were isolated in all groups. *Leuconostoc* spp. was isolated from all groups except from K10 (4°C, 3 d), K11 (4°C, 6 d) and K12 (4°C, 9 d). Lower averages of general acceptance scores were obtained for K10, K11 and K12. It can be concluded that incubations at 25°C for 18 h and also 24 h can be accepted as the most suitable values for producing traditional kefir. On the other hand, according to the unique pH value, the group incubated at 30°C in 24 h (K8) can be considered as the most suitable one.

Introduction

Kefir is a refreshing fermented dairy beverage with an alcoholic flavour and a specific aroma. It is easy to digest and rich in probiotic microbiota (Beshkova *et al*., 2003; Satır and Seydim, 2016). Beside the desired aroma of kefir, this fermented beverage has health-promoting influences for both humans and animals (Vinderola *et al*., 2005; Urdanate *et al*., 2007). Numerous health benefits are also associated with the composition of naturally occurring probiotics and prebiotics in kefir. Sabir *et al*. (2010) studied the probiotic properties of lactic acid bacteria (LAB) isolated from kefir and they demonstrated that all *Lactobacillus* spp., *Lactococcus*s spp., and *Pediococcus* spp. strains show good probitic activity because they were all able to survive at low pH, at different bile salt concentrations, and were able to auto aggregate and co-aggregate with *Escherichia coli* (*E. coli*). Similarly in another study, Santos *et al*. (2003) reported that *Lactobacillus acidophilus* (*L. acidophilus*) and *Lactobacillus kefiranofaciens* (*L. kefiranofaciens*) also demonstrated good probiotic characteristics. Kefir can be produced by fermenting milk with commercial freeze-dried kefir starter culture or traditional kefir grains. These kefir grains contain combination of different microorganisms, some of which are listed as follows: LAB (*Lactobacillus brevis*, *L. acidophilus*, *Lactobacillus casei*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii*), yeasts (*Kluyveromyces* spp., *Candida* spp., *Torulopsis* spp. and *Saccharomyces* spp.), streptococci (*Streptococcus salivarius*), Lactococci (*Lactococcus lactis* ssp. *lactis*, *Leuconostoc cremoris*, *Leuconostoc mesenteroides*) and occasionally acetic acid bacteria are also included (Simova *et al*., 2011). Microorganisms exist in a matrix composed of polysaccharide referred as kefiran (Bosch *et al*., 2006; Zajsek and Gorsek, 2010). According to a study conducted by Fontan *et al*. (2006), the quality of kefir depends on the concentration and mixture of flavour compounds such as lactic acid, acetaldehyde, ethanol, acetoin, diacetyl and carbon dioxide which are produced in milk by the help of the microbiota found on the kefir grains.

The species and the amount of various microorganisms show significant variation based either on its region of origin or the methods of production, fermentation conditions and the substrates used (Pintoda *et al*., 1996; Beshkova *et*

*Corresponding author.
Email: kolayisim@gmail.com

al., 2003; Cetinkaya and Elal-Mus, 2012; Altay *et al*., 2013). Fermentation conditions such as time and temperature are important parameters that influence the microbiota of kefir grain and also kefir produced by this grain (Guzel-Seydim *et al*., 2010; Altay *et al*., 2013). Kaptan *et al*. (1990) studied the effect of incubation temperature on some properties of kefir. In that study; pH, titratable acidity, viscosity, whey separation, amounts of acetaldehyde, alcohol and carbon dioxide as well as sensorial properties were compared in kefir samples incubated at 20 ± 1°C, 25 ± 1°C and 30 ± 1°C for 16 h, following 2 d of ripening. Leite (2012), produced kefir experimentally with incubation at 25°C, and every 6 h during fermentation, analysis was performed in order to evaluate the microbial community composition and chemical characteristics of a Brazilian milk kefir.

The purpose of the present work was to investigate microbiota changes under different incubation conditions. Generally, for the traditional production of kefir, fresh kefir grains are added to pasteurised milk at the rate of 2-3% (v/v) and incubated at 24-26°C for approximately 18-20 h, especially for homemade kefir. After incubation, the product is stored under cold chain (Guzel-Seydim *et al*., 2000). Based on these previous findings, in the present work, the incubation temperatures and periods were selected as 20 ± 1°C, 25 ± 1°C and 30 ± 1°C for 18, 24 and 48 h. In Turkey, there is a tradition to incubate the kefir in refrigerator for a longer incubation period. This is the reason why incubation at 4 ± 1°C for 3, 6 and 9 d was also introduced into the present work. Isolated microorganisms from the experimental samples were identified by using VITEK® 2 systems and confirmed by API.

Materials and methods

Reactivation of kefir grains before inoculation

The preparation and inoculation rate of kefir grains were modified from the method reported by Oner *et al*. (2010). Kefir grain was obtained from the Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Near East University. Pasteurised bovine milk was used in the experiment. Kefir grains were reactivated three times in milk. After each growth cycle at 25°C for 18 h, the grains were separated by using a sieve. Active kefir grains were inoculated into 12 sterile glass jars filled with milk at 20°C. Next, 3% (wet kefir weight/mL milk) kefir grains were inoculated into each milk samples in glass jars. After incubation, the grains were separated from kefir and washed with sterile water, then maintained at 4°C in sterile milk for a week for the second batch trial (Mistry, 2004; Oner *et al*., 2010).

Sampling plan of experimental kefir production

The experimental groups were incubated at 20°C, 25°C, 30°C and 4°C. Twelve different experimental groups were coded as follows: K1, K2 and K3 at 20°C for 18, 24 and 48 h, respectively. K4, K5 and K6 at 25°C for 18, 24 and 48 h, respectively. K7, K8 and K9 at 30°C for 18, 24 and 48 h, respectively. K10, K11 and K12 at 4°C for 3, 6 and 9 d, respectively. Care was taken to ensure that all conditions such as air flow, inoculating procedures, amount of milk, the grain and grain rate for inoculation, shapes and sizes of jars (except for the time periods and temperature) were the same for manufacturing of all kefir samples. All incubations were performed in a thermostatically controlled incubator. Following incubation, the products were cooled to 10°C, then microbiological, physico-chemical and sensorial analyses were conducted on each sample. The trials were performed in two replicates.

Enumeration of microorganisms

Kefir samples (10 mL) were weighed aseptically and homogenised in sterile Maximum Recovery Diluent (MRD, LAB103). Decimal dilutions were prepared in sterile tubes containing 9 mL of MRD. Standard culture methods were used to enumerate the microorganisms. *Lactobacillus* spp. counts were determined in De Man Rogosa Sharpe Agar (MRS Agar, LAB093), *Lactococcus* spp. in M17 Agar (MERCK, 115108) and yeast in Sabaroud Dextrose Agar (SDA, LAB 009) supplemented with chloramphenicol (LAB X009). *Lactobacilli* on MRS plates and *Lactococcus* spp. on M17 plates were incubated at 37°C for 48 h. Yeast on SDA plates were incubated at 25°C for 5 d. All microbiological analyses were conducted in duplicate (Harrigan and McCance, 1966). Microbiological data were transformed into logarithms of the number and presented as log colony forming unit (log CFU/mL).

Identification of isolated microorganisms

After enumeration, the colonies were selected for identification using VITEK® 2 Compact automated ID/AST instrument. After primary organism isolation, standardised inoculums were prepared for all selected colonies according to the manufacturer's instructions. VITEK®2 YST and VITEK® 2 GP (bioMérieux, France) Cards were used for identification. Carbohydrate confirmation was assessed by using commercially available API 20C AUX, API STAPH and API 20 STREP kits (bioMérieux, France). Microorganisms were identified to the species level

through the use of APILAB PLUS (Version 3.2.2., bioMérieux, France).

Physico-chemical analysis

The pH values of the kefir were measured with a pH meter (WTW inoLab pH7110). The total dry matter (%) and titratable acidity (lactic acid %) were determined according to the Turkish Standards Institute (TSE, 1988). All chemical analyses were carried out in duplicate.

Sensorial analysis

Sensorial evaluation was conducted with 10 untrained panellists (8 women and 2 men, age 25–30). Kefir samples (125 mL) were served and presented to the panellists in cups which were coded with random three-digit numbers. The panellists were all informed about the experimental product and the aim of the study before evaluation. However, they were not informed what the codes referred to. Evaluations were conducted in a room, under normal white fluorescent illumination. Panellists were asked to drink water and eat plain white bread before tasting each sample during the sensory sessions. The attributes considered were flavour (flavour intensity-FI, dairy taste-DT, sour taste-ST, bitter taste-BT and astringency-AST), odour (odour intensity-AI, milky odour-MO, fermented odour-FO and unacceptable odour-UO), consistency and general acceptability. These attributes were modified from the study of Irigoyen *et al*. (2005). Odour and flavour were scored on an increasing scale from 1 to 4, (1: very intense, 2: less intense, 3: undecided, 4: undetectable). For consistency evaluation, panellists were asked to mark one of the choices as liquid, normal or thick. For general acceptability panellists were asked to score the samples from 1 (unacceptable) to 5 (excellent).

Statistical analysis

All descriptive statistics of the research variables were calculated. For categorical variables frequency and percentage were calculated while for continuous variables arithmetic mean, standard deviation, median, minimum and maximum values were shown. Since the data did not meet parametric assumptions, non-parametric hypothesis tests were applied. For each variable (assay) studied in the present work, non-parametric hypothesis testing method was applied. Multiple groups were compared with Kruskal Wallis test and in the case of significance; Mann Whitney U test with Bonferroni correction was performed to test pairwise differences. SPSS (Demo Version 22.0) software was used for all analyses. The level of significance was accepted to be 0.05.

Results and discussion

Physico-chemical analyses

Median values for physico-chemical analysis are presented in Table 1. The lowest and the highest pH values were obtained for K9 and K10, respectively. Accordingly, the highest percentage of lactic acid was obtained for K9 and the lowest value was measured for K10. Kaptan *et al*. (1990) detected pH values of 4.58, 4.51 and 4.47 for the fermentation conditions at 20 ± 1°C, 25 ± 1°C and 30 ± 1°C for 16 h, respectively. In the present work, the most reduction of pH was observed at 30°C which is a similar result with Kaptan *et al*. (1990). Kefir was incubated until pH level was close to 4.5 as performed in the study conducted by Oner *et al*. (2010). They concluded that kefir has its unique properties at this pH value and they reached pH 4.5 at 25°C in 22 h. In the present work, pH 4.55 was achieved at 30°C in 24 h. The results demonstrated that, total dry matter (%) values of the samples were very close to each other and were not significantly affected ($p > 0.005$) by different fermentation conditions. In previous studies, the obtained total dry matter contents ranged between 8.88–16.73% (Ertekin and Guzel-Seydim, 2010; Uslu, 2010; Cetinkaya and Elal-Mus, 2012). The results obtained in the present study were also in the range of those values. Kaptan *et al*. (1990) fermented experimental kefir samples at 20 ± 1°C, 25 ± 1°C and 30 ± 1°C for 16 h, and similar to the results of the present work, they did not obtain a difference between dry matter values. Physico-chemical values of kefir are affected by factors such as the microbial quality of kefir grains, the grain to milk ratio, fermentation time and temperature, hygienic conditions and storage temperature (Altay *et al*., 2013).

Enumeration and identification of microorganisms

Lactobacillus spp. and yeast counts increased in K2 (20°C, 24 h) by nearly 1, 2, and 1 log, respectively. The difference between *Lactobacillus* spp. and yeast counts at 25°C was less than 1 log in all incubation periods. The difference between the yeast counts at 24 h and 48 h at 20°C was above 1 log. At 4°C, although the highest numbers for all microorganisms were determined on the sixth day, these values were lower than the values obtained in the other groups incubated at 4°C. Once the temperature trials were evaluated in accordance with the incubation periods, the highest counts for all microorganisms were obtained at 20°C for 48 h. From the same point of view, the lowest counts were obtained on the third day of incubation at 4°C. The results of enumerations are presented in Table 2.

Table 1: Physico-chemical analysis results.

Groups	pH		Total dry matter (%)		Titratable acidity (lactic acid %)	
	Median	Min-Max	Median	Min-Max	Median	Min-Max
K1	5.25	5.20-5.30	11.15	11.10-11.20	0.42	0.40-0.45
K2	5.20	5.10-5.30	11.05	11.00-11.10	0.45	0.45-0.45
K3	4.80	4.60-5.00	11.05	11.00-11.10	0.49	0.48-0.50
K4	4.85	4.80-4.90	11.30	11.20-11.40	0.46	0.45-0.47
K5	4.85	4.70-5.00	11.35	11.30-11.40	0.51	0.50-0.52
K6	4.30	4.30-4.30	11.40	11.30-11.50	0.61	0.61-0.61
K7	4.70	4.60-4.80	11.45	11.40-11.50	0.52	0.52-0.53
K8	4.55	4.50-4.60	11.50	11.50-11.60	0.47	0.45-0.48
K9	3.90	3.90-3.90	11.55	11.50-11.60	0.85	0.85-0.86
K10	5.65	5.50-5.80	11.10	11.10-11.10	0.31	0.30-0.32
K11	5.25	5.20-5.30	11.00	11.00-11.00	0.46	0.45-0.47
K12	5.25	5.15-5.35	11.30	11.20-11.40	0.45	0.43-0.47

Table 2: Microbiological analysis results (log10 CFU/mL).

Groups	*Lactobacillus* spp.		*Lactococcus* spp.		Yeast	
	Median	Max-Min	Median	Max-Min	Median	Max-Min
K1	6.04	6.80-6.17	6.85	6.79-6.90	6.09	6.04-6.14
K2	7.11	7.07-7.11	8.28	8.11-8.44	7.46	7.23-7.68
K3	7.54	7.27-7.69	8.78	8.72-8.84	7.53	7.34-7.72
K4	6.17	6.17-6.17	6.91	6.90-6.91	5.85	5.77-5.92
K5	6.47	6.47-6.47	6.87	6.83-6.90	5.67	5.44-5.90
K6	6.70	6.69-6.71	6.64	6.43-6.84	7.23	7.23-7.23
K7	6.37	6.14-6.60	6.68	6.36-7.00	6.15	6.00-6.30
K8	7.12	6.53-7.71	7.92	7.90-7.95	7.30	7.27-7.30
K9	6.29	6.11-6.47	7.78	7.72-7.84	6.43	6.17-6.69
K10	4.45	4.44-4.47	4.91	4.92-4.90	4.17	4.00-4.34
K11	5.70	5.60-5.79	6.47	6.17-6.77	6.04	6.04-6.04
K12	5.47	5.47-5.47	5.84	5.84-5.84	6.02	6.00-6.04

Kefir and kefir grains can be affected from the ratio of kefir grains to milk, the origin and method of cultivation (Beshkova *et al*., 2003; Fröhlich-Wyder, 2003; Mistry, 2004; Witthuhn *et al*., 2005; Cetinkaya and Elal-Mus, 2012). Koroleva (1991) described the microorganisms composition of properly prepared kefir and according to this description kefir's microbiota should contain homofermentative mesophilic lactic acid streptococci (10^8 –10^9 CFU/mL), thermophilic *lactobacilli* (10^5 CFU/mL), heterofermentative lactic acid streptococci (10^7 – 10^8 CFU/mL), yeasts (10^5 – 10^6 CFU/mL), acetic acid bacteria (10^5 – 10^6 CFU/mL). In the present work, *Lactococcus* spp. counts were higher than *lactobacilli* in all experimental groups. Bozkurt *et al*. (2010) reported the number of *Lactobacillus* spp. and *Lactococcus* spp. as log 7–8 CFU/mL and log 7–8 CFU/mL, respectively. These findings are also similar with the findings of the present work except with sample groups K10, K11 and K12 which were incubated at 4°C. Zanirati *et al*. (2015) found in that *Lactococcus* and *Lactobacillus* were predominant in their study. They also obtained *Leuconostoc* which is similar found in the present work except for groups K10, K11 and K12 which were incubated at 4°C (Table 3).

Enterococcus faecalis (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) are commonly found in milk and cheese products and have been found to have a positive influence on ripening of traditional cheeses (Franz *et al*., 1999; Foulquie Moreno *et al*., 2006). In addition to their technological properties, many strains of *enterococci*, mainly *E. faecalis* and *E. faecium*, may produce a variety of bacteriocins active against food-borne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium*

Table 3: VITEK® 2 Compact identification and API confirmation.

Microorganism	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12
Enterococcus durans	√*	√*	√*	√*	√*	√*	√*	√*	√*			
Enterococcus faecium					√	√	√	√				
Lactococcus lactis ssp. lactis	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*
Lactococcus garvieae		√	√			√		√		√	√	√
Kocuria rhizophila										√*	√*	√*
Kocuria kristinae										√*	√*	√*
Kocuria rosea										√*	√*	√*
Leuconostoc spp.	√*	√*	√*	√*	√*	√*	√*	√*	√*			
Micrococcus spp.	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*
Candida kefyr	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*	√*
Saccharomyces cerevisiae										√	√	√
Candida lipolytica (Y. lipolytica)		√		√*	√*	√*	√		√			

√: Identified by VITEK® 2 Compact

*: Confirmed by API System

botulinum, *Clostridium perfringens* and *Vibrio cholerae*. *Enterococci* are accepted as probiotics for humans or farm animals (Ogier and Serror, 2008). It should be noted that *Enterococci* are not considered as "Generally Recognized as Safe" (GRAS) and they can be an indicator of faecal contamination especially in water (Godfree *et al*., 1997). *Enterococci* presence in dairy products can either pose as a risk for human health (if in excessive numbers) or as a benefit by through the production of unique traditional sensorial properties and also in protecting against diverse spoilers and as a probiotic (Giménez-Pereira, 2005). In the present work, *Enterococcus durans* (*E. durans*) formed part of the microbiota profile of the samples fermented at 20°C, 25°C and 30°C but not in the samples that fermented at 4°C. This result was obtained by VITEK® 2 Compact and also confirmed by API systems. *E. faecium* was obtained at 25°C and 30°C by VITEK® 2 but not by using API systems. The microorganisms identified by VITEK® 2 Compact and the ones confirmed by API Systems are presented in Table 3.

In the present work, *Kocuria rhizophila*, *Kocuria kristinae*, *Kocuria rosea* were detected only in samples incubated at 4°C. *Kocuria* are classified as psychotropic bacteria (Patil and Gandhi, 2012; Mane and Gandhi, 2012), which supports the results obtained in the present work. *Kocuria* spp. has also been shown to be responsible for the spoilage of cold-stored dairy products. These species may produce heat-stable extracellular lipases which may cause spoilage and also a characteristic aroma in milk and milk products. Furthermore, *Kocuria rosea* may cause opportunistic infections in immune-compromised individuals (Mane and Gandhi, 2010, 2012). When the panellists' sensorial scores was analysed, it was found that incubation at 4°C for 3 d (K10) generated statistically significant different scores ($p < 0.005$) in flavour a stringency as compared to all other experimental groups. This may be due to the growth of *Kocuria* spp. in K10 (Table 3).

The yeasts commonly isolated from kefir grains are non-lactose fermenting *Saccharomyces cerevisiae* (*S. cerevisiae*) and lactose fermenting such as *Candida kefyr* (*C. kefyr*) (Angulo *et al*., 1993; Güzel-Seydim *et al*., 2000; Simova *et al*., 2002; Magalhaes *et al*., 2011). In the present work, *S. cerevisiae* was identified in only K10, K11 and K12 but *C. kefyr* was obtained in all groups. *Candida lipolytica* (*C. lipolytica*) which is re-named as *Yarrowia lipolytica* (*Y. lipolytica*) has been reported to be found in fermented milk products such as yoghurt, kefir and amasi (Fröhlich-Wyder, 2003; Groenewald *et al*., 2014). Usually, yeasts reported for kefir are the same as those species causing spoilage in other milk products (Fröhlich-Wyder, 2003). Groenewald *et al*. (2014) mentioned in their review that *Y. lipolytica* may be spoilage yeast in some cases, in dairy products such as cheese, due to off-flavour components. Frohlich-Wyder (2003) proposed as to whether all yeasts belonging to the specific kefir were normal flora or contaminants. In the present work, *C. lipolytica* was identified and also confirmed in the groups of K4, K5 and K6. However, it was not detected in the groups incubated at 4°C. In groups K2, K7 and K9, *C. lipolytica* was identified using the VITEK® 2 Compact but not by the API systems.

Sensorial analysis

Median values of panellists' scores on flavour and odour attributes are presented in Table 4 which demonstrates no significant differences in all groups incubated at 20°C and 25°C (K1, K2, K3, K4, K5 and K6) for all sensorial attributes. K9 was found to have significant difference in OI than K1 ($p < 0.05$). On the other hand, K10 incubated at 4°C for the shortest period, presented a significantly different DT as compared to groups K2, K4, K5, K6, K8 and K9, and also significantly different for odour intensity from groups K6 and K9. The general lack of significant differences in sensory evaluation conducted in the present work, especially between the groups incubated at 20°C and 25°C, may be due to similar physico-chemical properties between these groups. *S. cerevisiae* was only identified in the groups incubated at 4°C. This result may be responsible for the different sensorial scores obtained. The mean values of general acceptance scores are presented in Figure 1. According to the results, K4 (at 25°C for 18 h) yielded the highest score and was the most liked experimental group. All the experimental groups fermented at 25°C (K4, K5 and K6) had an average score of over 3 of 5, but the scores of the groups fermented at 4°C (K10, K11 and K12) had an average scores of equal and below 2. In the present work, *C. lipolytica* was identified in groups K4, K5 and K6. *C. lipolytica* has been reported to cause good organoleptic characteristics, in terms of aroma and texture of cheeses (Fröhlich-Wyder, 2003; Groenewald *et al*., 2014). This data can explain the high acceptance scores for those groups. The pH of these groups also reached the optimum for unique kefir properties.

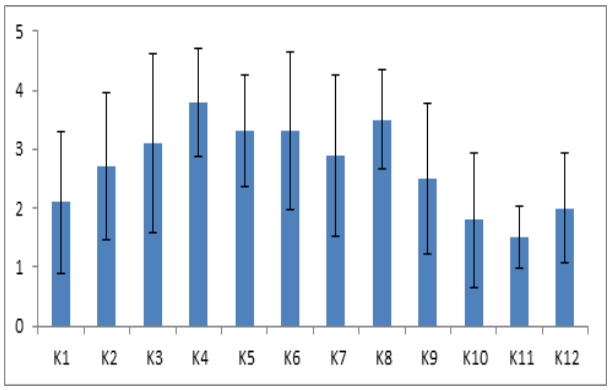

Figure 1: Mean values of general acceptance scores.

Table 4: Median values of panellists' scores on flavour and odour attributes.

Groups	Flavour Median (Min-Max)					Odour Median (Min-Max)			
	FI	DT	ST	BT	AST	OI	MO	FO	UO
K1	2.00 1.00-4.00	1.50 1.00-4.00	3.00 1.00-3.00	4.00 2.00-4.00	2.00 1.00-4.00	3.00 2.00-4.00	2.00 1.00-4.00	3.00 1.00-4.00	4.00 3.00-4.00
K2	2.00 1.00-4.00	1.00 1.00-3.00	3.00 1.00-4.00	4.00 2.00-4.00	2.00 1.00-4.00	2.00 1.00-4.00	2.00 1.00-3.00	3.50 1.00-4.00	4.00 2.00-4.00
K3	1.00 1.00-3.00	2.50 1.00-4.00	2.00 1.00-4.00	4.00 1.00-4.00	2.00 1.00-4.00	2.00 1.00-4.00	2.00 1.00-4.00	2.00 1.00-4.00	4.00 3.00-4.00
K4	1.50 1.00-3.00	2.00 2.00-4.00	3.50 1.00-4.00	4.00 1.00-4.00	2.00 1.00-3.00	1.50 1.00-4.00	2.00 1.00-4.00	1.50 1.00-4.00	4.00 3.00-4.00
K5	2.00 1.00-3.00	2.00 1.00-4.00	3.00 2.00-4.00	4.00 2.00-4.00	2.00 1.00-4.00	2.00 1.00-4.00	2.00 1.00-4.00	2.00 1.00-4.00	4.00 2.00-4.00
K6	1.00 1.00-3.00	3.00 2.00-4.00	1.50 1.00-4.00	4.00 1.00-4.00	1.00 1.00-3.00	1.50 1.00-2.00	3.00 1.00-4.00	1.00 1.00-4.00	4.00 3.00-4.00
K7	2.00 1.00-3.00	2.00 1.00-4.00	2.50 1.00-4.00	4.00 1.00-4.00	2.50 1.00-4.00	2.00 1.00-3.00	2.00 2.00-3.00	2.00 1.00-4.00	4.00 3.00-4.00
K8	1.00 1.00-3.00	4.00 [b] 2.00-4.00	2.50 1.00-4.00	4.00 1.00-4.00	2.00 1.00-3.00	1.00 2.00-4.00	1.50 1.00-4.00	2.00 1.00-4.00	4.00 3.00-4.00
K9	1.00 1.00-4.00	2.00 1.00-4.00	1.00 1.00-4.00	4.00 1.00-4.00	1.00 1.00-2.00	1.00[a] 1.00-2.00	2.50 1.00-4.00	2.00 1.00-4.00	4.00 1.00-4.00
K10	4.00 1.00-4.00	1.00 1.00-4.00	1.00 1.00-4.00	4.00 1.00-4.00	4.00[b,d,e,f,h,i] 3.00-4.00	4.00[f,i] 2.00-4.00	1.00[g] 1.00-2.00	4.00 2.00-4.00	4.00 3.00-4.00
K11	3.50 1.00-4.00	1.50 1.00-4.00	4.00 1.00-4.00	4.00 1.00-4.00	4.00 1.00-4.00	2.00 1.00-4.00	1.00 1.00-3.00	2.50 1.00-4.00	4.00 3.00-4.00
K12	2.00 1.00-4.00	1.50 1.00-4.00	4.00 1.00-4.00	3.00 2.00-4.00	1.50 1.00-4.00	2.00 1.00-4.00	1.50 1.00-3.00	3.50 1.00-4.00	4.00 3.00-4.00

Following letters were assigned to indicate statistically significant differences ($p < 0.05$);

a: from K1, b: from K2, c: from K3, d: from K4, e: from K5, f: from K6, g: from K7, h: from K8, i: from K9, j: from K10, k: from K11 and l: from K12

FI: flavour intensity, DT: dairy taste, ST: sour taste, BT: bitter taste, AST: astringency, OI: odour intensity, MO: milky odour, FO: fermented odour, UO: unacceptable odour

All the panellists' agreed that incubation at 4°C for 18 h and 24 h (K10 and K11) makes kefir liquid consistent. 100% panellists decided and marked as liquid for K10 and K11. 80% panellists decided that kefir was in liquid form in groups at 4°C for 48 h (K12). On the other hand, the groups incubated at 20°C for 18 h, 24 h and 48 h (K1, K2 and K3) were also decided to be liquid by panellists with ratios of 60%, 70% and 70%, respectively. Normal kefir consistency was observed at 30°C for 18 h (K7) by 80% panellists, and in all groups that incubated at 25°C (K4, K5 and K6) by 80%, 60% and 60% of the panellists, respectively. The panellists stated that incubation for 24 and 48 h at 30°C (K8 and K9) made the consistency of kefir thicker than normal. As Yaman (2011) mentioned in a review; kefir should have creamy consistency. In the present work, "normal consistency" referred to the creamy consistency intended.

Conclusion

The results demonstrated that, total dry matter (%) values of the samples were very close to each other and were not significantly affected ($p > 0.005$) by different fermentation conditions. It was also found that pH 4.55 was accepted as unique pH value for kefir at 30°C in 24 h. Incubation at 4°C was found not a suitable condition for producing unique properties of kefir. In Turkey, when preparing homemade kefir using kefir grains, incubation at 4°C is also being applied. That was the reason why we designed these experimental groups (K10, K11 and K12). Based on the microorganism counts, the experimental groups incubated at 4°C did not meet the expected values prescribed by the Codex Alimentarius (WHO/FAO, 2001) and Turkish Food Codex Regulation of Fermented Dairy Products (TGK, 2009) except for yeast counts. *Lactococcus lactis* ssp. *Lactis*, *Micrococcus* spp. and *Candida kefyr* were isolated in all kefir groups. *Leuconostoc* spp. was isolated in all groups but not in the groups incubated at 4°C. That may be one of the reasons why the kefir groups incubated at 4°C did not produce the unique properties of kefir. Accordingly, acidity and pH values of those groups were also not suitable for unique product properties. *Kocuria* spp. was found to be the dominant microflora in the groups incubated at 4°C but was not isolated in the groups incubated at 20°C, 25°C and 30°C. According to sensorial general acceptance average scores; the most liked group was K4 (25°C for 18 h). Microorganism counts, pH and acidity values were as expected. Consistency was also declared as normal by panellists. It can be concluded that incubations at 25°C for 18 h and also 24 h (K4 and K5) can be accepted as the most suitable values for producing traditional kefir. On the other hand, according to the unique pH value, the group incubated at 30°C for 24 h (K8) can also be considered as the most suitable one.

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