Journal of Pharmaceutical and Health Sciences 2017;5(1), 23-35.

Original Article

Effects of Starter Culture and Storage Temperature on Functional, Microbial and Sensory Characteristics of Kefir during Storage

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Abstract

The aim of this study was to investigate the effects of starters and storage temperature (4 ºC, 25 ºC) on microbiological and physicochemical properties, volatile compounds and sensory evaluation of kefir. Kefirs produced by KFA and Chr. Hansen starters were stored at 4 ºC and 25 ºC for 40 days. pH and acidity at 4 ºC did not change ($p \geq 0.05$), while at 25 ºC pH and total solid decreased as well as acidity. Concentrations of acetaldehyde and ethanol increased ($p < 0.05$). No significant differences ($p \geq 0.05$) were observed in protein and non-protein-nitrogen for both samples. Sensory evaluation revealed that storage did not affect texture and color of samples at 4 ºC. Kefir produced by Chr. Hansen starter stored at 4 ºC had the highest acceptability until 40-day storage and was preferred by the panelists.

Keywords: Fermented milk, Kefir, Functional properties, Microbiological analysis, Sensory evaluation

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Introduction

Kefir, a fermented milk product originated from Caucasian mountains, is a dairy beverage with moderate yeasty flavor and acidic taste that contains a small quantity of CO2 and alcohol. Kefir is a rich source of amino acids, vitamins (B2, B12, K, A, D), minerals (calcium, phosphorus, magnesium) and enzymes [5]. Although kefir is manufactured from bovine milk on a commercial scale, it may be produced also from the milk of sheep, ewe, goat and buffalo [21]. Production of kefir from non-dairy sources such as soy milk, coconut milk, peanut milk, juice, sugar and molasses solutions has been reported [15, 23]. Kefir is a fermented product of symbiotic community between bacteria and yeasts [16]. Kefir has many therapeutic effects such as stimulation of the immune system, inhibition of tumor growth, prevention of aging and allergies, reduction of cholesterol, and treatment of sleep disturbances [5].

There are many compounds in kefirs with bioactive properties such as exopolysaccharides and bioactive peptides. The distinct sensory characteristics of kafirs were attributed to the presence of some volatile flavor compounds such as acetaldehyde and ethanol. Kefir grains are formed by lactic acid bacteria, acetic acid bacteria, and yeasts. The coexistence between these microorganisms results in the production of metabolites conveying specific sensory characteristics to kefir [2].

The final quality and shelf-life of kefir can be affected by a variety of factors such as the type of milk, type of starter culture, the amount of inoculum, fermentation and storage time and temperature. Among the mentioned factors, type of starter culture and storage temperature are the most important parameters. Industrial production of kefir by kefir grain seems to be very difficult because of the complexity of the microbial flora, so the most reasonable way to obtain a suitable product is to use a certain type of starter culture [11, 23]. Storage temperature plays an important role on kefir characteristics. In most cases during transportation and storage, the product is stored at room temperature. Therefore, lack of comprehensive studies on the effect of storage temperature on kefir qualities encouraged us to investigate this factor in the present study.

One problem with kefir production in hot weather areas and summer time is that kefir has a shorter shelf-life when kept without refrigeration. This creates a problem for both the consumer and manufacturer. Therefore, selection of starter culture to enhance kefir shelf-life especially at room temperature was the objective of the present study. Kefir production method in Iran differs from how the kefir was produced originally. Brine is added to the final product of Iranian kefir. The aim of the present study was to investigate the effects of starter cultures (KFA and Christian Hansen (and storage temperature)4 ºC and 25 ºC(on microbiological, physicochemical and sensory characteristics of kefir produced according to Iranian industrial procedures. To our knowledge, no such study has been performed until now.

Materials and Method

Chemical materials

Sodium hydroxide (NaOH), potassium sulfate (K_2SO_4), copper sulfate ($CuSO_4$.5H2O), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), boric acid (H_3BO_3), trichloroacetic acid (CCl_3COOH), methyl red ($C_{15}H_{15}N_3O_2$), Bromo cresol ($C_{12}H_{14}Br_4O_5S$), phenolphthalein ($C_{20}H_{14}O_4$) were obtained from Sigma-Aldrich Co.

Microbial materials

MRS agar and plate count skim milk agar were obtained from Merck (Darmstadt, Germany). KFA starter culture was obtained from Micro milk Co. and Christian Hansen starter culture was purchased from Christian Hansen Co.

Equipment and materials

Filter papers, nitrate and nitrite free, 20 μm pore size (Wattman No. 1), Fossomatic (Foss-5000), Water bath (Memert), pH meter (Mettler Toledo), Humidity meter (Sartorius), Kjeldahl (Gerhard), Colony counter (Funke Gerber), Balance (EK-610i), Incubator (Memert), Autoclave (Webeco), MilcoScan (S-50), Homogenizer (APV), Pasteurizer (APV), Head-space GC (Varian CP-3800) were used in this study.

Sampling

In this study kefir was produced using two different starter cultures: 1) KFA starter culture (Streptococcus thermophilus, Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Leuconostoc mesenteroides subsp. cremoris, Lactococcus lactic subsp. lactis biovar diacetylactis, Debaryomyces hansenii) and 2) Christian Hansen starter culture (Lactobacillus acidophilus, Streptococcus thermophilus, Bifidobacterium, Lactococcus lactis subsp. cremoris, Leuconostoc, Lactococcus lactis subsp. lactis biovar diacetylactis, Lactococcus lactis subsp. lactis, Kluyveromyces marxianus subsp. marxianus). These two starter cultures were suggested by Micro milk Co. and Christian Hansen Co. for kefir production. Bovine raw milk free of antibiotics containing 2.5% fat was homogenized at 180 bar pressure. Afterward, the milk was pasteurized at 90 ºC during 15 min. In order to reach the optimum temperature for starter culture inoculation, milk temperature was cooled down to 35 ºC. After reaching optimum temperature, starter culture was added at a rate of 1.5 mL per 1 liter of milk. Samples were incubated at 35 ºC for about 16 hours. After incubation, pasteurized brine (brine preparation: 5 g of salt dissolved in 650 mL of water and pasteurized at 90 ºC for 15 min) was added to coagulate kefir and mixed together, thoroughly. It should be highlighted that the brine was added to the drink in order to produce a functional beverage in accordance with industrial production of kefir in Iran. Prepared kefir was packed and stored at 4 ºC and 25 ºC up to 40 days. Samples were taken at 1, 10, 20, 30 and 40 days of storage.

Microbiological Analysis

Microbiological analysis was performed according to Kok-Tas et al [14]. For counting lactic acid bacteria (LAB), MRS agar medium was used. 1 mL of well-mixed sample was added to 9 mL sterile peptone water. After preparation of serial dilutions, 1 mL of each dilution was transferred to plate, then plates were incubated under an anaerobic condition at 37 ºC for 72 h. Results were reported as CFU mL-1. Counting bacteria in raw milk was performed on plate count skim milk agar medium. 1 mL of raw milk was added to 9 mL sterile peptone water in order to prepare dilutions. Afterward, 1 mL of each dilution was added to plate and plates were incubated at 30 ºC for 72 h. Results were reported as CFU mL-1.

Physicochemical Analysis

Some physicochemical parameters such as pH, titratable acidity, dry matter, protein, non-protein-nitrogen were determined according to AOAC International Methods (1992).

Volatile compounds

Acetaldehyde and ethanol contents of kefir samples were measured according to Guzel-Seydim et al [8]. with some modifications: 5 mL of samples was transferred into 20 mL vials. The vials were preheated at 85 ºC for 5 min using a headspace autosampler (GC/FID Varian CP-3800). Samples were injected onto a 30 m CP-Sil 13 CB column. The temperature was maintained at 40 ºC. The oven temperature was programmed from -20 ºC to 30 ºC

increased at 5 ºC min-1 and 30 ºC to 200 ºC increased at 10 ºC min-1. Nitrogen was used as the carrier gas with 30 mL min-1 flow. GC/MS was used for verification of peak identification. Standard solutions of acetaldehyde and ethanol were prepared with distilled-deionized water.

Sensory evaluation

Sensory analysis was evaluated by 5 trained panelists using 9-point hedonic scales, in which 1 represented the lowest score and 9 indicated the highest score. The panelists had already previous experience in evaluation of dairy products. The panelists evaluated the products for taste, odor, texture, appearance and overall acceptance. Samples were coded and sensory analysis was carried out at room temperature. Panelists rinsed their mouth with pure, room temperature water between each sample testing [12].

Statistical analysis

In order to find a logical relationship between variables and analyze data, a variance analysis (ANOVA) was performed. In addition, for evaluating the significant effect on variables, GLM procedure was performed. SPSS 22.0 software (SPSS Inc, IBM, Chicago, IL) was used. All experiments were performed in duplicate.

Results and Discussion

Physicochemical and microbiological analysis of raw milk

According to Iranian National Standard No. 164, pH, titratable acidity, dry matter, protein, lactose and somatic cells of raw milk must be 6.6-6.8, 14-16 °D, 8% w/w, 3-3.3 %, 3.8 to 5.3 %, up to 500 million cells per mL, respectively. Since kefir is a fermented milk product, the presence of any microbial inhibitors and antibiotics influences the growth and reproduction of microorganisms and ultimately affects product quality. The results of physicochemical and microbiological analysis related to raw milk are presented in Table 1. With regard to these results and comparing them to national standards, we ensured that the quality of raw milk used in this study was appropriate for kefir production.

Table 1: Physicochemical and microbiological analysis of raw milk (Mean ±SD*)

Investigated factor	Reported amount
pH	6.7±0.007
Titratable acidity (ºDornic)	14.5±0.000
Dry matter (g/100mL)	11.060±0.130
Protein (g/100 mL)	3.063±0.025
Lactose (g/100 mL)	4.430±0.120
Somatic cells (Cell mL-1)	22875±4250
Total bacterial count (CFU mL-1)	6.250±0.016

**SD: Standard Deviation*

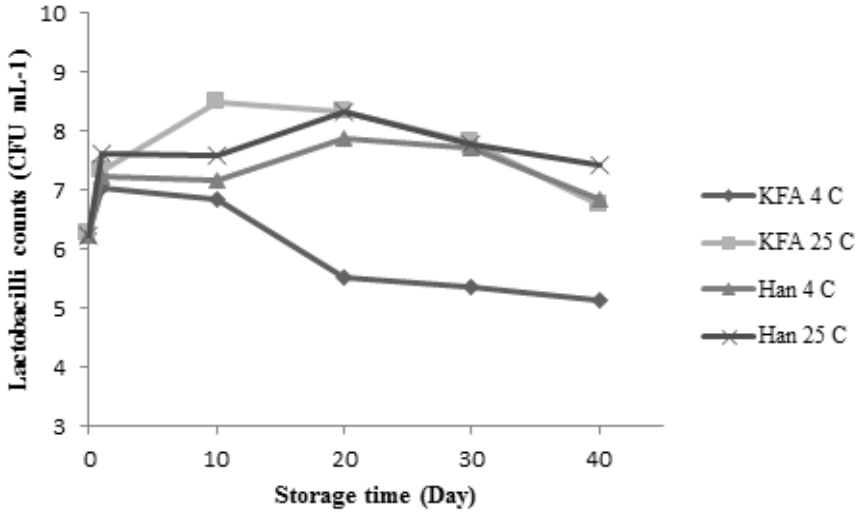

Figure 1. Changes in Lactobacilli population in kefir samples during storage

♦: Kefir samples prepared with KFA starter culture and stored at 4 ºC

■: Kefir samples prepared with KFA starter culture and stored at 25 ºC

▲:Kefir samples prepared with Christian Hansen starter culture and stored at 4 ºC

×: Kefir samples prepared with Christian Hansen starter culture and stored at 25 ºC

Microbiological analysis

As shown in Figure 1 lactic acid bacteria (LAB) in samples of kefir stored at 25 ºC were more abundant. These results were coherent with the mesophilic and thermophilic nature of LAB. At the end of the storage period, samples of kefir prepared with Christian Hansen starter culture stored at 25 ºC had the highest number (107 CFU mL-1) of LAB and samples of kefir prepared with KFA starter culture stored at 4 ºC (105 CFU mL-1), had the lowest number of LAB. The obtained data of the present study remains in agreement with what was reported by Irigoyen et al [10]. They stated that until the second day of storage population of LAB in kefir was 108 CFU mL-1, then the LAB declined with the maximum reduction equivalent to approximately 1.5 log unit between day 7 and 14. LAB then remained constant up to 28 days. In the survey conducted on Norwegian kefir, the number of LAB significantly decreased after 4 weeks and remained stable until the end of the storage period [6]. The bacterial count in kefir produced from the milk of bovine, sheep, and goat diminished during 7 days of storage at 4 ºC. The maximal number of LAB was observed in caprine milk at the end of the storage (107 CFU mL-1). The count of LAB in kefir samples (prepared from bovine milk and soy milk) decreased, but the reduction was greater after 14 days of storage. The number of Lactobacilli was inferior to 105 CFU mL-1 during storage [13]. Our findings remain also in agreement with those reported by Guzel et al [7] who observed the reduction of Lactobacilli in kefir samples stored at 4 ºC for 21 days. This may be due to the fact that LAB is mesophilic and grow in the temperature range 10-45 ºC, the optimum temperature for their growth being 30-40 ºC. Therefore, during storage time when the temperature increases the count of LAB will also increase.

Physicochemical analysis

Results of pH changes in kefir samples are presented in Figure 2. As can be seen, pH of kefir samples from both starter cultures after incubation for 16 h at 35 ºC have radically

decreased from 6.7 to 4.0. This result was in agreement with the results obtained by Motaghi et al [19] and Fontan et al [4], who studied the physicochemical properties of kefirs. The result of this study indicates that storage temperature and type of starter culture used in the manufacturing process of the kefir affects pH. Storage at higher temperatures led to products with a lower pH.

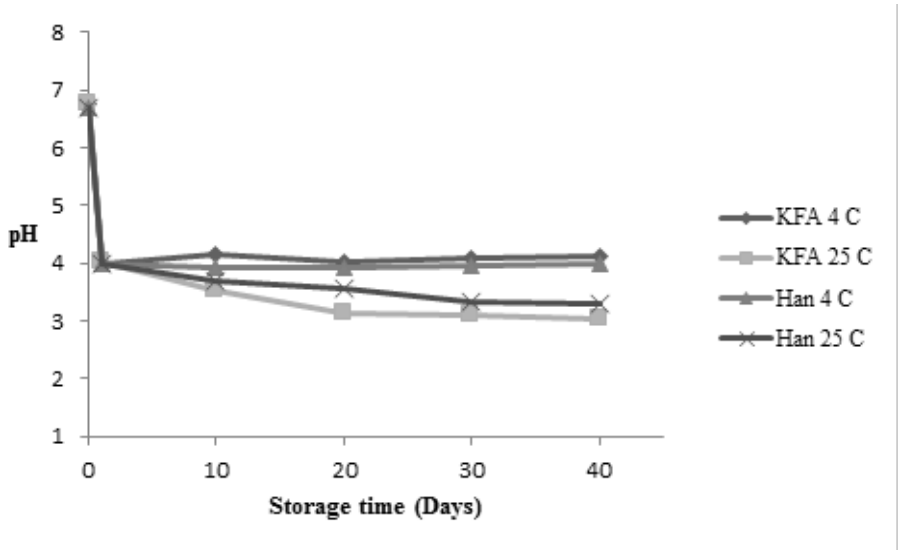

Figure 2: Changes in pH in kefir samples during storage

♦: Kefir samples prepared with KFA starter culture and stored at 4 ºC

■: Kefir samples prepared with KFA starter culture and stored at 25 ºC

▲:Kefir samples prepared with Christian Hansen starter culture and stored at 4 ºC

×: Kefir samples prepared with Christian Hansen starter culture and stored at 25 ºC

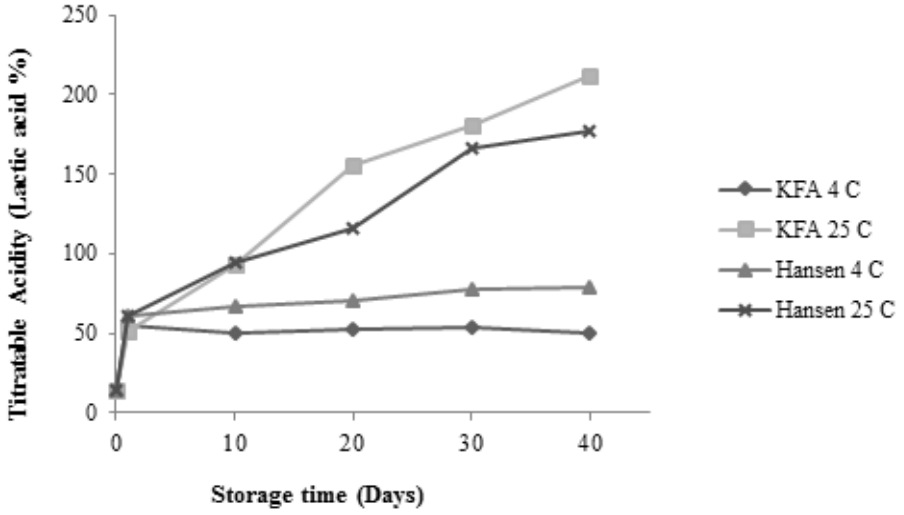

Figure 3: Changes in titratable acidity in kefir samples during storage.

♦: Kefir samples prepared with KFA starter culture and stored at 4 ºC

■: Kefir samples prepared with KFA starter culture and stored at 25 ºC

▲:Kefir samples prepared with Christian Hansen starter culture and stored at 4 ºC

×: Kefir samples prepared with Christian Hansen starter culture and stored at 25 ºC

The acidity of the kefir samples produced using both starter cultures (KFA and Christian Hansen), strongly increased during incubation from 14.5 °D to about 60 °D. As shown in Figure 3, changes in acidity in samples stored at 4 ºC are relatively constant, while the amount of acidity in samples stored at 25 ºC increased. The result of this study indicates that temperature and type of starter culture used to produce kefir significantly ($p<0.05$) affected the acidity values. Kok-Tas et al [14] also reported that titratable acidity of kefir samples did not significantly change during storage at 4 ºC over 21 days. In another study, it was shown that the acidity of kefir samples within 72 h of fermentation at 25 ºC increased over time [19].

Changes in dry matter values of kefir samples showed a decreasing trend (Table 2). The loss of dry matter in samples stored at 25 ºC was higher compared to the samples stored at 4 ºC

Table 2: Changes in dry matter value (g/100 mL) in kefir samples produced using KFA and Christian Hansen starter cultures and stored at two different temperatures (mean± SE) from each culture

Starter culture	**KFA**		**Christian Hansen**	
Temperature Day	4 ºC	25 ºC	4 ºC	25 ºC
1	6.165 ± 0.155^{Ab}	6.340 ± 0.010^{Ab}	7.340 ± 0.160^{Aa}	7.595 ± 0.105^{Aa}
10	6.075 ± 0.025^{Ab}	6.585 ± 0.015^{Aa}	6.650 ± 0.180^{Bb}	5.705 ± 0.095^{Bb}
20	6.250 ± 0.070^{Aa}	5.630 ± 0.070^{Bb}	6.270 ± 0.230^{Ba}	5.340 ± 0.160^{Bb}
30	6.330 ± 0.070^{Aa}	5.520 ± 0.060^{Bb}	5.870 ± 0.060^{Bb}	4.630 ± 0.150^{Cc}
40	6.165 ± 0.055^{Aa}	5.130 ± 0.070^{Bb}	5.750 ± 0.250^{Ba}	4.350 ± 0.150^{Cc}

** Values with different small letters in a row and with different capital letters in a column are statistically significant at $P < 0.05$*

($p<0.05$). The comparison between samples produced with one kind of starter culture at different temperatures (4 ºC and 25 ºC) revealed significant differences ($p<0.05$). The result of this study indicates that Kefir produced with KFA starter culture and stored at 4 ºC had the highest amount of dry matter and kefir produced by Christian Hansen starter culture stored at 25 ºC had the lowest dry matter. Kok-Tas et al [14] also stated that the dry matter in samples prepared from kefir grains and starter culture, and stored at 4 ºC for 21 days, decreased and dropped to the amount of 4.29 % and 4.35 %. It should be noted that the low dry matter content in this study in comparison to other studies is due to the use of brine in kefir preparation.

During storage the protein content of the samples that were prepared with the same starter culture but were held at different temperatures did not change significantly ($p\geq0.05$), while samples produced with different starter cultures at the same temperature showed significant ($p<0.05$) differences in the protein content in the final days of storage (Table 3). Samples prepared with Christian Hansen starter culture compared to samples prepared with KFA starter culture had a greater amount of protein. Similar results were obtained by Kok-Tas et al [14] who also reported that the type of starter cutler had a significant effect on the protein content.

Non-protein nitrogen content did not differ during storage except for day 40 when its level

Table 3: Changes in protein value (g/100 mL) in kefir samples made using two kinds of starter cultures (KFA and Christian Hansen) and stored at two different temperatures (mean± SE) from each culture

Starter culture	KFA		Christian Hansen	
Temperature Day	4 ºC	25 ºC	4 ºC	25 ºC
1	1.938 ± 0.009^{Ab}	1.965 ± 0.000^{Aab}	1.983 ± 0.018^{Aa}	1.956 ± 0.009^{Aab}
10	1.911 ± 0.000^{Ab}	1.947 ± 0.018^{Aab}	1.992 ± 0.027^{Aab}	2.028 ± 0.027^{Aa}
20	1.911 ± 0.000^{Ab}	1.894 ± 0.018^{Ab}	1.992 ± 0.027^{Aa}	2.001 ± 0.018^{Aa}
30	1.903 ± 0.009^{Ab}	1.903 ± 0.027^{Ab}	2.010 ± 0.009^{Aa}	2.010 ± 0.009^{Aa}
40	1.911 ± 0.000^{Ab}	1.911 ± 0.018^{Ab}	2.036 ± 0.018^{Aa}	2.028 ± 0.009^{Aa}

** Values with different small letters in a row and with different capital letters in a column are statistically significant at $P < 0.05$*

increased significantly ($p<0.05$). The type of starter culture used in this study had no significant effect on the non-protein nitrogen content (Fig. 4). The starter cultures used in this study had low proteolytic activity; therefore protein breakdown took place more slowly during storage resulting in less non-protein nitrogen formation due to free amino acids and peptides. The type of starter culture and temperature did not affect the non-protein nitrogen during storage in this study.

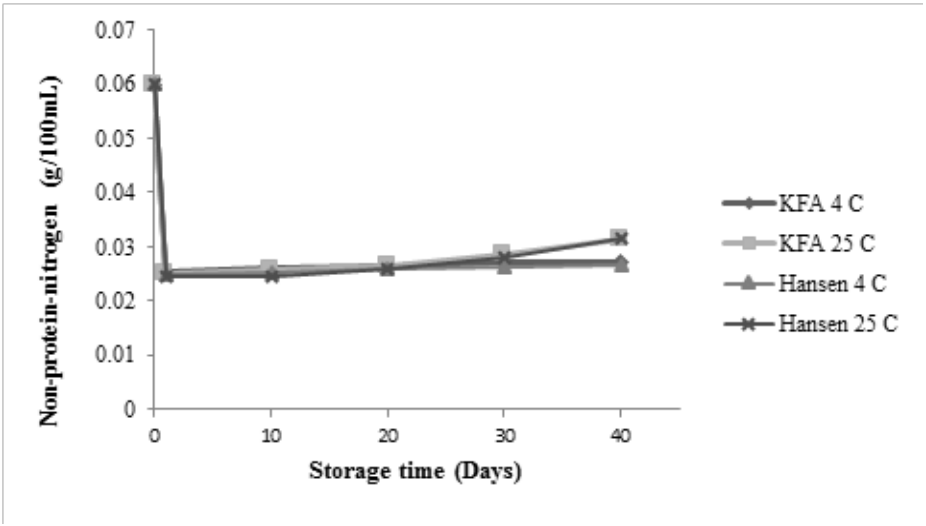

Figure 4. Changes in non-protein-nitrogen values in kefir samples

♦: Kefir samples prepared with KFA starter culture and stored at 4 ºC

■: Kefir samples prepared with KFA starter culture and stored at 25 ºC

▲:Kefir samples prepared with Christian Hansen starter culture and stored at 4 ºC

×: Kefir samples prepared with Christian Hansen starter culture and stored at 25 ºC

Table 4: Changes in acetaldehyde value (ng mL-1) in kefir samples made using two kind of starter cultures (KFA and Christian Hansen) and stored at two different temperatures (mean ± SE) from each culture

Starter culture	KFA		Christian Hansen	
Temperature Day	4 ºC	25 ºC	4 ºC	25 ºC
1	11.399 ± 0.002^{Aa}	9.099 ± 0.001^{Ab}	0^{Ad}	0.099 ± 0.001^{Ac}
10	12.301 ± 0.001^{Bb}	14.902 ± 0.002^{Ba}	11.799 ± 0.002^{Bc}	3.451 ± 0.151^{Bd}
20	14.001 ± 0.001^{Cc}	19.899 ± 0.002^{Ca}	11.102 ± 0.002^{Cc}	2.699 ± 0.002^{Cd}
30	17.702 ± 0.002^{Dd}	23.702 ± 0.002^{Da}	17.151 ± 0.051^{Dc}	5.401 ± 0.001^{Dd}
40	28.199 ± 0.002^{Ee}	32.800 ± 0.001^{Eb}	33.100 ± 0.001^{Ea}	29.399 ± 0.002^{Ec}

** Values with different small letters in a row and with different capital letters in a column are statistically significant at P < 0.05*

Volatile compounds

Table 4 represents the acetaldehyde values in kefir samples produced by two starter cultures kept at different temperatures. The result of this study indicates that temperature had a significant ($p<0.05$) effect on the production of acetaldehyde. Also, the amount of acetaldehyde in kefir samples produced with different starter cultures and stored at the same tempera-

Table 5: Changes in ethanol value (ng/mL) in kefir samples made using two kind of starter cultures (KFA and Christian Hansen) and stored at two different temperatures (mean ± SE) from each culture

Starter culture	KFA		Christian Hansen	
Temperature Day	4 ºC	25 ºC	4 ºC	25 ºC
1	0.170 ± 0.001^{Ab}	0.489 ± 0.002^{Aa}	0.099 ± 0.002^{Ad}	0.111 ± 0.001^{Ac}
10	0.329 ± 0.002^{Bc}	0.519 ± 0.001^{Ba}	0.371 ± 0.001^{Bb}	0.249 ± 0.002^{Bd}
20	0.601 ± 0.001^{Cb}	0.842 ± 0.002^{Ca}	0.409 ± 0.002^{Cc}	0.329 ± 0.001^{Cd}
30	0.679 ± 0.001^{Db}	0.881 ± 0.001^{Da}	0.551 ± 0.001^{Dc}	0.481 ± 0.001^{Dd}
40	0.719 ± 0.001^{Dc}	0.909 ± 0.002^{Da}	0.879 ± 0.001^{Eb}	0.509 ± 0.002^{Ed}

** Values with different small letters in a row and with different capital letters in a column are statistically significant at P < 0.05.*

Table 6: Sensory analysis in kefir samples made using two kinds of starter cultures (KFA and Christian Hansen) and stored at two different temperatures (mean ± SE) from each culture

	Starter culture and Storage temperature	Days of storage				
		1	10	20	30	40
Taste	KFA / 4 ºC	6.200±0.663^{A}	7.000±0.707^{A}	6.600±0.600^{A}	6.400±0.600^{A}	5.400±1.208^{A}
	KFA / 25 ºC	6.200±0.374^{A}	3.600±0.894^{B}	2.600±0.510^{B}	3.000±0.707^{B}	2.600±0.678^{B}
	Christian Hansen / 4 ºC	6.600±1.166^{A}	0.678^{A}±7.600	0.678^{A}±6.600	1.483^{AB}±6.000	6.600±0.510^{A}
	Christian Hansen / 25 ºC	1.030^{A}±6.400	0.800^{A}±6.200	0.980^{A}B±4.600	1.049^{AB}±4.000	0.583^{AB}±4.200
Texture	KFA / 4 ºC	7.200±0.663^{A}	0.600^{A}±7.600	7.000±0.632^{A}	7.400±0.748^{A}	6.400±0.812^{A}
	KFA / 25 ºC	7.000±0.548^{A}	0.748^{A}±7.400	7.200±0.663^{A}	7.200±0.663^{A}	6.600±0.678^{A}
	Christian Hansen / 4 ºC	0.374^{A}±7.800	0.548^{A}±8.000	0.600^{A}±7.600	0.583^{A}±7.800	0.374^{A}±7.800
	Christian Hansen / 25 ºC	0.400^{A}±7.600	0.548^{A}±8.000	0.400^{A}±7.600	0.447^{A}±7.000	0.400^{A}±7.400
Odor	KFA / 4 ºC	6.200±0.735^{A}	6.800±0.860^{A}	7.000±0.707^{A}	6.600±0.245^{A}	5.000±0. 707^{B}
	KFA / 25 ºC	6.000±0.548^{A}	5.200±0.860^{A}	3.800±0.374^{B}	3.25±0.860^{B}	2.60±0.600^{C}
	Christian Hansen / 4 ºC	0.735^{A}±7.200	0.583^{A}±7.800	0.632^{A}±7.000	1.049^{A}±7.000	0.645^{A}±7.500
	Christian Hansen / 25 ºC	0.707^{A}±7.000	0.970^{A}±6.800	1.140^{AB}±5.000	1.241^{A}B±5.200	0.632^{BC}±4.000
Color	KFA / 4 ºC	7.200±0.374^{A}	7.600±0.600^{A}	7.400±0.748^{A}	7.400±0.748^{A}	6.800±0.663^{A}
	KFA / 25 ºC	7.200±0.374^{A}	7.600±0.600^{A}	7.400±0.748^{A}	7.200±0.663^{A}	6.800±0.663^{A}
	Christian Hansen / 4 ºC	0.510^{A}±7.400	0.548^{A}±8.000	0.748^{A}±7.400	1.114^{A}±7.200	0.374^{A}±7.800
	Christian Hansen / 25 ºC	0.600^{A}±7.600	0.548^{A}±8.000	0.400^{A}±7.600	0.447^{A}±7.000	0.490^{A}±7.200
Total acceptability	KFA / 4 ºC	6.200±0.663^{A}	6.800±0.860^{AB}	6.000±0.707^{A}	6.600±0.600^{A}	5.400±1.030^{AB}
	KFA / 25 ºC	6.000±0.548^{A}	4.600±0.748^{B}	3.200±0.370^{B}	4.400±0.244^{B}	2.400±0.400^{C}
	Christian Hansen / 4 ºC	1.020^{A}±6.800	0.812^{A}±7.400	1.020^{A}±6.800	0.730^{A}±6.800	0.245^{A}±6.400
	Christian Hansen / 25 ºC	0.735^{A}±6.200	0.927^{AB}±6.400	1.068^{AB}±5.200	0.374^{B}±4.200	1.158^{BC}±3.800

** Values with different small letters in a row and with different capital letters in a column are statistically significant at $P < 0.05$.*

ture showed a significant difference. Maximum and minimum amounts of acetaldehyde were observed in samples of kefir produced by Christian Hansen starter (33.100 ± 0.001 ng mL-1) and in samples prepared with KFA starter (28.199 ± 0.002 ng mL-1), respectively. According to the results of this study, it can be stated that both storage temperature and type of starter culture affected the acetaldehyde production during storage. Similar results have been reported in other studies. In traditional Tunisian kefir, acetaldehyde was produced after 15 h of fermentation and slowly increased until the end of storage [20].

According to the results of previous studies, it can be concluded that type of starter culture and production process significantly affect the production of acetaldehyde. In this study,

mixing kefir with brine resulted in lower acetaldehyde concentration in comparison with other studies. In some studies, it has been reported that the amount of acetaldehyde decreased during storage due to the conversion of acetaldehyde to ethanol by alcohol dehydrogenase [17], whereas in this study, acetaldehyde did not decrease while ethanol increased.

The production of ethanol in all kefir samples increased significantly ($p<0.05$) during storage (Table 5). The maximum and minimum amounts of ethanol were identified in samples of kefir prepared with KFA starter stored at 25 ºC (0.9091 ng mL-1) and in samples of kefir prepared with Christian Hansen starter stored at 25 ºC (0.509 ng mL-1), respectively. From the results of this study, it may be concluded that both storage temperature and starter culture affect ethanol content. Similar results were obtained in other studies. During tests conducted on kefir made from bovine milk and a commercial starter culture, the amount of ethanol increased and a significant difference was observed after 48 h of fermentation until the end of fermentation. The final ethanol concentration was 0.018 % (w/w) [4]. Zajsek and Gorsek [22] reported that ethanol in fermented samples at different temperatures increased significantly with temperature and reached 7.2 g L-1 at 23 ºC and 10.7 g L-1 at 31 ºC. Guzel et al stated that the amount of ethanol in kefir samples during a 21-day storage period increased and reached a final amount of 0.08 % [7]. In this study, the addition of brine caused a lower ethanol production than that observed in other studies.

Sensory analysis

A sensory evaluation carried out on samples of kefir showed that texture and color of samples did not significantly ($p\geq0.05$) differ during storage. According to the results of this study (Table 6), both storage temperatures and type of starter culture influence sensory properties of kefir. The results of sensory evaluation of samples were consistent with the results of pH, acidity, and bacterial population. In comparison with samples stored at 4 ºC, samples stored at 25 ºC had a more sour taste due to the greater number of LAB which resulted in lower pH and higher acidity. In a survey similar to this study, kefir produced using probiotic microbial flora had desirable sensory properties during cold storage. It was stated that differences in organoleptic characteristics of kefir samples made from the same raw material may be related to the type of inoculated microorganism [18]. In another study, kefir inoculated with 1 % kefir grains had a more distinct flavor than samples inoculated with 5 % kefir grains. The difference was observed after 14 days. Taste of samples increased sharply during storage. The bitterness of the samples increased during storage. Results showed that panelists preferred dairy kefirs with a distinct milky flavor and that bitter, sour and yeasty flavors exerted detrimental effects on the perception of the quality of kefir [10]. In another recent study, sensory evaluation of samples produced with kefir grains and kefir starter culture was performed on days 1, 7 and 14 of storage. Overall evaluation of sensory characteristics showed that samples inoculated with starter culture compared with samples inoculated with kefir grains had higher rating [14].

Conclusions

The results of this study revealed that starter culture and storage temperature had a significant effect on microbiological, physicochemical and sensory characteristics of kefir. Sensory evaluation revealed that storage did not significantly affect the texture and color of the samples and kefir samples stored at 4 ºC had more pleasant taste and odor in comparison to samples stored at 25 ºC. Overall acceptability scores revealed that kefir samples produced by Christian Hansen starter stored at 4 ºC had the highest acceptability (6.40 ± 0.25) until 40-day storage and were awarded by the panelist as a delightful product.

This study showed that starter culture and storage temperature affected the microbiological, physicochemical and sensory characteristics of kefirs produced according to Iranian procedures. KFA starter culture is a suitable starter for commercial production of kefir. By using KFA starter and storing kefir at 4 ºC, it is possible to produce an acceptable product with longer shelf-life which will help food manufacturer to capture the market as well as a consumer to drink a functional beverage with beneficial health properties. According to the results of this study and the results presented in the literature, the best temperature for kefir storage is 4 ºC and in these conditions, kefir shelf-life can be up to 40 days with good microbiological, physicochemical and sensory properties. For achieving this goal, transport and storage of the product must be done at 4 ºC.

ACKNOWLEDGEMENTS

The support of Iran Dairy Industries Company (Pegah) and Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) are gratefully acknowledged.

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