PAPER

ASSESSMENT OF DURABILITY AND CHARACTERISTICS OF CHANGES IN KEFIR MADE FROM COW'S AND GOAT'S MILK

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ABSTRACT

Present study was aimed to determine the changes and the best consumption time of kefir samples made from cow and goat milk using starter culture during the storage at the refrigerator (+4°C for 35 days). It was found that goat kefir (GK) samples had higher pH and lipolysis values during storage while cow kefir (CK) samples had higher values of viscosity. The counts of lactococci, lactobacilli, leuconostoc, total mesophilic aerobic bacteria, acetic acid bacteria and yeast decreased during the storage. Amounts of lactic, acetic, citric, pyruvic and oxalic acids were higher in GK samples. Acetaldehyde, diacetyl and acetoin contents were higher in CK samples. The highest sensory scores were obtained for CK samples on 14th day and for GC sample on 21th day of the storage.

Keywords: cow milk, goat milk, kefir, shelf-life, storage

1. INTRODUCTION

Kefir is a fermented dairy product obtained by lactic acid and alcohol fermentation (WSZOLEK *et al*., 2001). It is originated from Caucasia region and believed that the word "kefir" is derived from the Turkish word "keyf", "keyif", "kefi" or "kef" (YAYGIN, 1994). In the Great Turkish Dictionary of the Turkish Language Institution (2019), it is reported that the word "kef" means "pleasure" and "foam", the word "keyif" means "body well-being", "health and vitality", the word "keyf" means "comfortable" and the word "kefi" means "foam". It is also known called kefyr, kephir, kefer, kiaphur, knapon, kepi and kippi in different regions (RATTRAY and O'CONNELL, 2011). However, it is called "kefir" in many languages now.

Kefir is defined as "the yogurt of the 21st century" (WSZOLEK *et al*., 2006). Kefir is traditionally produced with kefir grains. Nowadays, commercial freeze-dried kefir starter culture and the product that remains after the removal of kefir grains in addition to kefir grains are used in the production of commercial kefir (BENSMİRA and JIANG, 2012). But, kefir produced with kefir grains due to the different microflora of each kefir grain shows significant variation in taste, aroma and texture. Moreover, there is a risk of microbial contamination due to the use of kefir grains many times and necessity to separate the kefir grains from the kefir produced after fermentation in traditional kefir production. Furthermore, the kefir produced by the method has a short shelf life. Therefore, kefir grain is not suitable for industrial kefir production (GÜZEL-SEYDİM *et al*., 2010; KİM *et al*., 2018). Commercial kefir is produced using pure starter cultures that contain a mixture of limited number of bacteria and yeasts, which give a similar flavor to the traditional kefir, but do not cause bulge or leakage (O'BRIEN *et al*., 2016). The most common method in the production of commercial kefir is the use of commercial concentrated lyophilized kefir starter cultures (YAMAN, 2011).

Because of the health benefits of kefir, people's interest onto kefir has been increasing day by day. In the dairy industry, kefir is produced mostly from cow's milk (PURNOMO and MUSLİMİN, 2012). However, the researchers used goat milk (PURNOMO and MUSLİMİN, 2012; KACZYŃSKİ *et al*., 2018), sheep milk (WSZOLEK *et al*., 2001; CAİS-SOKOLİŃSKA *et al*., 2008), camel milk (KAVAS, 2015), buffalo milk (GUL *et al*., 2015), whey (MAGALHÃES *et al*., 2011), coconut milk (ISMAİEL *et al*., 2011), rice milk (NURLİYANİ *et al*., 2015), soy milk (KESENKAŞ *et al*., 2011; ISMAİEL *et al*., 2011), oat milk (DİNKÇİ *et al*., 2015) or peanut milk (BENSMİRA and JİANG, 2012) in addition to cow's milk in the production of kefir.

It has been interested in kefir produced from goat milk since the αs_1-casein, which has an important responsibility in cow's milk allergy is very low in goat milk. The studies on goat kefir produced with commercial culture mostly focused on physical, chemical and microbiological properties. However, it has been determined that there are not enough studies on the biochemical, organic acid and aroma profile of kefir produced by commercial culture.

Present study was aimed to determine the changes in chemical, physical, biochemical, microbiological, sensorial properties and also, organic acids and flavor components in kefir samples made from cow and goat milk fermented by kefir starter culture during storage at refrigerated condition. Also, the best consumption time of kefir during refrigerated storage was determined in this study.

2. MATERIALS AND METHODS

2.1. Materials

Cow and goat milk were used in production of the experimental kefir samples. Raw cows' milk was purchased from a factory and raw goats' milk from a farmer in Bolu. Kefir starter culture which is consisted of *Lactococcus lactis* ssp., *Leuconostoc* ssp., *Streptococcus thermophilus*, *Lactobacillus* ssp., kefir yeast, and kefir grain microflora according to the product information were obtained from Danisco Biolacta (Kefir DC1, Olsztyn, Poland). Sterilized bottles made of high-density polyethylene (HDPE) material were used for the preservation of the kefir samples.

2.2. Kefir Manufacture

The milk was transported to the research and development laboratory of Department of Food Engineering, Bolu Abant İzzet Baysal University. While the milk was transferred to the pasteurizer, it was filtered through cloth and steel strainer. Then, the temperature of the milk was increased to 55°C in the pasteurizer and a portion of the milk was passed through the cream separator to obtain skim milk. The resulting skimmed milk was used to adjust the fat content of the kefir milk to 3.1 %. Fat standardized milks were pasteurized at 90°C for 10 min and cooled to 28°C and starter culture was added to both the milks at a level of 0.0065 g L^{-1}. Afterwards, inoculated milk was filled into the sterilized HDPE bottles (1 L). All procedures were carried out aseptically as much as possible. After inoculation, kefir milk samples were incubated at 28°C until the pH reached 4.60. After incubation, kefir curd was broken by shaking down 10 times. The stirred kefir samples were kept at a refrigerator (4°C) for 35 days. The chemical, physical, biochemical, microbiological, sensorial properties and also organic acids and flavor components in kefir were determined on 1st, 7th, 14th, 21th, 28th and 35th day of storage period. Cow and goat milk kefir were coded as CK and GK, respectively.

2.3. Determination of chemical properties

Analysis of pH, titratable acidity, dry matter, fat and protein contents of kefir samples were done as described by KURT *et al*. (1993). The pH of milk and kefir samples was directly measured using a calibrated pH meter (WTW 720, Germany).

2.4. Determination of physical properties

Apparent viscosity of kefir samples was determined at 15°C, using a sine-wave vibro-viscometer SV-10 (A&D Company, Japan). The syneresis of kefir samples was determined by a procedure modified from Sodini, Montella, & Tong (2005). A sample of about 25 g of kefir (A) was centrifuged for 10 min at 1250*g* at 4°C. The expelled whey (B) was weighed. The syneresis (%) was calculated as: Syneresis (%) = (B/A)x100. A color measurement device (Konica Minolta CR400, Japan) was used to measure color values as CIE L*, a* and b* of the milk and kefir samples.

2.5. Determination of the lipolytic and proteolytic activity

The lipolysis as acid degree value (ADV) of kefir samples was determined by titrimetric method according to CASE *et al*. (1985). The proteolysis values in the milk and kefir samples were determined according to HULL (1947).

2.6. Enumeration of microorganisms

The kefir samples were dispersed and further diluents were made in Ringer solution (¼ strength, Merck, Germany). The Lactobacilli counts were determined on MRS medium under microaerophilic condition with Anaerocult C (Merck, Germany) at 30°C for 3 days (IRIGOYEN *et al*., 2005). The Lactococci counts were determined on M 17 medium under microaerophilic condition with Anaerocult C at 30°C for 2 days (IRIGOYEN *et al*., 2005). The Leuconostoc counts were determined on MSE agar, a selective medium for Leuconostoc, under aerobic condition at 25°C for 5 days (GARCÍA FONTÁN *et al*., 2006). The total mesophilic aerobic bacteria (TMAB) counts were determined on PCA agar under aerobic condition at 30°C for 2 days (MAINVILLE *et al*., 2001). The acetic acid bacteria counts were determined on APM agar under aerobic condition at 25°C for 5 days (WITTHUHN *et al*., 2005). The yeast counts were determined on YGC agar under aerobic condition at 25°C for 5 days (WITTHUHN *et al*., 2005).

2.7. Determination of organic acids

Lactic, citric, acetic, pyruvic, orotic and oxalic acid in milk and kefir samples were determined by HPLC (Perkin Elmer Flexar, USA) according to GÜZEL-SEYDİM (2000b) with some modifications. Four grams of the milk was diluted into 25 mL of 0.013N H_2SO_4 while four grams of kefir samples were diluted into 25 mL of 0.010N H_2SO_4. The mixture was vortexed for 1 min and centrifuged at 7000 *g* at 4°C for 7 min. The supernatant was passed through filter having a pore diameter of 0.45 μm (Millipore) and put into vial. The organic acids were separated in an isocratic system using a C 18 column (Perkin Elmer, 5μm, 250mm x 4.6mm i.d) at a column oven temperature of 50±1°C. The wavelengths of the UV detection were performed at 210 nm using Photodiode Array Detector. The mobile phase was carried out with 10 mM KH_2PO_4 adjusted to pH 2.5 with phosphoric acid. The flow rate of the mobile phase was adjusted at 0.5 mL min^{-1}. The amount of organic acids was determined on standard curves of individual organic acids.

2.8. Determination of volatile flavor components

The acetaldehyde, diacetyl, acetoin and ethanol contents of the kefir samples were analyzed at the research center of YENİGIDAM of Bolu Abant İzzet Baysal University, according to the method given by GÜZEL-SEYDİM *et al*. (2000b). The volatile flavor components in the product were determined by GC (Shimadzu GC 2010, Japan) with Flame Ionization Detector. The kefir samples (5 g) were weighed into 20 mL headspace vials. The prepared vials were heated in a dry block heater at 85°C for 5 min. At the end of the heating period, the air in the headspace of the vial using a gas-tight syringe (Supelco) was injected into the capillary column (Agilent, DB-23, 0.25 id 0.25mm x 60m). The column oven temperature was maintained at 40°C for 1 min and then, increased to 250°C and kept at this temperature for a further 3 minutes. The temperature rise time was 35 minutes. The

carrier gas was helium and the flow rate was 0.6 mL min^{-1}. The amount of the volatile components was determined on standard curves of individual volatile components.

2.9. Determination of sensory properties

Sensory evaluation of the samples was determined by modifying the hedonic scale system (DRAKE, 2009) and was performed by 11 trained panelists. The scoring was based on 5 points in three categories: 1) structure, consistence and texture, 2) taste and smell and 3) general appreciation.

2.10. Statistical Analysis

The CK and GK samples were manufactured two times and all the analyses were performed in two parallels. The differences between the characteristics of cow and goat milk kefir samples were determined by t-test and Mann Whitney U test according to the availability of the dependent variable in each group showing normal distribution. The changes occurred in the kefir samples during storage were analysed by one-way ANOVA based on the Tukey HSD test (DEVORE and PECK, 1993). Statistical analysis of all obtained data was performed with SPSS 20.0 package program (SPSS Inc., Chicago, IL) at the significance level 0.05.

3. RESULTS AND DISCUSSION

Some properties of both cow and goat milk used in kefir production were presented in Table 1. As seen from the table, dry matter, fat and protein contents of them were close to each other. The pH, viscosity and lipolysis values of goat milk were higher than that of cow milk. Moreover, L* value (lightness) of goat milk was higher than the value of cow milk. Goats convert carotene from green fodder completely into vitamin A, and therefore color of goat milk is whiter than cow milk (GÜRSOY, 2007). The tyrosine value was higher in cow milk.

Table 1. Some properties of standardized milk used in the study (n=2).

Analyses	Cow milk ($\bar{x}$)	Goat milk ($\bar{x}$)
Dry matter (%)	11.04	11.09
Fat (%)	3.00	3.06
Protein (%)	2.96	3.02
pH	6.60	6.64
Acidity (LA, %)	0.16	0.15
Viscosity (mPa.s)	2.032	2.064
Color L*	79.378	79.727
Color a*	-3.212	-3.121
Color b*	5.482	5.911
Lipolysis (meq KOH/100 g fat)	0.359	0.935
Proteolysis (mg tyrosine/5 mL milk)	0.171	0.143

$\bar{x}$: Mean of two repetitions, n: number of repetitions L*: lightness (0= black, 100= white), a*: green (-) or red (+), b*: blue (-) or yellow (+).

3.2. Chemical changes

Some chemical changes in the kefir samples made from cow and goat milk during 35-day storage were given in Table 2. The kefir samples made from goat milk (GK) had higher general mean dry matter content than the kefir samples made from cow milk (CK) ($P<0.05$). Similar results were observed in cow kefir (KAVAS, 2015) and goat kefir samples (KACZYŃSKİ *et al*., 2018). During storage, dry matter contents in kefir samples did not change ($P>0.05$). A similar result was obtained by ERTEKIN and GÜZEL-SEYDiM (2010).
There was no difference ($P>0.05$) between the general mean fat values of CK and GK samples. This was thought to be related with the standardization of the fat content of the milk used in the production of both samples. During storage, fat contents in kefir samples did not change ($P>0.05$).
The general mean protein contents of CK and GK samples were close to each other ($P>0.05$). During the storage, the change in protein values of kefir samples was found to be significant ($P<0.05$) in GK samples, but not significant ($P>0.05$) in CK samples.
The acidity as lactic acid (%) was higher in CK than GK samples in general ($P<0.05$). The reason for the slower development of acidity in GK samples compared to the CK sample might be related to the fact that the non-protein nitrogen content of goat milk and its buffering capacity are higher than cow milk (TRATNIK *et al*., 2006). In general, the acidity increased during storage and the change was significant ($P<0.05$) in both samples.
It was observed that the pH values of the CK samples were lower than that of the GK samples ($P<0.05$). The pH values of CK samples were like those determined by ERTEKIN and GÜZEL-SEYDİM (2010) while the pH value of GK samples were similar to those reported by KACZYŃSKI *et al*. (2018). This is related to the high buffering capacity of goat milk (TRATNIK *et al*., 2006). The pH value of the samples on the first day of storage was lower than 4.60. This decline was due to the lactic acid bacteria in the sample continued to produce lactic acid from lactose while the internal temperature of the samples was reaching from incubation temperature (28°C) to 4°C. The pH value of CK samples decreased until the 14th day of storage, there was a slight increase on the 21st day of storage, and then decreased again until the 35th day of storage. In the case of GK samples, the pH decreased until the 14th day of storage and then increased slightly up to 35th day of storage. In both kefir samples, the difference between day 1 and day 35 of storage was significant ($P<0.05$). The pH value of kefir did not change very fast during storage. This is related to yeasts found in kefir (O'BRIEN *et al*., 2016). Some yeast species assimilate lactic acid and cause to rise pH (RATTRAY and O'CONNELL, 2011).

3.3. Physical properties

The changes in some physical properties of the kefir samples during storage and statistical analysis results of these changes were given in Table 3. The general mean viscosity value of CK samples (64.80 mPa.s) was higher than GK samples (9.46 mPa.s) and the difference was significant ($P<0.05$). The viscosity value of CK samples was like those determined by YILDIZ-AKGÜL *et al*. (2018) while the viscosity value of GK samples was similar to those reported by GÜNEŞER and KARAGÜL-YÜCEER (2010). Similarly, TRATNIK *et al*. (2006) and GÜNEŞER and KARAGÜL-YÜCEER (2010) reported that the viscosity values of kefir samples made from goat milk were lower than the viscosity values of kefir samples made from cow milk. The reason for this is that the main fraction of goat milk casein is β-casein while the main fraction of cow milk casein is α_{s1}- and β-casein (HUMA *et al*., 2018). Goat milk protein micelles form softer and more brittle gel. As a result, weak texture occurs in

goat fermented dairy products (GÜNEŞER and KARAGÜL-YÜCEER, 2010). In addition, it has been thought that the difference between the acidity values of the samples affect viscosity of the samples. In general, viscosity values of both CK samples ($P<0.05$) and GK samples ($P>0.05$) increased during storage. Variability in viscosity during storage might be related to the production of exopolysaccharides and degradation of exopolysaccharides into monomers by microorganisms and enzymes in kefir.
The general mean syneresis value of GK samples was higher than that of CK samples ($P<0.05$). Compositional differences of goat milk such as low α_{s1}-casein concentration and smaller diameter fat globule lead to softer structure in fermented dairy products made from goat milk and more syneresis in these products (MILANI and WENDORFF, 2011). The changes in syneresis values during storage were found to be nonsignificant ($P>0.05$) in GK samples and significant ($P<0.05$) in CK samples. There was an inverse relationship between syneresis and viscosity values of the kefir samples during storage.
The general mean L*, a* (negative) and b* values of CK samples were higher than that of GK samples and the difference was significant ($P<0.05$). L* values of the kefir samples were higher than L* values of milk used in the production of the samples. In general, the L* values of both kefir samples tended to decrease during storage. The changes in L* and b* values of CK and GK samples was found to be significant ($P<0.05$) during storage. During storage, a* values of CK and GK samples showed fluctuant. However, this change was not significant ($P>0.05$). GUL *et al*. (2018) reported that milk variety has an effect on a* and b* values while no effect L* value.

3.4. Lipolysis and proteolysis

The changes in lipolysis and proteolysis of the kefir samples during storage and statistical analysis results of these changes were shown in Table 4. The lipolysis values of GK samples were higher than CK samples ($P<0.05$) and this may be related with some post-milking issues, such as milking time and storage type of goat milk. Because, when examining Fig. 1, it was seen that the amount of lactic and acetic acids in goat milk was higher than cow milk. This suggested that goat milk had been exhibited high lipolytic activity before reaching to the laboratory. WSZOLEK *et al*. (2001) reported that milk type used in the production of kefir influences the amount of free fatty acids. The lipolysis values of CK and GK samples increased during storage ($P<0.05$). CAIS-SOKOLIŃSKA *et al*. (2008) reported similar results.
The general mean values of proteolysis were found to be 0.597 mg tyrosine $5g^{-1}$ kefir for CK samples and 0.397 mg tyrosine $5g^{-1}$ kefir for GK samples ($P<0.05$) and this might be because of the number of microorganisms in kefir, especially the number of lactic acid bacteria. Microorganisms can obtain the amino acids needed from proteins and peptides through proteolytic systems (DİNKÇİ *et al*., 2015). Proteolysis values of CK and GK samples increased during storage and the increase was significant ($P<0.05$). DİNKÇİ *et al*. (2015) reported that proteolytic activity increased as long as storage time increased, and storage time affected proteolytic activity.

Table 2. Chemical changes in cow and goat kefir samples during storage.

Properties	Kefir	Storage time (Days) ($\bar{x}$±SD) (n=2)						
		1	7	14	21	28	35	General mean
Drymatter (%)	CK	10.78±0.006^{a*}	10.76±0.041^{a}	10.73±0.027^{a}	10.73±0.065^{a}	10.74±0.057^{a}	10.72±0.011^{a}	**10.74±0.038^{B*}**
	GK	11.13±0.029^{a}	11.14±0.011^{a}	11.19±0.062^{a}	11.15±0.044^{a}	11.09±0.092^{a}	11.13±0.008^{a}	**11.14±0.048^{A}**
Fat (%)	CK	3.10±0.071^{a}	3.14±0.159^{a}	3.09±0.059^{a}	3.12±0.057^{a}	2.94±0.035^{a}	3.01±0.124^{a}	**3.07±0.100^{A}**
	GK	3.18±0.035^{a}	3.15±0.005^{a}	3.15±0.071^{a}	3.13±0.009^{a}	3.13±0.053^{a}	3.04±0.018^{a}	**3.13±0.054^{A}**
Protein (%)	CK	3.10±0.013^{a}	3.10±0.020^{a}	3.09±0.000^{a}	3.06±0.047^{a}	3.07±0.008^{a}	3.06±0.002^{a}	**3.08±0.026^{A}**
	GK	3.06±0.003^{c}	3.10±0.003^{a}	3.10±0.009^{a}	3.10±0.004^{a}	3.09±0.012^{ab}	3.07±0.002^{bc}	**3.09±0.018^{A}**
Acidity (%)	CK	0.87±0.006^{c}	0.87±0.006^{bc}	0.90±0.003^{b}	0.94±0.001^{a}	0.94±0.000^{a}	0.95±0.013^{a}	**0.91±0.034^{A}**
	GK	0.80±0.003^{b}	0.82±0.003^{ab}	0.84±0.017^{a}	0.84±0.004^{a}	0.84±0.003^{a}	0.82±0.066^{ab}	**0.82±0.019^{B}**
pH	CK	4.31±0.011^{a}	4.27±0.020^{ab}	4.26±0.001^{bc}	4.27±0.009^{ab}	4.23±0.007^{bc}	4.21±0.014^{c}	**4.26±0.033^{B}**
	GK	4.47±0.002^{a}	4.44±0.009^{ab}	4.38±0.014^{c}	4.40±0.014^{bc}	4.41±0.007^{bc}	4.42±0.012^{bc}	**4.42±0.030^{A}**

CK: Cow kefir, GK: Goat kefir, $\bar{x}$: Mean, n: Number of repetitions, SD: Standard deviation, ^{a,b}: Means in each row show statistically difference (P>0.05 or P<0.05) among storage days for each property of the samples. ^{A,B}: Means in the same column show statistically difference between kefir samples in terms of related property (P<0.05).

Table 3. Physical changes in cow and goat kefir samples during storage.

Properties	Kefir	Storage time (Days) ($\bar{x}$±SD) (n=2)						
		1	7	14	21	28	35	General mean
Viscosity (mPa.s)	CK	42.17±2.165^{c*}	52.12±1.539^{c}	48.43±0.798^{c}	64.62±9.117^{bc}	93.23±10.438^{a}	88.24±3.906^{ab}	**64.80±20.916^{A*}**
	GK	8.44±0.188^{a}	8.58±0.119^{a}	10.37±0.268^{a}	9.66±0.285^{a}	10.13±0.502^{a}	9.61±1.493^{a}	**9.46±0.904^{B}**
Syneresis (%)	CK	46.26±0.837^{a}	42.42±0.338^{cd}	45.30±0.414^{ab}	43.83±0.219^{bc}	41.49±0.417^{d}	43.68±0.605^{bc}	**43.83±1.723^{B}**
	GK	62.04±0.250^{a}	61.58±0.799^{a}	55.61±1.848^{a}	59.19±0.609^{a}	58.80±0.219^{a}	60.80±4.270^{a}	**59.67±2.676^{A}**
Color L*	CK	82.810±0.057^{a}	82.723±0.025^{ab}	82.680±0.042^{ab}	82.583±0.081^{b}	82.530±0.050^{b}	82.685±0.057^{ab}	**82.668±0.104^{A}**
	GK	81.849±0.014^{a}	81.498±0.122^{b}	81.606±0.049^{ab}	81.562±0.064^{ab}	81.549±0.110^{ab}	81.447±0.035^{b}	**81.585±0.145^{B}**
Color a*	CK	-3.298±0.032^{a}	-3.338±0.032^{a}	-3.258±0.004^{a}	-3.318±0.110^{a}	-3.228±0.025^{a}	-3.358±0.025^{a}	**-3.299±0.060^{B}**
	GK	-3.103±0.040^{a}	3.047±0.013^{a}	-3.189±0.014^{a}	-3.189±0.023^{a}	-3.152±0.030^{a}	-3.174±0.077^{a}	**-3.142±0.062^{A}**
Color b*	CK	6.535±0.000^{c}	6.640±0.028^{bc}	6.743±0.025^{ab}	6.815±0.028^{a}	6.63±0.046^{bc}	6.830±0.035^{a}	**6.700±0.112^{A}**
	GK	5.741±0.191^{ab}	5.535±0.052^{b}	5.878±0.032^{ab}	5.960±0.042^{a}	5.891±0.044^{a}	5.804±0.043^{ab}	**5.801±0.157^{B}**

CK: Cow kefir, GK: Goat kefir, $\bar{x}$: Mean, n: number of repetitions, SD: Standard deviation, ^{a,b}: Means in each row show statistically difference (P>0.05 or P<0.05) among storage days for each property of the samples. ^{A,B}: Means in the same column show statistically difference between kefir samples in terms of related property (P<0.05). L*: lightness (0= black, 100= white), a*: green (-) or red (+), b*: blue (-) or yellow (+)

Table 4. Biochemical changes in cow and goat kefir samples during storage.

Properties	Kefir	Storage time (Days) ($\bar{x}$SD) (n=2)						
		1	7	14	21	28	35	General mean
Lipolysis (meqKOH 100g fat^{-1})	CK	0.45±0.016^{b*}	0.46±0.012^{b}	0.73±0.045^{ab}	1.03±0.018^{ab}	1.16±0.209^{a}	1.23±0.310^{a}	**0.84±0.349^{B*}**
	GK	0.99±0.002^{c}	1.12±0.050^{c}	1.33±0.001^{b}	1.63±0.000^{a}	1.43±0.086^{b}	1.42±0.060^{b}	**1.32±0.224^{A}**
Proteolysis (mg tyrosine 5g kefir^{-1})	CK	0.440±0.019^{d}	0.489±0.045^{cd}	0.582±0.031^{bc}	0.624±0.009^{b}	0.645±0.013^{b}	0.804±0.006^{a}	**0.597±0.124^{A}**
	GK	0.353±0.018^{b}	0.388±0.012^{ab}	0.412±0.014^{a}	0.407±0.009^{a}	0.404±0.009^{a}	0.422±0.006^{a}	**0.397±0.025^{B}**

CK: Cow kefir, GK: Goat kefir, $\bar{x}$: Mean, n: number of repetitions, SD: Standard deviation, ^{a,b}: Means in each row show statistically difference (P>0.05 or P<0.05) among storage days for each property of the samples. ^{A,B}: Means in the same column show statistically difference between kefir samples in terms of related property (P<0.05).

3.5. Microbial Profile

The changes in some microbial properties of the kefir samples during storage and statistical analysis were shown in Table 5. As seen from the table, there was no significant ($P>0.05$) difference in the counts of lactococci between the samples of CK and GK. Lactococci counts of the samples were consistent with the results obtained by WSZOLEK *et al*. (2001). The highest number of lactococci (~9 log cfu g^{-1}) was determined on the first day of storage in both kefir samples. The change in lactococci count between the 1st and 7th days of storage was significant in CK samples ($P<0.05$). In GK samples, the change in the lactococci count was statistically significant between the first and last day of storage ($P<0.05$). The number of lactococci was above 8 log units for both samples during storage. TEMIZ and KEZER (2015) reported that the decrease in the number of lactococci was less than about 1.25 log units after 28-day storage.

The general mean count of lactobacilli in CK samples was higher (6.74 log cfu g^{-1}) than that of GK samples (6.45 log cfu g^{-1}) and the difference was significant ($P<0.05$). WSZOLEK *et al*. (2001) reported the similar results. Lactobacilli counts of CK and GK samples decreased during storage ($P<0.05$). It was determined that 2.7 log-decrease in CK samples and 2.4 log-decrease in GK samples occurred on the 7th day of storage ($P<0.05$). IRIGOYEN *et al*. (2005) and GRØNNEVIK *et al*. (2011) reported that there was an approximately 2 log decrease in the number of lactobacilli in kefir samples on day 28 of storage when compared with the first day of storage.

The general mean count of leuconostocs of CK samples (6.74 log cfu g^{-1}) was higher than that of GK samples (6.50 log cfu g^{-1}), but not significant ($P>0.05$). WSZOLEK *et al*. (2001) reported the similar findings. The leuconostoc counts of the kefir samples decreased during storage and the decrease was significant ($P<0.05$). A statistically significant decrease was found in the leuconostoc count of CK samples on the 7th day of storage. On the other hand, a statistically significant decrease was found on the 1st, 7th and 14th days of storage of GK samples. At the end of storage, the number of leuconostoc of both kefir samples was over 6 log units. Some researchers reported that the number of leuconostoc in the kefir samples decreased below 6 log units at the end of storage (GRØNNEVIK *et al*., 2011; GUL *et al*., 2015).

The general mean counts of TMAB of each sample were found close to each other ($P>0.05$). TMAB counts of both samples decreased throughout storage time and the decrease was significant ($P<0.05$) for CK samples but not significant ($P>0.05$) for GK samples. In CK samples, the decrease was statistically significant between day 1st and day 14th of storage ($P<0.05$). TEMIZ and KEZER (2015) reported that storage time had effect on TMAB counts.

It was determined that the general mean counts of AAB of CK samples was higher than that of GK samples and this was significant ($P<0.05$). The similar findings were reported by IRIGOYEN *et al*. (2005). During storage, the number of AAB decreased in both samples ($P<0.05$). Approximately 1.2 log reduction ($P<0.05$) occurred between the 1st and 14th days of storage in GK samples. However, the counts of AAB in the GK samples remained almost constant ($P>0.05$) from the 14th day of storage to the end of storage. LEITE *et al*. (2013) reported a 0.6 log unit reduction in the counts of AAB during 28-day storage.

The general mean counts of yeast of CK and GK samples were 1.95 and 1.10 log cfu g^{-1}, respectively and the difference between them was significant ($P<0.05$). In general, kefir contains yeasts between 3-6 log cfu g^{-1} (ERTEKİN and GÜZEL-SEYDİM, 2010; DİNKÇİ *et al*., 2015).

Table 5. Microbial changes in cow and goat kefir samples during storage.

Properties	Kefir	Storage time (Days) ($\bar{x}$SD) (n=2)						
		1	7	14	21	28	35	General mean
Lactococci (log cfu g^{-1})	CK	9.23±0.057^{a*}	8.64±0.201^{b}	8.54±0.062^{b}	8.60±0.108^{b}	8.49±0.070^{b}	8.55±0.037^{b}	**8.67±0.275^{A*}**
	GK	8.98±0.008^{a}	8.65±0.054^{ab}	8.78±0.038^{ab}	8.68±0.154^{ab}	8.77±0.116^{ab}	8.49±0.043^{b}	**8.72±0.168^{A}**
Lactobacilli (log cfu g^{-1})	CK	9.04±0.163^{a}	6.33±0.341^{b}	6.28±0.031^{b}	6.22±0.047^{b}	6.26±0.003^{b}	6.28±0.151^{b}	**6.74±1.082^{A}**
	GK	8.62±0.054^{a}	6.20±0.237^{b}	5.96±0.054^{b}	5.96±0.136^{b}	5.96±0.170^{b}	6.01±0.143^{b}	**6.45±1.022^{B}**
Leuconostoc (log cfu g^{-1})	CK	8.33±0.231^{a}	6.53±0.112^{b}	6.41±0.082^{b}	6.39±0.011^{b}	6.42±0.023^{b}	6.35±0.364^{b}	**6.74±0.759^{A}**
	GK	8.44±0.001^{a}	6.61±0.103^{b}	5.92±0.108^{c}	6.01±0.067^{c}	5.94±0.126^{c}	6.11±0.052^{c}	**6.50±0.938^{A}**
TMAB (log cfu g^{-1})	CK	9.24±0.107^{a}	8.66±0.229^{ab}	8.64±0.137^{b}	8.57±0.021^{b}	8.36±0.144^{b}	8.26±0.173^{b}	**8.62±0.345^{A}**
	GK	9.35±0.100^{a}	8.55±0.013^{a}	8.71±0.078^{a}	8.20±0.730^{a}	8.64±0.161^{a}	8.20±0.067^{a}	**8.61±0.466^{A}**
AAB (log cfu g^{-1})	CK	7.37±0.012^{a}	6.36±0.146^{b}	6.11±0.040^{bc}	6.02±0.010^{bc}	6.03±0.001^{bc}	5.92±0.155^{c}	**6.30±0.525^{A}**
	GK	6.98±0.378^{a}	6.12±0.308^{ab}	5.77±0.128^{b}	5.81±0.083^{b}	5.76±0.161^{b}	5.72±0.218^{b}	**6.03±0.498^{B}**
Yeast (log cfu g^{-1})	CK	2.39±0.017^{a}	2.12±0.232^{a}	1.76±0.086^{a}	1.39±0.144^{a}	1.67±0.048^{a}	2.35±0.657^{a}	**1.95±0.443^{A}**
	GK	1.81±0.096^{a}	1.47±0.172^{a}	0.94±0.060^{a}	0.67±0.180^{a}	0.78±0.042^{a}	0.93±0.690^{a}	**1.10±0.477^{B}**

CK: Cow kefir, GK: Goat kefir, $\bar{x}$: Mean, n: number of repetitions, SD: Standard deviation, TMAB: Total mesophilic aerobic bacteria, AAB: Acetic acid bacteria, ^{a,b}: Means in each row show statistically difference ($P>0.05$ or $P<0.05$) among storage days for each property of the samples. ^{A,B}: Means in the same column show statistically difference between kefir samples in terms of related property ($P<0.05$).

However, some researchers reported yeast count between 0.5-3 log cfu g^{-1} during storage in kefir samples produced from cow and goat milk by using different commercial starter cultures (WSZOLEK *et al*., 2001; GARCIA FONTÁN *et al*., 2006; KESENKAŞ *et al*., 2011). In Codex standard for fermented milks (CODEX STAN 243-2003), it is stated that the number of yeasts in kefir should be at least 10^4 cfu g^{-1}. Yeasts produce ethyl alcohol and CO_2. The high number of yeasts in commercial kefir has caused packaging problems. In addition, due to consumer demand, low yeast counts have been generally preferred in kefir production in some countries. The difference of yeast number in kefir is varied depending on the type of culture used, the inoculation rate of the culture and the number of yeasts of the culture used. The number of yeasts in both CK and GK samples decreased until the 21st day of storage period. Then, the yeast count of the samples increased towards the end of the storage. During storage, the number of yeasts in both CK and GK samples was less than 2.5 log. The yeast count of CK samples was higher than that of GK samples during storage. Decrease in yeast count of both samples was nonsignificant ($P>0.05$) during storage DİNKÇİ *et al*. (2015) reported that storage time had no effect on yeast number.

3.6. Organic acid content

The changes in some organic acid contents of the kefir samples during storage were shown in Fig. 1.

General average lactic acid content in GK samples was greater than that of CK samples (Fig. 1a) and this was significant ($P<0.05$). TÜRKER *et al*. (2014) found that the lactic acid content of kefir samples from goat milk was higher than that of kefir samples from cow milk. The changes of lactic acid content in CK and GK samples were statistically significant ($P<0.05$) during storage. It was believed that fluctuations in the amount of lactic acid during storage were related to the amount of lactic acid produced by lactic acid bacteria and the assimilation of lactic acid by some yeast species (RATTRAY and O'CONNELL, 2011).

The average acetic acid content of GK samples was approximately 4 times higher than the values of CK samples (Fig. 1a) ($P<0.05$). TÜRKER *et al*. (2014) and GUL *et al*. (2015) reported that milk variety affected the amount of acetic acid in kefir. The acetic acid content of CK and GK samples were similar with values determined by MUIR *et al*. (1999) and GRØNNEVIK *et al*. (2011). The acetic acid content of CK samples was highest on day 1st of storage. The difference in the amount of acetic acid between the 1st and 7th days of storage was significant ($P<0.05$). GRØNNEVIK *et al*. (2011) reported that the change in the amount of acetic acid of kefir samples during storage was not significant. This was thought to be related to the fact that acetic acid is an intermediate product (LEITE *et al*., 2013).

The cow and goat milk used for kefir production in this study showed citric acid content of 1175 $\mu g\ g^{-1}$ and 433 $\mu g\ g^{-1}$, respectively. While the average amount of citric acid was 108.34 $\mu g\ g^{-1}$ in the GK samples, CK samples contained no citric acid (Fig. 1b). Citrate is a preferred substrate for the formation of acetoin and diacetyl by some lactic acid bacteria (GÜZEL-SEYDiM *et al*., 2000a). GRØNNEVIK *et al*. (2011) reported that there was more than 90 % reduction in the amount of citrate in kefir milk during fermentation and it was converted to other volatile components. ISMAIEL *et al*., (2011) could not detect citric acid in kefir produced under different fermentation conditions. The amount of citric acid in the GK samples increased up to the 14th day of storage ($P<0.05$), then suddenly decreased on the 21st day ($P<0.05$) and reached 35 $\mu g\ g^{-1}$. Although there was an increase in the amount of citric acid from the 21st day of storage to the end of storage, this increase was not

significant (P>0.05). KESENKAŞ *et al*. (2011) reported that the storage time affected the amount of citric acid in kefir.

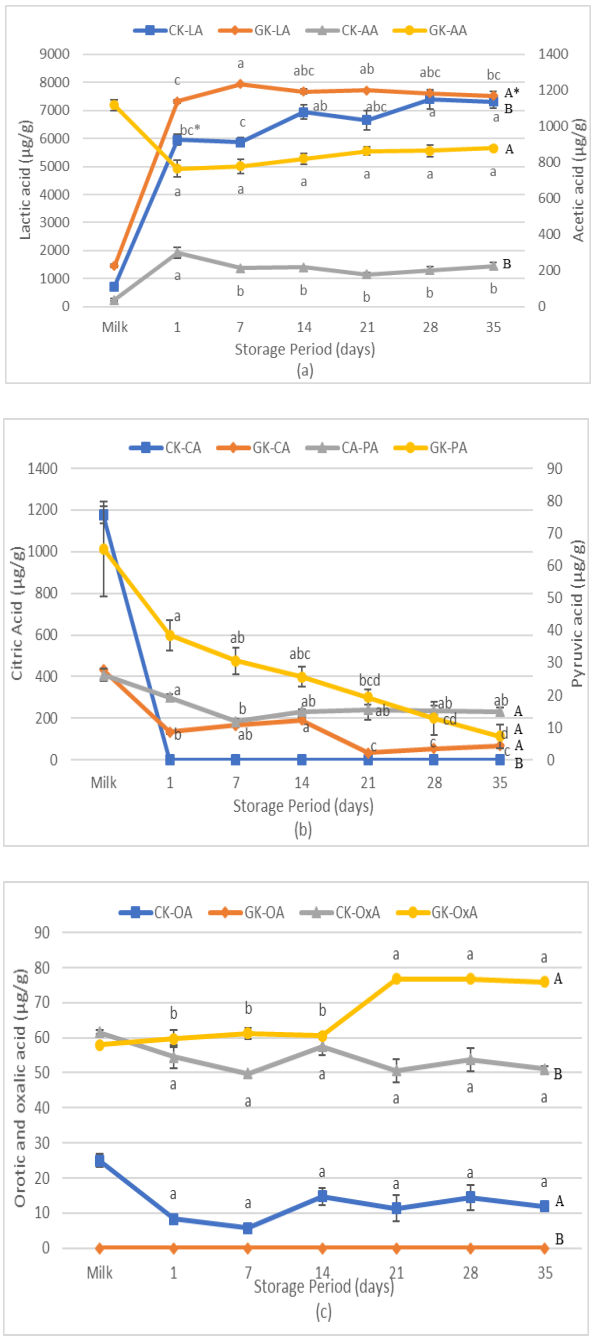

Figure 1. Lactic and acetic acid (a), citric and pyruvic acid (b), and orotic and oxalic acid (c) content in cow and goat kefir samples during storage (CK: Cow kefir, GK: Goat kefir, LA: Lactic acid, AA: Acetic acid, CA: Citric acid, PA: Pyruvic acid, OA: Orotic acid, OxA: Oxalic acid, ^{a,b,c,d}: show statistically difference (P>0.05 or P<0.05) among storage days for each property of the samples. ^{A,B}: show statistically difference between kefir samples in terms of related property (P<0.05).)

General mean pyruvic acid content of GK samples (22.39 $\mu g\ g^{-1}$) was higher than that of CK samples (15.31 $\mu g\ g^{-1}$) (P>0.05) (Fig. 1b). Pyruvic acid levels of both CK and GK samples were lower than the milk used in the production. MUIR *et al*. (1999) reported higher amounts in traditional and commercial kefir (47 and 60 $\mu g\ g^{-1}$). GÜZEL-SEYDİM *et al*. (2000b) and BESHKOVA *et al*. (2003) reported that pyruvic acid was completely consumed during storage. The amount of pyruvic acid significantly (P<0.05) decreased in both kefir samples during storage. Reduction in pyruvic acid might be due to the conversion to other organic compounds during storage (GÜZEL-SEYDİM *et al*., 2000b).
Orotic acid content was determined as 25 $\mu g\ g^{-1}$ in cow milk used in the production of CK samples and as 5.71-14-74 $\mu g\ g^{-1}$ in CK samples during storage (Fig. 1c). The values of CK samples were lower than findings of MUIR *et al*. (1999). On the other hand, no orotic acid was detected in both goat milk used in the production of the GK samples and GK samples during storage. In a study, while the amount of orotic acid decreased during fermentation of kefir, it increased during storage (GÜZEL-SEYDİM *et al*., 2000a,b). During storage time, no significant changes were observed in CK samples (P>0.05).
Overall mean content of oxalic acid of the GK samples was higher (P<0.05) than that of the CK samples (Fig. 1c). ISMAIEL *et al*. (2011) could not detect oxalic acid in kefir produced under different fermentation conditions. TÜRKER *et al*. (2014) reported that milk variety affects the amount of oxalic acid in kefir. oxalic acid might be found up to 169.15 mg L^{-1} in cow kefir and to 119.37 mg L^{-1} in goat kefir. During storage, fluctuations in the amount of oxalic acid in the kefir samples were determined. The changes in CK samples were not significant (P>0.05) during storage. In GK samples, the increase in the amount of oxalic acid on day 21st of storage was significant (P<0.05).

3.7. Aroma content

The changes in acetaldehyde, diacetyl, acetoin and ethanol content in the kefir samples during storage were shown in Fig. 2. The general mean value of acetaldehyde of CK samples was relatively higher, but insignificant (P>0.05). WSZOLEK *et al*. (2001) reported similar results. During storage, the amount of acetaldehyde decreased in both samples and the values were between 1.52-2.92 $\mu g\ g^{-1}$ in CK samples and 1.83-2.44 $\mu g\ g^{-1}$ in GK samples. These values were in agreement with values obtained by GRØNNEVIK *et al*. (2011). Differences in the amount of acetaldehyde in kefir may vary depending on milk fat ratio, starter culture type, the microbial diversity of the culture, the rate of use of culture, temperature and duration of incubation and storage (ERTEKIN and GÜZEL-SEYDIM, 2010; YILDIZ-AKGÜL *et al*., 2018). The changes in acetaldehyde values of kefir samples during storage were found to be significant only in the CK samples (P<0.05). BESHKOVA *et al*. (2003) reported a similar trend in amount of acetaldehyde during storage. The decrease in the amount of acetaldehyde is related to the conversion of acetaldehyde to ethyl alcohol by the enzyme, which called alcohol dehydrogenase.
Diacetyl content of the CK samples was higher than that of GK samples (Fig. 2a) (P<0.05). Diacetyl values obtained in this study were in accordance with the values determined by WSZOLEK *et al*. (2001) and BESHKOVA *et al*. (2003). In some studies, diacetyl was not detected during fermentation and storage of kefir (GÜZEL-SEYDİM *et al*., 2000a,b). During storage, the amount of diacetyl in CK samples increased up to the 14th day of storage and thereafter declined until the end of storage. In the GK samples, it increased until the 21st day of storage and decreased in the following days. The change in diacetyl values of kefir samples during storage was found to be significant (P<0.05) only in the CK samples. It has been reported that the optimum flavour balance for kefir is to be achieved when the ratio

of diacetyl to acetaldehyde is 3:1 (GRØNNEVIK *et al*., 2011). The highest ratio of diacetyl to acetaldehyde during storage was determined to be on the 14th day in the CK samples and on the 21st day in the GK samples. However, these rates were below 3. Actually, some of researchers found this ratio between 0-2 in their studies (WSZOLEK *et al*., 2001; GRØNNEVIK *et al*., 2011; YILDIZ-AKGÜL *et al*., 2018).

Acetoin content was determined to be between 13.86-17.26 $\mu g\ g^{-1}$ in the CK samples and 7.81-9.00 $\mu g\ g^{-1}$ in the GK samples during storage (Fig. 2b). General mean value of acetoin of CK samples was higher than that of GK samples ($P<0.05$). The values of the CK samples were similar with the values obtained (16-25 $\mu g\ g^{-1}$) by GÜZEL-SEYDİM *et al*. (2000b). BESHKOVA *et al*. (2003) were unable to detect acetoin in kefir samples in their study. Acetoin value of the samples tended to decrease during storage. The change in the amount of acetoin was significant ($P<0.05$) only in CK samples especially on day 21 during storage. GRØNNEVIK *et al*. (2011) reported that the amount of acetoin formed in kefir samples decreased during storage. The decrease in the amount of acetoin may be associated with further degradation, which results in 2.3-butanediol (GRØNNEVIK *et al*., 2011).

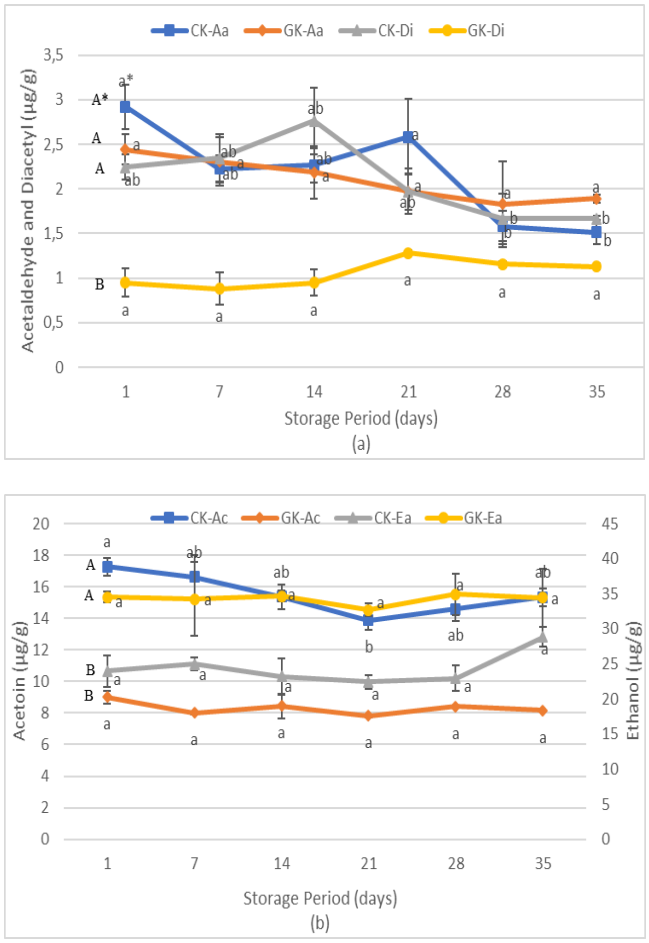

Figure 2. Acetaldehyde - diacetyl (a), acetoin - ethanol (b) contents in cow and goat kefir samples during storage (CK: Cow kefir, GK: Goat kefir, Aa: Acetaldehyde, Di: Diacetyl, Ac: Acetoin, Ea: Ethanol, ^{a,b,c,d}: show statistically difference ($P>0.05$ or $P<0.05$) among storage days for each property of the samples. ^{A,B}: show statistically difference between kefir samples in terms of related property ($P<0.05$).)

General mean value of ethanol was higher in the GK samples and this difference was significant (P<0.05) (Fig. 2b). The values obtained in this study were consistent with values reported by GRØNNEVIK *et al*. (2011) and GUL *et al*. (2015). Yeasts are primarily responsible for alcohol production in kefir, and some heterofermentative lactobacilli, such as *Lactobacillus kefir*, can also produce ethanol (GÜZEL-SEYDİM *et al*., 2000a; RATTRAY and O'CONNELL, 2011). In some studies, ethanol content in kefir was determined to vary between 0-9700 $\mu g\ g^{-1}$ (GÜZEL-SEYDİM *et al*., 2000b; WSZOLEK *et al*., 2001; GARCÍA FONTÁN *et al*., 2006; PURNOMO and MUSLIMIN, 2012; TEMIZ and KEZER, 2015; YILDIZ *et al*., 2018). The amount of ethanol in kefir varies greatly depending on the type of culture used, the inoculation rate of the culture and the microbial diversity of the culture. While a considerable change did not occur in the amount of ethanol in the CK samples until the 28th day of storage period, a sudden increase happened on the last day. The amount of ethanol in the GK samples did not change much during storage. Changes in the amount of ethanol in both samples were not significant (P>0.05). The increase in ethanol content of the CK samples on the last day of storage can be attributed to the high number of yeasts on the same day. WSZOLEK *et al*. (2001) found that storage time did not affect the amount of ethyl alcohol in kefir samples, like our results.

3.8. Sensory properties

The overall average scores of structure, consistence and texture, taste and smell, and general appreciation of CK samples was higher than the average values of GK samples (Fig. 3). While the difference between the structure, consistence and texture, and general appreciation scores of both samples were significant (P<0.05), the difference between taste and smell scores were nonsignificant (P>0.05). In terms of all sensory characteristics, the highest sensory scores were obtained on the 14th day of storage in the CK samples while the lowest sensory scores were on the 35th day of storage. The highest sensory scores were obtained on the 21st day of storage in the GK samples and lowest sensory scores were obtained on the 7th day of storage. In both samples, the change in sensory properties during storage period was significant (P<0.05) except for taste and smell characteristic of GC sample. Panelists reported that the GK samples had low consistence, did not give full sensation in the mouth and showed distinct the goat smell.

Figure 3. Sensory changes in cow and goat kefir samples during storage.

4. CONCLUSIONS

Based on the results of this study, it can be said that kefir samples produced from both cow and goat milk can be stored up to 35 days at refrigerator temperature. All samples had sensory scores above 3 during storage. However, the highest scores by sensory analyses and the highest diacetyl/acetaldehyde ratio in the kefir samples were obtained on the 14th day of storage in CK samples and the 21st day of storage in GC samples.

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