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Determination of rheological properties of kefir produced with buffalo milk and other milk mixtures*

Manda sütü ve diğer süt karışımlarıyla üretilen kefirlerin reolojik özelliklerinin belirlenmesi

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ABSTRACT

Objective: The objective of this study was to develop the product and to determine the rheological model and sensorial properties of kefir.

Material and Methods: The shear rate ($\dot{\gamma}$) and shear stress (τ) values measured at 15-20°C using the rheological properties of kefir produced with different types of ruminant milk are characterized by mathematical models (Newton, Power law, Bingham), consistency coefficient (K), flow behavior index (n), threshold shear stress (τ_0) and apparent viscosity (μ_{app}) values were calculated. Sensory assessment was carried out using facial expression sensory evaluation scale and scoring method.

Results: As a result of rheological analysis, the Power law model appears to be suitable for describing the flow behavior of kefir types as indicated by high correlation coefficient (R^2) values in this study. The flow diagrams of kefir processed with different milk species exhibit shear-thinning non-Newtonian behavior. Sensory analysis showed that the samples did not receive a great deal of appreciation about organoleptic properties.

Conclusion: Shelf life should be 28 days. Hence, kefir preserves the sensory properties until end of shelf life and to keep rheological properties compatible with each other throughout shelf life.

ÖZ

Amaç: Bu çalışmada farklı ruminant sütlerinin çeşitli kombinasyonları ile kefir üretmek üzere ürün geliştirilmesi ve kefirin reolojik modellere uyumu ve duyuşal özelliklerinin belirlenmesi amaçlanmıştır.

Materyal ve Yöntem: Farklı ruminant süt türleri ile üretilen kefirin reolojik özellikleri 15-20°C'de ölçülen kayma hızı ($\dot{\gamma}$), kayma gerilimi (τ), kıvam katsayısı (K), akış davranış indeksi (n), eşik kayma gerilimi (τ_0) ve görünür viskozite (μ_{app}) değerleri matematiksel modeller (Newton, Power law, Bingham) ile karakterize edilerek hesaplanmıştır. Yüz ifadeli duyuşal değerlendirme formu ve puanlama yönteminde yararlanarak duyuşal değerlendirme gerçekleştirilmiştir.

Araştırma Bulguları: Reolojik analizler sonucunda, yüksek korelasyon katsayısı (R^2) değerleri ile gösterilen kefir türlerinin akış davranışlarını açıklamak için Power-law modelinin uygun olduğu belirlenmiştir. Farklı süt türleri ile işlenen kefirin akış diyagramları, Newtonian olmayan kayma incelenmesi davranışı sergilemektedir. Duyusal analiz sonuçlarına göre, örneklerin duyuşal olarak çok fazla takdir almadığı görülmektedir.

Sonuç: Raf ömrünün 28 gün olması gerektiği sonucuna varılmıştır. Bu sayede hem duyuşal özellikler raf ömrünün sonuna kadar korunabilir hem de reolojik özellikleri raf ömrü boyunca birbirleriyle uyumlu kalabilir.

INTRODUCTION

Fermented milk products are of importance as a human nutrition and are considered as basic foodstuffs. In recent years, fermented dairy products received a great deal of attention for human health and as a result of this, their consumption increased significantly. (Demirgul et al., 2018).

Kefir is an important fermented dairy product and referred to as a probiotic since it contains more than 10^7 cfu/g of beneficial and specific microorganisms (Kesenkas et al., 2013). Kefir is known for its refreshing effect in the mouth, typical yeast taste and unique aroma. The word "kefir" is derived from the Turkish word "keyif (pleasure)," which means "feeling good" after drinking (Lopitz-Otsoa et al., 2006; Tamime & Robinson, 2007). Although cow's milk is most commonly used for its production, it can also be produced from the milk of other animals such as goats, sheep, buffalo and camels as well as from vegetable sources such as soybean, rice, and coconut milk. Kefir can be also prepared from pasteurized whole, semi-skimmed or skimmed milk (Otlés & Cagindi, 2003; Rosa et al., 2017).

Studies conducted in the past indicated that there are many probiotic microorganisms in the microbiota of kefir. Studies also revealed that various bacteria and yeasts isolated from kefir are resistant to low pH and bile acids can adhere to the intestinal mucosa, have a strong antagonistic effect against pathogens, and have positive effects on health (Golowczyc et al., 2008; Diosma et al., 2014; Zanirati et al., 2015). Various studies reported that kefir has antimicrobial, anti-inflammatory, antiallergenic, and anticarcinogenic effects. Furthermore, the product is effective in controlling the body weight, glycemic response, blood pressure, and blood lipids and can be used against digestion problems such as lactose intolerance and constipation.

In this study, K value was 4.65 Pa.s, n value was 0.35, and μ_{apparent} was 0.32 Pa.s in all samples and during storage. In the study by Ergin et al. (2017) on homogenized kefir, the K value was lower (0.021-0.545 Pa.s) and the n value was 0.56-0.98. The consistency coefficient was determined to be slightly higher in the present study due to the difference in raw materials and the lack of homogenization process, whereas there was a decrease in the flow behavior index toward the end of the shelf life in the study by Ergin et al. (2017). The study conducted by Gurbuz & Sheifel (2008) examined the power law model and determined the n value to be in the range of 0.35-0.4. In a study on kumis, the n values determined at temperatures of 4-10-20°C were 0.74-0.79-0.75, respectively, and the K values were 0.015-0.010-0.008 Pa.s, respectively (Sabancı et al., 2016).

On the other hand, the rheological properties are particularly important to determine different interactions in new kefir formulation (Gul et al., 2018). Dairy beverages are marketed with different rheological properties and qualities. Especially, maintaining the texture can be a problem in the commercial manufacture of alternative fermented dairy products. To guarantee the quality of the final product, manufacturers rely on pre-fermentation processing such as increasing milk solids and/or supplementation or additives for product stability (Duboc & Mollet, 2001; Rimada & Abraham, 2006). Rheological parameters have been considered as an analytical tool to provide fundamental insights on the structural organization of food and play an important role in fluid heat transfer.

Knowing the rheological properties of kefir is important in determining the processes to be carried out during the storage and packaging process (Assil et al., 1991; Ahmed et al., 2007). Rheology has many applications in the field of food acceptability, food processing and packaging and the relationship between consumer preferences and rheological properties of foods is a key role of the science of rheology. The major factors affecting the rheological properties of kefir are the chemical composition of the milk used for its production, the starter culture, the type of milk and/or milk mixtures the incubation temperature, the thermal processing of milk, etc. (Kayacier & Dogan, 2006). Especially fat and protein

constitute one of the most important fractions of milk. Fat globules play an eminent role in the technology and properties of dairy products (Elzeini, 2006). Size of fat globules is of particular importance in such processes as separation of milk, churning of cream, cheese making as well as the functionality of cheese (Metzger & Mistry, 1995; Gunasekaran & Ding, 1999; Ma & Barbano, 2000; Everett & Olson, 2003; Rowney et al., 2003). It determines the amount of protein absorbed per unit of interface area, and products emulsion stability and their optical and rheological properties (color and viscosity) (Walstra & Jenness, 1984; Walstra, 1995) as well as conductivity and elastic constant. Studies on the rheological properties of kefir in the literature are limited (Wszolek et al., 2001; Paraskevopoulou et al., 2003; Bensmira et al., 2010) and there are no research on the effects of processing circumstances on kefir quality (Dogan, 2011). So far, most of the studies conducted on kefir concerned with its microbiology. Hence a study was conducted and the objective of this study was evaluating the effect of different ratios of milk mixtures on the time needed for the pH to reach the 4.4 value and the rheological behavior and sensorial properties of kefir.

MATERIALS and METHODS

Materials

Buffalo, cow, sheep, and goat milk

The entire buffalo milk and a part of the sheep milk used as raw materials in the study were obtained from a private facility, cow and goat milk was obtained from the farm of the Ege University Faculty of Agriculture Farm. All of the cow and goat milk required for the study was obtained from internal sources.

Kefir starter culture

The kefir starter culture used in the production process was prepared using Micromilk KFA 1 culture mixture (Micromilk, Italy) (30 % *Streptococcus thermophilus*, 20 % *Lactococcus lactis* subsp. *lactis*, 20 % *Lactococcus lactis* subsp. *cremoris*, 10 % *Debarymyces hansenii*, 10 % *Leuconostoc mesenteroides* subsp. *cremoris* and 10 % *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*).

Methods

Kefir production

Raw buffalo, cow, sheep, and goat milk were heat-treated at 90°C for 15 minutes. In order not to negatively affect the growth of microorganisms in kefir culture, a single pasteurization process was performed, and double pasteurization was not applied, the milk was pasteurized one by one and mixed in proper proportions and culture was inoculated. Then, 100 % buffalo, 70 % buffalo-30 % cow, 70 % buffalo-30 % sheep, 70 % buffalo-30 % goat and 70 % buffalo-30 % cow-sheep-goat milk were cooled down to 25-30°C and added with Micromilk KFA 1 kefir culture at a rate of 2.5 %-3 % separately. The main idea for choosing milk with 70% buffalo and 30% cow/sheep/goat ratios was to determine the effect of high amount of buffalo milk on sensory quality and rheological properties. The incubation was terminated when the pH value reached 4.7. at the end of the incubation, in order for the product to be suitable for storage under refrigerator conditions, it was kept at room temperature for 30 minutes, and after the temperature was reduced, bottling and it was stored for 24 hours in the refrigerator. The results were statistically evaluated once the pilot analyses were conducted in two replicates. The shelf life was determined as 28 days and 1., 7., 14., 21. and 28. days rheological and sensorial analyzes were made.

The basic components of milk and kefir samples were determined and they are tabulated in Table 1 and Table 2, respectively.

Table 1. Basic component of milks**Çizelge 1.** Sütlerin temel bileşenleri

Components/Value	Milks			
	Buffalo	Cow	Sheep	Goat
Dry matter %	18.70±0.21	12.92±0.12	20.86±0.06	13.29±0.09
Fat %	7.00±0.03	3.60±0.01	8.00±0.01	4.16±0.06
Protein %	5.70±0.04	3.48±0.01	5.95±0.01	3.21±0.01
Lactose %	5.40±0.01	5.04±0.02	5.90±0.01	5.02±0.09
Ash %	0.60±0.01	0.80±0.01	1.01±0.02	0.90±0.01
Lactic acid %	0.14±0.01	0.16±0.01	0.19±0.01	0.15±0.01
pH value	6.57±0.01	6.55±0.01	6.55±0.01	6.54±0.01

Table 2. Basic component of kefir**Çizelge 2.** Kefirlerin temel bileşenleri

Components/Value	Kefirs				
	100 % buffalo	70 % buffalo-30 % cow	70 % buffalo-30 % sheep	70 % buffalo-30 % goat	70 % buffalo-30 % cow-sheep-goat
Dry matter %	16.64±0.02	14.42±0.02	17.65±0.04	14.58±0.04	15.69±0.10
Fat %	6.75±0.07	5.95±0.07	6.85±0.07	6.05±0.07	6.10±0.14
Protein %	4.30±0.02	4.04±0.02	4.70±0.01	4.05±0.04	4.15±0.04
Lactose %	4.84±0.16	3.61±0.16	5.20±0.70	3.74±0.16	5.44±0.88
Ash %	0.75±0.01	0.82±0.02	0.90±0.07	0.74±0.04	0.98±0.04
Lactic acid %	0.95±0.05	0.95±0.05	1.04±0.02	0.94±0.06	0.91±0.04
pH value	4.71±0.01	4.69±0.01	4.60±0.01	4.62±0.01	4.60±0.02

Rheological measurements

Rheological properties were determined with a Brookfield LVDV-II Pro (ABD) brand viscometer. Rheological calculations were made taking into account the measurements that give torque values in the range of 0 to 200 rpm, and torque values between 10% and 90%. The models and equations are given in Table 3.

Table 3. Compatibility of milks with rheological models**Çizelge 3.** Sütlerin reolojik modellere uyumluluğu

Milk	Criteria	Rheological Model Compatibility		
		Newtonian Model	Power Law Model	Bingham Model
Buffalo	R ²	0.912±0.003	0.972±0.003	0.982±0.002
	RMSE	3.533±0.003	0.292±0.003	0.263±0.001
	χ ²	13.623±0.012	0.097±0.012	0.078±0.007
Cow	R ²	0.963±0.002	0.954±0.002	0.932±0.003
	RMSE	0.028±0.002	0.089±0.002	0.415±0.011
	χ ²	0.001±0.001	0.012±0.002	0.258±0.004
Sheep	R ²	0.955±0.001	0.995±0.001	0.963±0.002
	RMSE	0.073±0.003	0.029±0.003	0.078±0.003
	χ ²	0.005±0.001	0.001±0.001	0.007±0.001
Goat	R ²	0.932±0.003	0.963±0.003	0.955±0.001
	RMSE	1.602±0.004	0.077±0.004	0.323±0.001
	χ ²	2.704±0.001	0.006±0.001	0.116±0.002

The models' equations are given in Equations (1-6). Accordingly, μ is the Newtonian fluid viscosity (Pa.s), τ is the shear stress (Pa), τ_0 is the initial shear stress (Pa), κ is the coefficient of consistency (Pa.s), σ is the shear rate (1/s), n is the fluid behavior index (Saygılı et al., 2022). The apparent viscosity values, consistency coefficient, initial shear stress and flow behavior index values of kefir samples produced with various combinations of buffalo, cow, sheep and goat milk were determined. Apparent viscosity values (μ_{apparent}) are used for non-Newtonian fluids. In the study, the apparent viscosity values were calculated depending on the K and n values 0.05 for 100 rpm (Equation 1).

$$\mu_{\text{apparent}} = K \cdot \dot{\gamma}^{n-1} \quad (1 - \text{Apparent Viscosity})$$

$$\tau = \mu \cdot \dot{\gamma} \quad (2 - \text{Newtonian Model})$$

$$\tau = K \cdot (\dot{\gamma})^n \quad (3 - \text{Power Law Model})$$

$$\tau = \tau_0 + (K \cdot \dot{\gamma}) \quad (4 - \text{Bingham Model})$$

$$RMSE = \left[\left(\frac{1}{N} \sum_{i=1}^n (\text{Theoretical value}_i - \text{Experimental value}_i)^2 \right)^{0.5} \right] \quad (5 - \text{Root Mean Square})$$

$$\chi^2 = \frac{\sum_{i=1}^n (\text{Theoretical value}_i - \text{Experimental value}_i)^2}{N-n} \quad (6 - \text{Chi Square})$$

Post-production samples were stored in the refrigerator. Analyzes were made with three parallels. Preliminary experiments were made in rheological analyzes and spindle 18 and spindle 34 were used for this purpose (Saygılı et al., 2022).

Sensory analysis

Kefir samples were presented to the panelists at 20°C for tasting. By tickle, approximately 20 g of product was put into plastic glasses and ready to tasting.

All samples, until the product was deemed not acceptable, were evaluated by at 8 assessors trained in evaluating dairy products. The attributes considered were: odour intensity, milky odour, fermented odour, vegetable odour, mouth odour, viscosity, flavour intensity, dairy taste, sour taste, bitter taste, milky taste, astringency, and acceptability. All panellists had regular previous experience of sensory testing milk products and were trained to judge various product formulations of differing textures. Each attribute was scored on an increasing scale of 1 (not present) to 7 (very intense).

Statistical analysis

IBM SPSS Statistics package program was used for the evaluation of experimental data. The significance of the differences between rheological properties and sensory properties in terms of different milk ratios in the mean values ($p < 0.05$) were determined by the One-way ANOVA multiple comparison test in the Duncan's test. The kefir production and analysis were applied 2 different times.

RESULTS and DISCUSSION

The present study aimed to characterize the experimental shear stress-shear velocity data using Newtonian, power law, and Bingham models to determine the rheological characteristics of different milk and kefir samples, and to investigate the changes during storage. In this regard, linear and nonlinear regression analyses were achieved, and the results were statistically interpreted. The results of the study and its comparison with other studies are as follows.

The highest R^2 values and the lowest χ^2 and RMSE values were considered as the criteria in choosing the model that best expresses the rheological characteristics of buffalo, cow, sheep, and goat milk (Table 3). Although the Newtonian character is the most common fluid type for milk, non-Newtonian time-independent fluid behavior is also observed in some milk varieties (Gurbuz et al., 2008; Dogan,

2011). The results indicated that all milk samples can be characterized with high regression coefficient and low error level using the models examined. It was determined that the rheological properties of buffalo milk were characterized using both Bingham and power law models while the power law model was the most suitable model for sheep and goat milks with the lowest statistical error. In addition, cow milk was characterized by both Newtonian and power law models, similarly. It was aimed to compare the rheological properties of all milks in the base of the same rheological model with low statistical errors. Thus, the common rheological model characterizing the rheological properties of all milk samples with high compatibilities was selected as the power law model (Table 5). The rheological coefficient values determined for the power law model are given in Table 4. K (consistency coefficient), n (flow behavior index), and apparent viscosity (μ_{apparent}) values for all kefir samples show low similarity with each other, whereas the K value and μ_{apparent} value were similar in cow and sheep milk samples.

Table 4. Rheological coefficient values of milks determined according to Power law fluid model

Çizelge 4. Sütlerin Üssel akışkan modele göre belirlenen reolojik katsayı değerleri

Milk	K (Pa.s ⁿ)	n (-)	μ_{apparent} (Pa.s)
Buffalo	5.489±0.004 ^C	0.282±0.004 ^a	0.290±0.003 ^z
Cow	0.001±0.001 ^A	1.100±0.006 ^d	0.001±0.001 ^x
Sheep	0.025±0.004 ^A	0.646±0.005 ^c	0.005±0.002 ^x
Goat	1.692±0.011 ^B	0.403±0.007 ^b	0.146±0.003 ^y

Similarly, the compatibility of rheological models to the experimental data for kefir samples produced by using the different combinations of milks was characterized by using Bingham, power law and Newtonian models (Icier et al., 2008). The highest R² values, the lowest χ^2 and root mean square error (RMSE) values were taken as the criteria for choosing the model that best expressed the rheological characteristics of the kefir samples. The statistical error levels of kefir no.1 (100 % buffalo milk), which were determined based on the rheological models are given in Table 5.

Table 5. Compatibility of buffalo milk (1) kefir with rheological models

Çizelge 5. Manda sütü (1) kefirinin reolojik modellere uyumluluğu

Days	Criteria	Rheological Model Compatibility		
		Newtonian Model	Power Law Model	Bingham Model
1	R ²	0.112±0.001	0.996±0.003	0.948±0.001
	RMSE	2.357±0.009	0.156±0.037	0.545±0.017
	χ^2	5.849±0.047	0.030±0.013	0.331±0.021
7	R ²	0.261±0.025	0.998±0.001	0.965±0.003
	RMSE	1.264±0.034	0.073±0.004	0.277±0.010
	χ^2	1.687±0.092	0.006±0.001	0.085±0.006
14	R ²	0.381±0.001	0.998±0.001	0.982±0.015
	RMSE	6.715±0.284	0.291±0.026	1.046±0.357
	χ^2	47.921±3.818	0.097±0.016	1.505±0.828
21	R ²	0.424±0.025	0.998±0.002	0.974±0.002
	RMSE	1.213±0.018	0.067±0.014	0.257±0.012
	χ^2	1.550±0.046	0.005±0.002	0.074±0.006
28	R ²	0.061±0.001	0.999±0.001	0.948±0.001
	RMSE	1.757±0.055	0.004±0.001	0.377±0.009
	χ^2	3.258±0.202	0.030±0.013	0.158±0.007

It was determined that the rheological properties of kefir no. 1 during the storage period were best characterized by the power law model, which had the lowest error level among the studied models. The statistical error levels of kefir no. 2 (70 % buffalo-30 % cow), no. 3 (70 % buffalo-30 % sheep), no. 4 (70 % buffalo-30 % goats), and no. 5 (70 % buffalo-30 % cow, sheep, goat) determined based on the examined rheological models were given in Tables 5-9. Similarly, it was found that the rheological characteristics of kefir nos. 2, 3, 4, and 5 in all storage processes can be characterized using the power law model with the least statistical error level. Previous studies reported that the kefir samples exhibited a non-Newtonian fluid character in the range of 10°C-30°C (Icier et al., 2008). The mathematical model explaining this behavior was reported as the Herschel-Bulkley model that combines the Bingham and power law fluid behaviors.

Table 6. Compatibility of buffalo-cow milk (2) kefir with rheological models

Çizelge 6. Manda-inek sütü (2) kefirinin reolojik modellere uyumluluğu

Days	Criteria	Rheological Model Compatibility		
		Newtonian Model	Power Law Model	Bingham Model
1	R ²	0.229±0.086	0.998±0.002	0.963±0.003
	RMSE	1.927±0.060	0.139±0.006	0.443±0.031
	χ ²	4.075±0.247	0.020±0.002	0.218±0.031
7	R ²	0.265±0.122	0.997±0.001	0.960±0.007
	RMSE	1.594±0.062	0.096±0.038	0.372±0.051
	χ ²	2.678±0.209	0.011±0.008	0.155±0.042
14	R ²	0.061±0.035	0.998±0.001	0.947±0.001
	RMSE	9.498±0.395	0.375±0.034	2.183±0.159
	χ ²	95.045±7.896	0.157±0.028	5.309±0.774
21	R ²	0.103±0.001	0.999±0.001	0.952±0.002
	RMSE	1.688±0.009	0.049±0.001	0.388±0.013
	χ ²	2.999±0.032	0.002±0.001	0.167±0.011
28	R ²	0.007±0.002	0.989±0.006	0.887±0.086
	RMSE	3.022±0.140	0.149±0.086	0.537±0.088
	χ ²	9.625±0.891	0.029±0.029	0.324±0.105

Table 7. Compatibility of buffalo-sheep milk (3) kefir with rheological models

Çizelge 7. Manda-koyun sütü (3) kefirinin reolojik modellere uyumluluğu

Days	Criteria	Rheological Model Compatibility		
		Newtonian Model	Power Law Model	Bingham Model
1	R ²	0.038±0.003	0.996±0.003	0.945±0.021
	RMSE	1.969±0.002	0.115±0.057	0.465±0.102
	χ ²	4.085±0.084	0.016±0.015	0.246±0.106
7	R ²	0.003±0.001	0.996±0.001	0.962±0.002
	RMSE	5.120±1.104	0.218±0.038	0.699±0.360
	χ ²	28.293±11.81	0.054±0.018	0.616±0.557
14	R ²	0.064±0.004	0.988±0.002	0.970±0.001
	RMSE	2.444±0.618	0.244±0.165	0.350±0.210
	χ ²	6.616±3.221	0.084±0.093	0.167±0.170
21	R ²	0.049±0.002	0.988±0.007	0.949±0.002
	RMSE	4.061±1.639	0.225±0.017	0.495±0.155
	χ ²	18.895±13.96	0.058±0.010	0.291±0.168
28	R ²	0.019±0.003	0.988±0.005	0.927±0.029
	RMSE	9.402±2.175	0.410±0.196	0.948±0.127
	χ ²	95.559±43.06	0.208±0.179	1.007±0.256

Table 8. Compatibility of buffalo-goat milk (4) kefir with rheological models**Çizelge 8.** Manda-keçi sütü (4) kefirinin reolojik modellere uyumluluğu

Days	Criteria	Rheological Model Compatibility		
		Newtonian Model	Power Law Model	Bingham Model
1	R ²	0.099±0.002	0.986±0.012	0.979±0.008
	RMSE	2.283±0.266	0.159±0.098	0.208±0.042
	χ ²	5.609±1.279	0.034±0.035	0.064±0.020
7	R ²	0.087±0.003	0.994±0.008	0.931±0.013
	RMSE	1.712±0.001	0.133±0.091	0.460±0.122
	χ ²	3.086±0.036	0.024±0.027	0.243±0.125
14	R ²	0.128±0.003	0.998±0.002	0.959±0.015
	RMSE	10.407±0.09	0.463±0.117	2.040±0.255
	χ ²	114.029±1.94	0.246±0.120	4.660±1.156
21	R ²	0.145±0.001	0.997±0.003	0.960±0.012
	RMSE	1.691±0.001	0.077±0.029	0.326±0.036
	χ ²	3.012±0.001	0.007±0.005	0.118±0.026
28	R ²	0.128±0.003	0.997±0.001	0.931±0.014
	RMSE	3.060±0.095	0.088±0.010	0.465±0.082
	χ ²	9.867±0.611	0.008±0.002	0.244±0.085

Table 9. Compatibility of buffalo-cow-sheep-goat milk (5) kefir with rheological models**Çizelge 9.** Manda-inek-koyun-keçi sütü (5) kefirinin reolojik modellere uyumluluğu

Days	Criteria	Rheological Model Compatibility		
		Newtonian Model	Power Law Model	Bingham Model
1	R ²	0.094±0.003	0.947±0.022	0.985±0.004
	RMSE	5.064±1.316	0.370±0.280	0.256±0.070
	χ ²	27.957±13.95	0.196±0.231	0.760±0.041
7	R ²	0.127±0.001	0.989±0.001	0.977±0.002
	RMSE	1.934±0.002	0.181±0.049	0.252±0.048
	χ ²	4.020±0.007	0.039±0.020	0.075±0.027
14	R ²	0.182±0.004	0.983±0.012	0.969±0.015
	RMSE	2.483±0.224	0.132±0.063	0.174±0.090
	χ ²	6.651±1.124	0.022±0.020	0.040±0.037
21	R ²	0.144±0.002	0.944±0.021	0.910±0.093
	RMSE	1.825±0.048	0.144±0.089	0.189±0.094
	χ ²	3.602±0.172	0.029±0.031	0.047±0.042
28	R ²	0.077±0.022	0.977±0.012	0.943±0.001
	RMSE	2.617±0.479	0.179±0.053	0.278±0.054
	χ ²	7.516±2.676	0.039±0.023	0.093±0.036

Since all samples were noted to be compatible with the power law model, the rheological coefficient values K (consistency coefficient) and n (flow behavior index) and apparent viscosity (μ_{apparent}) values were calculated for this model. The apparent viscosity equation for the power law model was determined by using Equation 1 (Karatas, 2014). The variation between shear stress and shear rate for non-Newtonian fluids is not linear, and the mechanical shear rate value in the mouth is experimentally assumed to be 60 1/sec (Steffe, 1996). Hence, the apparent viscosity values were calculated by employing the rheological constants in Equation 1 and taking the shear stress as 60 1/sec. The rheological coefficient and apparent viscosity values derived for the power law model are given in Table 10.

$$\mu_{\text{apparent}} = K^* \dot{\gamma} n^{-1} \quad (1)$$

Table 10. Rheological coefficient values of kefir characterized by the Power Law fluid model**Çizelge 10.** Üssel akışkan modeli ile karakterize edilen kefirlerin reolojik katsayı değerleri

Samples	Days	K (Pa.s ⁿ)	n (-)	μ_{apparent} (Pa.s)
1 (100 % Buffalo)	1	2.453±0.084 ^B	0.403±0.010 ^c	0.208±0.003 ^y
	7	1.349±0.028 ^A	0.426±0.006 ^c	0.129±0.001 ^x
	14	7.856±0.121 ^C	0.449±0.005 ^c	0.932±0.007 ^t
	21	1.175±0.031 ^A	0.453±0.007 ^c	0.129±0.003 ^x
	28	2.022±0.030 ^B	0.373±0.004 ^b	0.152±0.002 ^x
2 (Buffalo-Cow)	1	2.022±0.108 ^B	0.421±0.015 ^c	0.190±0.002 ^x
	7	1.621±0.120 ^A	0.428±0.021 ^c	0.155±0.002 ^x
	14	11.06±0.494 ^D	0.397±0.004 ^b	0.936±0.025 ^t
	21	1.770±0.001 ^A	0.403±0.001 ^{bc}	0.153±0.002 ^x
	28	3.999±0.268 ^{BC}	0.251±0.018 ^a	0.185±0.005 ^x
3 (Buffalo-Sheep)	1	2.096±0.015 ^B	0.391±0.004 ^b	0.172±0.002 ^x
	7	6.781±1.360 ^C	0.323±0.021 ^{ab}	0.421±0.048 ^z
	14	3.825±0.538 ^B	0.364±0.031 ^b	0.286±0.077 ^y
	21	5.739±1.576 ^C	0.332±0.007 ^{ab}	0.371±0.091 ^y
	28	14.72±5.255 ^D	0.201±0.091 ^a	0.539±0.004 ^z
4 (Buffalo-Goat)	1	3.432±0.409 ^B	0.356±0.030 ^b	0.244±0.001 ^y
	7	1.770±0.012 ^A	0.401±0.001 ^b	0.152±0.002 ^x
	14	12.412±0.011 ^D	0.380±0.008 ^b	0.995±0.004 ^t
	21	1.840±0.001 ^A	0.382±0.002 ^b	0.146±0.001 ^x
	28	3.971±0.176 ^B	0.269±0.012 ^a	0.198±0.002 ^x
5 (Buffalo-Cow-Sheep-Goat)	1	7.977±2.980 ^C	0.228±0.107 ^a	0.332±0.019 ^y
	7	2.768±0.347 ^B	0.431±0.048 ^b	0.268±0.019 ^y
	14	4.674±0.165 ^{BC}	0.273±0.020 ^a	0.238±0.027 ^y
	21	3.897±0.070 ^C	0.247±0.002 ^a	0.178±0.012 ^x
	28	5.205±1.420 ^C	0.289±0.077 ^a	0.277±0.011 ^y

Note: The difference between kefir samples with different letters was significant ($p < 0.05$).

A,B,C,D: Expresses the differences in consistency coefficients of the kefir samples, which is characterized by the power law model in the related column, based on the storage days.

a,b,c: Expresses the differences in the flow behavior index of the kefir samples, which is characterized by the power law model in the relevant column, based on the storage days.

x,y,z,t: Expresses the differences in apparent viscosity of the kefir samples, which is characterized by the power law model in the related column, based on the storage days.

Examination of kefir samples characterized by the power law fluid model revealed that there were changes in the K, n and μ_{apparent} values during storage.

The investigation of μ_{apparent} of the samples revealed that buffalo milk (1) kefir was similar for 7, 21, and 28 days and varied between 0.129 and 0.152 Pa.s., while it was 0.208 Pa.s for Day 1. For Day 14, it was determined to be 0.932 Pa.s, which was a higher value than the other days of storage.

Kefir no. 2 was estimated to be 0.936 Pa.s (which indicates a high viscosity) for Day 14, which was similar to sample no. 1. For Days 1, 7, 21, and 28, it was determined to have a viscosity between 0.153 and 0.190 Pa.s. The product had a very close viscosity at the beginning and end of its shelf life. Although its viscosity fluctuated, it did not change much.

The viscosity of kefir of buffalo-sheep milk (3) for Day 1 was 0.172 Pa.s, which was lower than the other days. It was 0.421-0.539 Pa.s and 0.286-0.371 Pa.s for Days 7 and 28 and Days 14 and 21, respectively. The observed viscosity of sample no. 3 at the end of its shelf life was considered to be due

to the fact that it was produced using a mixture of buffalo and sheep milk containing high dry matter and fat content.

Buffalo milk (4) kefir was similar for Days 7, 21, and 28 and ranged from 0.146 to 0.198 Pa.s. while it was 0.244 Pa.s for Day 1. For Day 14, it was determined to be 0.995 Pa.s, (similar to sample nos. 1 and 2), which was higher than that of other storage days.

It was discerned that buffalo-cow-sheep-goat milk (5) kefir remained unchanged throughout the entire shelf life, except for Day 21. It was found to be 0.178 Pa.s for Day 21. It can be said that sample no. 5 had a decreasing viscosity over the shelf life, and the reason for this could be the use of milk containing different composition elements together.

The analysis of all samples revealed that nos. 2 and 3 had similar viscosity to nos. 1, 4, and 5 for all analysis days. The evaluation of all the samples during the storage period revealed that sample nos. 1, 2, and 4 (made from buffalo milk kefir, buffalo-cow milk kefir, and buffalo-goat milk kefir, respectively) had similar rheological characteristics. Similarly, sample nos. 3 and 5 (made from buffalo-sheep milk and buffalo, cow, sheep, and goat milk) showed similar rheological characteristics. The product with the highest consistency was sample no. 3. In addition to this, the consistency of sample no. 5 remained similar throughout the shelf life.

In this study, K value was 4.65 ± 0.07 Pa.s, n value was 0.35 ± 0.21 , and μ_{apparent} was 0.32 ± 0.03 Pa.s for all samples during storage. In a similar study (Ergin et al., 2017) on homogenized kefir, the K value was lower (0.021-0.545 Pa.s) and the n value was 0.56-0.98. The consistency coefficient was determined to be slightly higher in the present study due to the difference in raw materials and the lack of homogenization process, whereas there was a decrease in the flow behavior index toward the end of the shelf life in the study by Ergin et al. (2017). Similarly, Gurbuz & Sheifel (2008) examined the power law model and determined the n value to be in the range of 0.35-0.4. In another study on kumis, the n values at temperatures of 4-10-20°C were found to be 0.74-0.79-0.75, respectively, and the K values were 0.015-0.010-0.008 Pa.s, respectively (Sabancı et al., 2016).

In the study on viscosity measurement in kefir, it was concluded that when the sample was between 15°C and 20°C, it could be characterized by non-Newtonian fluid behavior and was compatible with power law model.

It can be stated that generally, buffalo and goats' milk have larger casein micelles than cows' milk because their protein networks have smaller pores, higher density and higher gel strength (Gomes et al., 2013). Additionally, the surface area of the fat globules is another parameter (Nguyen et al., 2014). Also, Nguyen et al. (2014) reported that a decrease in yoghurt water holding capacities (WHC) depended on the increase in the surface area of the fat globules of cows' milk. However, Menard et al. (2010) reported a low surface area for the fat globules of buffalo milk: 1.78 vs 1.97 m²/g of fat for cows' milk. Lucey et al. (1998) reported that the rheological properties of fermented dairy products can be affected by acidity, total solids and milk and culture types used. Additionally, Hassan (2008) stated that EPS content could improve the WHC of yoghurt by interacting between proteins and micelles. In this study, the type of milk and culture used for kefir production had a significant factor in the samples, as revealed by the rheological analysis results. The high gel stability in kefir from buffalo milk and buffalo milk mixtures could be attributed to its relatively high levels of protein and fat contents. Ramchandran & Shah (2009) found that a decrease in fat content can result in fragile gel stability due to the weaker protein gel in fermented milk. Additionally, Michalski et al. (2002) found a positive relationship between fat globule size and the mechanical properties of fermented milk gels. Therefore, higher gel stability values in kefir from buffalo and buffalo milk mixtures can be also explained by the larger size of fat globules in buffalo milk as compared to cows' milk (Menard et al., 2010). In addition, as 1 casein plays a very important role in gel formation, namely a higher as 1 casein content can cause a strong texture (Michalski et al., 2002). Buffalo milk has a higher as 1 casein content

(1.42 g 100/mL milk) than cows' milk, which has values of around 1.08 g 100/ mL milk (Hussain et al., 2012). In another study reported by Tamime & Robinson (2007), increasing gel structure was explained by fat globules in the protein network improving WHC, as casein fat globule membrane interactions caused an increase in viscosity due to more stable gel formation. As a result, it can be said that higher protein content increases the elastic character of a gel due to the increased number of protein interactions and bonds. The bonds that comprise the protein matrix are strong secondary bonds (the casein aggregates are linked to each other with secondary bonds rather than chemical ones) like hydrogen, electrostatic and hydrophobic bonds (Lucey et al., 1998). These bonds contribute to increased elasticity and as a consequence, to reduced viscous behavior of the samples.

Sensory evaluation

The consistency of favorable kefir should be fluid, and its appearance should be homogeneous and shiny. When consumed, kefir should have a slight yeast taste and be slightly sour. It should provide a refreshing effect (AOAC, 2000). Studies conducted to determine the sensory characteristics of kefir revealed the information that the type of milk used for production and the storage time exert more pronounced effects than the starter culture. In addition, it has been determined that the starter culture affects the flavor and viscosity of the product (Wszolek et al., 2001).

Sensory evaluation was performed with a panel of 8 people for Days 1, 7, and 14 of the production using the discrimination tests (Meilgaard et al., 1999). Sensory evaluation was not done for Days 21, and 28, since kefir, which can be easily oxidized, showed a change in sensory properties on every analysis day owing to its production from high-fat milk.

The form of sensory evaluation made in a specified way is given in Appendix 1. The evaluation for scoring results are provided in Table 11, and the facial expression in Table 12.

Table 11. Result of sensory evaluation scale for scoring

Çizelge 11. Puanlamaya yönelik duyuusal değerlendirme sonuçları

Storage days	100 % Buffalo (1)	Buffalo-Cow (2)	Buffalo-Sheep (3)	Buffalo-Goat (4)	Buffalo-Cow-Sheep-Goat (5)
1	6.21±1.56 ^{Aa}	6.66±2.06 ^{Ad}	5.77±1.39 ^{Bf}	6.55±1.13 ^{Ad}	7.00±1.41 ^{Ag}
7	5.00±1.58 ^{Bb}	5.20±2.16 ^{Bb}	6.60±2.30 ^{Ad}	5.60±1.81 ^{Bf}	5.80±1.92 ^{Bf}
14	3.60±1.14 ^{Cc}	4.20±0.83 ^{Ce}	5.00±1.22 ^{Bb}	5.30±1.09 ^{Bbf}	5.40±1.67 ^{Bbf}

Note: The difference between kefir samples with different letters was significant ($p < 0.05$)

a,b,c,d,e,f,g: Refers to the sensory differences for scoring for the kefir sample in the related column.

A,B,C: Refers to the sensory differences for scoring for the kefir sample in the related line.

Table 12. Result of facial expression sensory evaluation scale

Çizelge 12. Yüz ifadeli duyuusal değerlendirme sonuçları

Storage days	100 % Buffalo (1)	Buffalo-Cow (2)	Buffalo-Sheep (3)	Buffalo-Goat (4)	Buffalo-Cow-Sheep-Goat (5)
1	3.20±1.09 ^{Aa}	3.44±1.42 ^{Aa}	3.44±0.58 ^{Aa}	3.77±0.44 ^{Bc}	4.00±0.71 ^{Bd}
7	2.30±0.97 ^{Cb}	2.70±1.30 ^{Cb}	3.80±1.64 ^{Bc}	3.00±1.41 ^{Aab}	3.30±1.48 ^{Aa}
14	2.50±0.70 ^{Cb}	2.50±0.50 ^{Cb}	3.60±0.54 ^{Bc}	3.20±0.83 ^{Aa}	4.10±0.54 ^{Bd}

Note: The difference between kefir samples with different letters was significant ($p < 0.05$).

a,b,c,d: Expresses the sensory differences in facial expression for the kefir sample in the related column.

A,B,C: Expresses sensory differences with facial expressions for kefir example in the related line.

The results of the sensory evaluation within the scope of the present study indicated that kefir (1) produced using buffalo milk was not particularly pleasant in terms of smell and taste. Kefir (2) produced using buffalo and cow milk was not highly appreciated in terms of smell and taste, but its consistency was quite favorable. Kefir (3) produced using buffalo and sheep milk was considered to be the most delicious product by the panelists who loved high-fat foods. It was determined that kefir (4) produced using buffalo and goat milk was favorable in odor and taste but had some problems in terms of consistency. Kefir (5) produced using a mixture of buffalo, cow, sheep, and goat milk was considered as the most admired product by the panelists in terms of smell, taste, and consistency. In this study, the most popular product was kefir (5) produced using a mixture of buffalo, cow, sheep, and goat milk, followed by kefir (3) produced using buffalo and sheep milk, kefir (4) produced using buffalo and goat milk, kefir (2) produced using buffalo and cow milk, and kefir (1) produced using buffalo milk. Other studies have reported that kefir produced using sheep's milk received the highest score from the consumers in terms of taste, followed by kefir produced from cow and goat milk (Wszolek et al., 2001). In a study using cow's milk and fruit mixtures, odor, taste, texture, and appearance were determined based on a 30-point scale. The results showed that during the 14-day storage period, the samples initially scored close to 30 points and then 14.4 points, along with sensory differentiation toward the end of the shelf life. The sensory characteristics of kefir tend to decline toward the end of its shelf life. It is considered that underdevelopment of aroma and acetic acid bacteria, incubation conditions, amount and type of starter culture, and excessive gas formation caused by the overgrowth of yeasts are the main reasons of flavor defects that may adversely affect the sensory evaluation (Altınayar, 1994).

The rheological and sensorial properties were influenced depending upon milk types like buffalo, cow, goat and sheep and its proportion. The power-law model appears to be suitable for describing the flow behavior of kefir types as indicated by high correlation coefficient (r) values. All of the flow diagrams of kefir processed with different milk species exhibit shear-thinning non-Newtonian behavior.

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Appendix 1

Sensory Evaluation Form

Name Surname:

Date:

Products: Buffalo milk and buffalo milk base kefir

Facial Expression Sensory Evaluation Scale



Sensory Evaluation Scale for Scoring

Liking Scale			
Likely extremely	9	Dislike slightly	4
Like very much	8	Dislike moderately	3
Like moderately	7	Dislike very much	2
Like slightly	6	Dislike extremely	1
Neither like or dislike	5		

Sensory Evaluation Form for Scoring

Product	Consistency	Odor	Taste	General Evaluation
1				
2				
3				
4				
5				