

## An innovative approach: cow/oat milk based kefir

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### Abstract

The aim of this study was to evaluate the potential of kefir production using cow-oat milk mixture. Therefore kefir samples with 20, 40 and 60 % of oat milk were produced. The effect of oat-milk ratio on physicochemical, rheological, microbiological and sensory characteristics of the kefir samples was studied during 21 days storage at refrigerated conditions. Increasing oat milk concentration affected the whey-off and apparent viscosity by higher whey off and lower viscosity results. Also the proteolytic activity of the samples was decreased by raising the oat milk concentrations. Increase of the oat milk concentration led to a decrease of pH of the samples. Statistical analysis showed that the lactococci and lactobacilli viable cell counts differed among the samples while the highest count was detected in sample with the highest amount of oat milk. The control sample (without oat milk) had higher yeast count during the storage period. The final product with 20 % of oat-milk and without addition of flavour was found to be the most acceptable by the sensory panellists. The results indicate the possibility for a new cow/oat milk based kefir.

*Key words:* kefir, oat milk, sensory analysis, rheology, microbiology

### Introduction

Since the late twentieth century, modern life forced the over-use of refined food products as well as the preference to foods of animal origin. Along with high stress factors and awareness of the relationship between diet and health, there is a growing consumers' demand for a new generation of food products - the "functional foods" (Bekers et al., 2001; Cirak, 2009). Designer foods, pharma-foods, medicinal foods, nutraceuticals, therapeutic foods, super foods, foodiceuticals, and medi-foods are all known as functional foods (Soccol et al., 2010). Generally, functional foods contain some health promoting components along with basic nutrients (Karagül-Yüceer and Avşar, 2009). Several components of food are known to be particularly beneficial to human health such as the probiotic bacteria, dietary fibers, polyunsaturated fatty acids, oligosaccharides,

minerals and antioxidants (Bekers et al., 2001; Jelicic et al., 2008).

Many researchers have recognized the beneficial effects of consuming oat and oat based food products. Oat has been reported to be effective in moderating the effects of hypertension, lowering the total serum- and LDL-cholesterol, regulating blood glucose and insulin levels, controlling weight and promoting gastrointestinal health. Many of these effects were attributed to the presence of dietary fibers, principally the  $\beta$ -glucans (Bekers et al., 2001; Lambo et al., 2005).

Kefir is a soured, frothy and mildly alcoholic dairy drink produced by the combination of acid and alcohol fermentation. For many years it was produced and used in Middle Asian and Eastern European countries, Russia and Caucasia for many years. Microbiological and chemical composition of

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kefir provides a complex probiotic effect due to the inherent lactic acid bacteria and yeasts (Kesenkaş et al., 2011a; Pogacic et al., 2013). Lactobacilli are present as the largest portion of this microbial population with lactococci, acetic acid bacteria and yeasts making up the remaining part of the microorganisms contained in kefir (Kesenkaş et al., 2011b). Although the research results on the probiotic properties of kefir microflora are limited in comparison to milks fermented with classical probiotic bacteria (Stepaniak and Fetlinski, 2002), kefir has functional properties including reduction of lactose intolerance symptoms, stimulation of the immune system, hypocholesteremic, antimutagenic and anticarcinogenic properties (Guzel-Seydim et al., 2011).

The use of oat in functional foods is receiving increased attention. Some recent studies have shown that oat is a suitable substrate for various kinds of lactic acid bacteria and functionality of fermented products, mainly yogurts, could be improved by production of oat-based milk or cow-oat milk mixtures (Fernandez-Garcia et al., 1998; Bekers et al., 2001; Martensson et al., 2001). There is no reported research on the properties of cow/oat milk based kefir produced from grains. So the aim of this study was to investigate the potential for production of kefir using cow-oat milk mixtures in various ratios (80:20, 60:40, 40:60). In order to determine the effects of the oat-milk ratio on some physical, chemical, microbiological and sensory properties of the produced kefir samples, the analyses were conducted during 21 day long storage period.

## Materials and methods

### *Production of kefir samples*

The rolled oats (Hahne, Germany) were floured and then used to prepare oat milk with a concentration of 13 g in 100 mL water. Whole fat cow milk and oat milk mixtures in 4 different ratios were used for kefir production (100 % cow milk (K), 80 % cow milk - 20 % oat milk (A), 60 % cow milk - 40 % oat milk (B) and 40 % cow milk - 60 % oat milk (C)). Samples were pasteurized for 10 min at 90 °C, immediately cooled down to 25 °C and inoculated with 3 % (g/mL) kefir grains (Ege University Dept. of Dairy Technology, Turkey). The samples were incubated at 25 °C until pH 4.55-4.60 without stir-

ring about 20 h. The grains were separated from the fermented milk mixtures by filtering them through a sieve. Samples were taken into 250 mL glass bottles and stored at 4±1 °C for 21 days. Physicochemical, microbiological and sensorial tests were performed on the 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days of storage.

### *Physicochemical analysis*

The pH value was determined by a pH meter (Hanna Instruments 211, Germany). The titratable acidity, contents of total solids and fat contents were measured by the Soxhlet-Henkel method, drying at 105 °C until constant mass and Gerber method respectively, according to AOAC (2000), Renner (1993) and Oysun (2001). The protein content was determined by Kjeldahl method using a Gerhardt Turbotherm digestion system and distillation unit Gerhardt Vapodest (Germany). A multiplication factor 6.38 was used to convert the percentage of the nitrogen to the percentage of the protein. The proteolysis in the samples was determined by measuring liberated amino acids and peptides using the O-phthaldialdehyde (OPA) method as described by Donkor et al. (2007). The proteolytic activity of the samples was expressed as the amount of  $\alpha$ -amino groups measured as difference in absorbance values.

### *Rheological analysis*

In order to monitor the stability against sedimentation (whey-off), 100 mL of the samples were added to measuring cylinders (100 mL) and stored at 4 °C. On each analyse date, the serum layer was monitored visually and the whey-off values (%) were calculated according to Paraskevopolou et al. (2003).

The apparent viscosity of the samples was measured with a rotational Brookfield DV-II+Pro viscometer (Middlebro, MA, USA). All viscosity measurements were carried out with the LV2 spindle at a spindle rotation of 10.00 rpm and temperature of the samples was 10 °C. The data were evaluated using Rheocalc V3.2 Build 46-1 (Brookfield Engineering Labs).

### *Sensory characteristics*

The sensory evaluation of the test samples was carried out by 8 experienced panellists from Department of Dairy Technology who were familiar with kefir. The kefir samples were organoleptically

examined for taste, odour, consistency and overall acceptability. The panellists rated the samples on the basis of the nine point's hedonic scale (Bodyfelt et al., 1988). The sensory scores stated; Dislike extremely = 1, Dislike very much = 2, Dislike moderately = 3, Dislike slightly = 4, Neither like nor dislike = 5, Like slightly = 6, Like moderately = 7, Like very much = 8, Like extremely = 9. The samples were coded with three digit random numbers and served to the panellists randomly. Water, plain crackers and bread were served to the panellists for rinsing between samples.

### Microbiological analysis

The samples for microbiological analyses were taken in aseptic conditions on every analysed date. A quantity of 10 g from each sample was emulsified in 90 mL of sterile 2 % (w/v) tri-sodium citrate solution in a laboratory blender (Colworth Stomacher 400, Seward, UK). Subsequently serial dilutions were made with Ringers' solution. The enumeration of microorganisms was carried out using the methods described by Irigoyen et al. (2005). Lactococci count was carried out on M17 medium (pH  $7.2 \pm 0.2$ ) at an incubation temperature of 30 °C under aerobic conditions for 2 days. Lactobacilli count was performed on MRS medium (pH  $6.5 \pm 0.2$ ) at an incubation temperature of 30 °C under anaerobic conditions for 3 days. Cycloheximide (200 mg/L) was added to the two above media to inhibit yeast growth. Yeasts were grown on OGYE medium (pH  $7.0 \pm 0.2$ ) added with 1% oxytetracycline at 25 °C for 5 days.

### Statistical analysis

The effect of oat-milk ratio on some physical, chemical, microbiological and sensory properties of the produced kefir samples was carried out by Analysis of Variance (ANOVA) and the mean differences were analysed using Duncan's Multiple Range Test when a 0.05 % difference in the level of these values was verified. Also the effect of storage on the samples was determined by the same method using SPSS® 15.0 for Windows. All experiments were conducted in triplicate.

## Results and discussion

### Chemical properties and composition

Table 1 presents the results for composition as total solids, fat and protein contents of kefir samples. The data showed that fat and protein contents were affected by the cow/oat milk ratios used in the production. The total solids content of kefir samples with 20, 40 and 60 % of oat milk decreased slightly. The protein content ranged between 1.53 % and 3.48 %. These differences could be due to different ratios of oat milk added into cow milk. Other researches with plain kefir had different results. Irigoyen et al. (2005) produced kefir samples using 1 % and 5 % kefir grains and obtained thereby the fat and total solids contents of 3.20-3.59 % and 11.3-11.6 % respectively. Dinç (2008) demonstrated the mean values of 2.7 % and 13.3 % for fat and total solid contents respectively. Moreover, Muir et al. (1999) reported the protein content of traditional kefir samples between 3.22 and 4.54 %.

Table 1. Chemical composition of kefir samples (n=3,  $\pm$ SD)

Samples	Total solids, %	Fat, %	Protein, %
A	10.99 $\pm$ 0.10 <sup>b</sup>	2.50 $\pm$ 0.00 <sup>c</sup>	2.78 $\pm$ 0.00 <sup>c</sup>
B	10.94 $\pm$ 0.04 <sup>b</sup>	2.05 $\pm$ 0.00 <sup>b</sup>	2.15 $\pm$ 0.02 <sup>b</sup>
C	10.76 $\pm$ 0.01 <sup>a</sup>	1.50 $\pm$ 0.00 <sup>a</sup>	1.53 $\pm$ 0.10 <sup>a</sup>
K	11.02 $\pm$ 0.04 <sup>b</sup>	2.83 $\pm$ 0.10 <sup>d</sup>	3.48 $\pm$ 0.02 <sup>d</sup>

<sup>a, b, c, d</sup>Means in the same column with different superscripts significantly differ (P<0.05). A: 80 % cow milk- 20 % oat milk kefir; B: 60 % cow milk- 40 % oat milk kefir; C: 40 % cow milk- 60 % oat milk kefir; K: Control sample (100 % cow milk kefir), SD: Standard deviation

### Acidity

Table 2 shows pH value and titratable acidity of kefir samples produced from cow milk and cow/oat milk blends. The average pH values at the 1<sup>st</sup> day of storage were in the range between 4.31 and 4.55. The pH of sample A was higher than the pH of the other samples, whereas the sample C had the lowest pH values at all storage periods (P<0.05). It could be concluded that the increase of oat milk concentration in kefir samples resulted in a decrease of the pH value.

Some previous studies suggested no significant differences in pH changes during the storage period (Irigoyen et al. 2005; Tratnik et al., 2006). However, it was reported that pH decreased significantly during the first day or week of storage depending on several factors such as the milk species, starter culture type, incubation time and temperature (Ertekin and Seydim, 2010; Öner et al., 2010; Gronnevik et al., 2011; Purnomo and Muslimin, 2012).

Lactic acid is extremely important for producing high-quality fermented milk products, and appropriate concentrations are needed to ensure proper flavour. According to the Turkish Codex, Standard for Fermented Milks (2001), the minimum titratable

acidity has to be 0.60 %. According to the presented results, already at the first day after the production, the concentration of lactic acid in kefir samples was significantly different. The kefir sample with the highest amount of oat milk had the lowest lactic acid concentration, while the control sample containing cow milk solely had the highest amount. No significant differences were observed during storage ( $P>0.05$ ). However the lactic acid concentrations in all samples, except the sample with the highest oat milk concentration, were found to be acceptable according to the Turkish Codex. One of the main reasons of lower acidity of cow/oat based kefir samples compared to cow milk kefir might be their lower buffering capacity (Martensson et al. 2000).

Table 2. Acidity of kefir samples during storage for 21 days ( $n=3$ ,  $\pm$ SD)

	Samples	Day 1	Day 7	Day 14	Day 21
pH	A	4.55 $\pm$ 0.01 <sup>dZ</sup>	4.27 $\pm$ 0.00 <sup>dX</sup>	4.27 $\pm$ 0.00 <sup>cX</sup>	4.35 $\pm$ 0.02 <sup>cY</sup>
	B	4.42 $\pm$ 0.01 <sup>bZ</sup>	4.18 $\pm$ 0.00 <sup>bX</sup>	4.19 $\pm$ 0.02 <sup>bX</sup>	4.30 $\pm$ 0.00 <sup>bY</sup>
	C	4.31 $\pm$ 0.00 <sup>aW</sup>	4.09 $\pm$ 0.00 <sup>aX</sup>	4.11 $\pm$ 0.01 <sup>aY</sup>	4.20 $\pm$ 0.00 <sup>aZ</sup>
	K	4.48 $\pm$ 0.00 <sup>cZ</sup>	4.21 $\pm$ 0.00 <sup>cX</sup>	4.20 $\pm$ 0.01 <sup>bX</sup>	4.28 $\pm$ 0.00 <sup>bY</sup>
Titratable acidity, %	A	0.70 $\pm$ 0.00 <sup>cXY</sup>	0.68 $\pm$ 0.01 <sup>cX</sup>	0.72 $\pm$ 0.01 <sup>cY</sup>	0.73 $\pm$ 0.00 <sup>cY</sup>
	B	0.60 $\pm$ 0.02 <sup>bX</sup>	0.59 $\pm$ 0.01 <sup>bX</sup>	0.62 $\pm$ 0.01 <sup>bX</sup>	0.62 $\pm$ 0.00 <sup>bX</sup>
	C	0.47 $\pm$ 0.00 <sup>aX</sup>	0.47 $\pm$ 0.00 <sup>aX</sup>	0.48 $\pm$ 0.02 <sup>aX</sup>	0.48 $\pm$ 0.00 <sup>aX</sup>
	K	0.88 $\pm$ 0.03 <sup>dX</sup>	0.85 $\pm$ 0.01 <sup>dX</sup>	0.84 $\pm$ 0.00 <sup>dX</sup>	0.87 $\pm$ 0.01 <sup>dX</sup>

<sup>a, b, c, d</sup>Means in the same column with different superscripts significantly differ ( $P<0.05$ )

<sup>X, Y, Z, W</sup>Means in the same row with different superscripts among kefir samples significantly differ ( $P<0.05$ )

SD: Standard deviation

A: 80 % cow milk- 20 % oat milk kefir; B: 60 % cow milk- 40 % oat milk kefir; C: 40 % cow milk- 60 % oat milk kefir; K: Control sample (100 % cow milk kefir)

Table 3. Whey-off values of kefir samples during storage for 21 days ( $n=3$ ,  $\pm$ SD)

	Samples	Day 1	Day 7	Day 14	Day 21
Whey off mL/100mL	A	23.00 $\pm$ 2.83 <sup>aX</sup>	30.25 $\pm$ 1.77 <sup>aXY</sup>	33.75 $\pm$ 1.77 <sup>aY</sup>	33.75 $\pm$ 3.89 <sup>aY</sup>
	B	36.50 $\pm$ 0.71 <sup>cX</sup>	42.00 $\pm$ 1.41 <sup>bY</sup>	43.75 $\pm$ 1.41 <sup>bY</sup>	43.50 $\pm$ 1.41 <sup>bY</sup>
	C	22.00 $\pm$ 0.71 <sup>aX</sup>	44.00 $\pm$ 2.21 <sup>bY</sup>	44.25 $\pm$ 2.12 <sup>bY</sup>	44.00 $\pm$ 1.41 <sup>bY</sup>
	K	28.75 $\pm$ 1.77 <sup>bX</sup>	35.50 $\pm$ 3.54 <sup>aX</sup>	33.25 $\pm$ 2.48 <sup>aX</sup>	32.50 $\pm$ 0.00 <sup>aX</sup>

<sup>a, b, c, d</sup>Means in the same column with different superscripts significantly differ ( $P<0.05$ )

<sup>X, Y, Z</sup>Means in the same row with different superscripts among kefir samples significantly differ ( $P<0.05$ )

SD: Standard deviation of means

A: 80 % cow milk- 20 % oat milk kefir; B: 60 % cow milk- 40 % oat milk kefir; C: 40 % cow milk- 60 % oat milk kefir; K: Control sample (100 % cow milk kefir)

### Whey-off

Whey-off results of kefir samples were given in Table 3. The obtained results ranged from 22 to 44.25 mL/100 mL during storage. Differences among the samples at the same storage period were significant ( $P < 0.05$ ). However, Duncan grouping showed that initially samples A and C had close values whereas sample A and K and, sample B and C had statistically similar results to each other at the rest of the storage period. Generally, values sharply increased in the first 7 days of storage for all samples and the storage period significantly affected whey off values except for the control sample ( $P < 0.05$ ). Surprisingly, sample C which contained the highest oat milk (60 %) concentration, had the lowest whey-off values at the first day of storage. such data could be attributed to the  $\beta$ -glucan found in oat milk which might have caused a decrease in whey-off. However, the observed increase during the rest of a storage period could have occurred as a result of the thermodynamic incompatibility between milk proteins and polysaccharides present in oat milk (Mejri and Sahli, 2014). Thus considering the whole storage period it appeared that addition of oat milk in concentrations above 20 % negatively affected the whey-off values of the kefir samples.

### Apparent viscosity

Table 4 presents the apparent viscosity values throughout the storage period. Flow properties of kefir samples made from cow's milk blended with different ratios of oat milk were similar. The shear-thinning behaviour was expected in fermented milks because the textures of these products are affected by weak physical bonds, electrostatic and hydrophobic interactions (Ertekin and Seydim,

2010). Table 4 contains results related to the apparent viscosity changes (mPa s) recorded at a shear rate of  $300 \text{ s}^{-1}$  during the storage period. The highest viscosity was observed in sample K besides, while the lowest viscosity was determined in sample B.

Apparent viscosity changes of sample B exposed no significant differences during storage ( $P > 0.05$ ). However significant differences ( $P < 0.05$ ) were determined during storage of samples A, C and K. Viscosity of samples A and C decreased until the 14<sup>th</sup> day of storage, whereas viscosity of samples K and C decreased until the 7<sup>th</sup> day of storage. All samples reached their highest viscosity values at the beginning of storage. Obviously, the apparent viscosity values of the samples were affected by the oat milk concentrations. Increase of oat milk content in cow/oat milk kefir negatively influenced its viscosity.

Garrote et al. (2001) reported that the apparent viscosity of different kefir samples (measured at shear rate  $629 \text{ s}^{-1}$ ) prepared with different kefir grains ranged between 7.5 and 15.4 mPa.s. Ertekin and Seydim (2010) stated that apparent viscosity in kefir containing different type of fat replacers varied from 1.83 to 3.12 Pa.s at 1<sup>st</sup> day of storage or 1.83 to 3.75 Pa.s at 7<sup>th</sup> day of storage. Also, it was reported that viscosity of cow milk kefir decreased to approximately  $158 \text{ s}^{-1}$  during the 1<sup>st</sup>, 5<sup>th</sup> and 10<sup>th</sup> day of storage (Tratnik et al., 2006).

### Proteolysis

The OPA based spectrophotometric assay detects released  $\alpha$  amino groups, which originate from the proteolysis of milk proteins, thus giving a direct measurement of proteolytic activity. Table 5 presents the proteolytic activity of the produced samples and

Table 4. Viscosity of kefir samples during storage for 21 days ( $n=3$ ,  $\pm$ SD)

	Samples	Day 1	Day 7	Day 14	Day 21
Viscosity mPa.s	A	141.25 $\pm$ 0.26 <sup>bW</sup>	110.58 $\pm$ 1.13 <sup>bY</sup>	103.63 $\pm$ 1.20 <sup>cX</sup>	123.53 $\pm$ 4.60 <sup>bZ</sup>
	B	28.29 $\pm$ 2.55 <sup>aX</sup>	20.95 $\pm$ 0.07 <sup>aX</sup>	24.30 $\pm$ 0.42 <sup>aX</sup>	24.40 $\pm$ 2.40 <sup>aX</sup>
	C	50.39 $\pm$ 4.38 <sup>aY</sup>	38.79 $\pm$ 2.40 <sup>aX</sup>	38.54 $\pm$ 13.4 <sup>bX</sup>	39.24 $\pm$ 1.20 <sup>aX</sup>
	K	501.29 $\pm$ 15.55 <sup>cY</sup>	402.42 $\pm$ 34.92 <sup>cX</sup>	416.31 $\pm$ 17.96 <sup>dX</sup>	438.36 $\pm$ 22.41 <sup>cXY</sup>

<sup>a, b, c, d</sup>Means in the same column with different superscripts significantly differ ( $P < 0.05$ )

<sup>x, y, z</sup>Means in the same row with different superscripts among kefir samples significantly differ ( $P < 0.05$ )

SD: Standard deviation of means

A: 80 % cow milk- 20 % oat milk kefir; B: 60 % cow milk- 40 % oat milk kefir; C: 40 % cow milk- 60 % oat milk kefir; K: Control sample (100 % cow milk kefir)



Table 5. Proteolytic activity in kefir samples during storage for 21 days (n=3, ± SD)

	Samples	Day 1	Day 7	Day 14	Day 21
Proteolysis (absorbance)	A	0.346±0.000 <sup>cX</sup>	0.506±0.007 <sup>cY</sup>	0.522±0.012 <sup>cY</sup>	0.555±0.000 <sup>cZ</sup>
	B	0.296±0.001 <sup>bX</sup>	0.421±0.010 <sup>bY</sup>	0.429±0.001 <sup>bY</sup>	0.452±0.007 <sup>bZ</sup>
	C	0.249±0.003 <sup>aX</sup>	0.335±0.008 <sup>aY</sup>	0.342±0.002 <sup>aY</sup>	0.367±0.004 <sup>aZ</sup>
	K	0.427±0.021 <sup>dX</sup>	0.547±0.005 <sup>dY</sup>	0.571±0.020 <sup>dY</sup>	0.644±0.008 <sup>dZ</sup>

<sup>a, b, c, d</sup>Means in the same column with different superscripts significantly differ (P<0.05)

<sup>X, Y, Z</sup>Means in the same row with different superscripts among kefir samples significantly differ (P<0.05)

SD: Standard deviation of means

A: 80 % cow milk - 20 % oat milk kefir; B: 60 % cow milk - 40 % oat milk kefir; C: 40 % cow milk - 60 % oat milk kefir; K: Control sample (100 % cow milk kefir)

is expressed as the amount of free amino groups measured as absorbance values at 340 nm.

The statistical analysis showed that the storage period significantly affected the amount of liberated amino acids in all kefir samples (K, A, B, C) (P<0.05) with the maximum absorbance values of 0.644, 0.555, 0.452 and 0.367 respectively measured at 21<sup>st</sup> day. The proteolysis in the control sample was significantly (P<0.05) higher than that of kefirs containing oat milks which could be clearly attributed to higher protein content as presented in Table 1. However, clearly appreciable proteolytic activity was observed in all kefir samples which explains the high cell density of lactic acid bacteria being dependent on a proteolytic system that can liberate essential amino acids from milk derived peptides (Donkor et al., 2007; Kesenkaş, 2011b).

### Sensory characteristics

The sensory results of kefir samples are presented in Table 6. Taste was evaluated as the most important factor in the sensory analysis. The effect of storage period on taste scores was insignificant (P>0.05). Oat milk left a cereal taste in mouth. Increase of oat milk concentration over 20 % had an adverse impact on taste scores (P<0.05). These scores slightly decreased at the 14<sup>th</sup> day of storage and increased again at the end of storage. During this period the most noted defect was a yeasty, cereal taste and flavour. However, the scores were very close to each other at the end of storage (P>0.05).

Odour profiles of the samples scored from 4.96 to 8.09 at the first day (table 6). The control sample had highest odour scores at all storage periods. Intense souring, smell of oat milk and inadequate aroma affected the odour characteristics of sample C. As presented in table 6, there were significant

differences among samples except on the 21<sup>st</sup> day of storage (P<0.05). Moreover, the statistical analysis showed that storage period significantly affected the odour scores only in kefir containing 40 % oat milk (P<0.05).

According to the obtained results, the essential factor affecting the consistency was oat milk used in kefir production. Consistency scores changed from 4.92 to 7.88. The differences between samples were significant at all storage periods with control sample having the highest scores again (P<0.05). The consistency scores of samples B and C increased throughout the storage, where sample A showed a slight decrease on the 14<sup>th</sup> day of storage. Also, the consistency scores of control sample decreased during storage. Nevertheless, the storage period effected only the consistency scores of sample B (P<0.05).

It appears that oat milk ratio in kefir samples significantly affected the overall acceptability (P<0.05). Similarly to other sensory characteristics, control sample had the highest overall acceptability scores while sample C had the lowest score. The results showed that taste and consistency were the most important parameters for the overall acceptability.

Kılıç et al. (1999) reported that sensory scores for all kefir characteristics decreased significantly with time. In contrast to result of the present study, Irigoyen et al. (2005) stated that kefir samples revealed the best acceptability level in the first days of storage. The sensory character of fermented milk products like kefir was mainly influenced by the type of milk and starter culture used and storage period (Wszloek et al., 2001).

Table 6. Sensory properties of kefir samples during the storage for 21 days (n=3,  $\pm$  SD)

	Samples	Day 1	Day 7	Day 14	Day 21
Taste	A	5.83 $\pm$ 0.00 <sup>cX</sup>	6.79 $\pm$ 0.30 <sup>cX</sup>	6.01 $\pm$ 0.18 <sup>cX</sup>	6.54 $\pm$ 0.41 <sup>aX</sup>
	B	5.00 $\pm$ 0.00 <sup>bX</sup>	5.65 $\pm$ 0.50 <sup>bX</sup>	5.01 $\pm$ 0.18 <sup>bX</sup>	5.46 $\pm$ 0.18 <sup>aX</sup>
	C	4.55 $\pm$ 0.18 <sup>aX</sup>	4.43 $\pm$ 0.40 <sup>aX</sup>	3.94 $\pm$ 0.08 <sup>aX</sup>	4.67 $\pm$ 0.12 <sup>aX</sup>
	K	8.09 $\pm$ 0.12 <sup>dX</sup>	7.79 $\pm$ 0.11 <sup>dX</sup>	6.82 $\pm$ 0.26 <sup>dX</sup>	7.21 $\pm$ 0.88 <sup>aX</sup>
Odour	A	6.92 $\pm$ 0.59 <sup>bX</sup>	7.22 $\pm$ 0.30 <sup>cX</sup>	6.94 $\pm$ 0.08 <sup>cX</sup>	6.88 $\pm$ 0.18 <sup>aX</sup>
	B	5.67 $\pm$ 0.00 <sup>aX</sup>	6.43 $\pm$ 0.20 <sup>bY</sup>	5.75 $\pm$ 0.00 <sup>bX</sup>	6.30 $\pm$ 0.18 <sup>Y</sup>
	C	4.96 $\pm$ 0.30 <sup>aX</sup>	5.57 $\pm$ 0.20 <sup>aX</sup>	4.82 $\pm$ 0.09 <sup>aX</sup>	6.04 $\pm$ 0.76 <sup>aX</sup>
	K	8.09 $\pm$ 0.12 <sup>cX</sup>	7.86 $\pm$ 0.21 <sup>cX</sup>	7.19 $\pm$ 0.44 <sup>cX</sup>	7.25 $\pm$ 0.35 <sup>aX</sup>
Consistency	A	<b>6.38<math>\pm</math>0.42<sup>bX</sup></b>	7.11 $\pm$ 0.05 <sup>cX</sup>	6.94 $\pm$ 0.27 <sup>cX</sup>	7.13 $\pm$ 0.53 <sup>bcX</sup>
	B	5.42 $\pm$ 0.12 <sup>aX</sup>	<b>5.93<math>\pm</math>0.10<sup>bY</sup></b>	<b>6.07<math>\pm</math>0.09<sup>bY</sup></b>	<b>6.17<math>\pm</math>0.23<sup>abY</sup></b>
	C	<b>4.92<math>\pm</math>0.12<sup>aX</sup></b>	5.00 $\pm$ 0.20 <sup>aX</sup>	5.44 $\pm$ 0.27 <sup>aX</sup>	5.46 $\pm$ 0.18 <sup>aX</sup>
	K	<b>7.88<math>\pm</math>0.06<sup>cX</sup></b>	7.65 $\pm$ 0.30 <sup>dX</sup>	7.50 $\pm$ 0.00 <sup>dX</sup>	7.42 $\pm$ 0.35 <sup>cX</sup>
Overall Acceptability	A	6.13 $\pm$ 0.42 <sup>bX</sup>	6.93 $\pm$ 0.30 <sup>cX</sup>	6.41 $\pm$ 0.13 <sup>cX</sup>	6.92 $\pm$ 0.35 <sup>bcX</sup>
	B	5.13 $\pm$ 0.18 <sup>aX</sup>	5.86 $\pm$ 0.40 <sup>bX</sup>	5.44 $\pm$ 0.08 <sup>bX</sup>	6.05 $\pm$ 0.18 <sup>abX</sup>
	C	4.88 $\pm$ 0.18 <sup>aX</sup>	4.72 $\pm$ 0.40 <sup>aX</sup>	4.32 $\pm$ 0.09 <sup>aX</sup>	5.05 $\pm$ 0.18 <sup>aX</sup>
	K	8.00 $\pm$ 0.24 <sup>cX</sup>	7.79 $\pm$ 0.11 <sup>cX</sup>	7.32 $\pm$ 0.26 <sup>dX</sup>	7.25 $\pm$ 0.59 <sup>cX</sup>

<sup>a, b, c, d</sup>Means in the same column with different superscripts significantly differ ( $P < 0.05$ )

<sup>X, Y</sup>Means in the same row with different superscripts among kefir samples significantly differ ( $P < 0.05$ )

SD: Standard deviation of means

A: 80 % cow milk - 20 % oat milk kefir; B: 60 % cow milk - 40 % oat milk kefir; C: 40 % cow milk - 60 % oat milk kefir; K: Control sample (100 % cow milk kefir)

### Microbiological characteristics

The viable cell counts of lactococci, lactobacilli and yeasts determined during storage period are presented in Table 7. The viable lactococci count for sample A and for control sample remained relatively constant until the end of storage, whereas the other two samples (B, C) had variable results ( $P < 0.05$ ). Although there were significant differences between samples for viable counts of lactococci during the first week of storage ( $P < 0.05$ ), these differences weren't significant and never declined less than 6.0 log cfu/mL afterwards.

As it can be seen from Table 7, storage period effected only the viable lactobacilli counts of sample A and C ( $P < 0.05$ ) while the control sample had the lowest count (6.03 log cfu/mL) throughout the storage. The differences between samples were found significant ( $P < 0.05$ ) at all periods except at the 21<sup>st</sup> day of storage. Essentially, kefir containing 60 % oat milk (C) attracted attention among the samples by having the highest count of both, lactococci and lac-

tobacilli, at all periods. Such data may be related to the ability of these bacteria to utilize maltose, which is the main fermentable carbohydrate present in the oat milk.

Yeasts play an important role during kefir fermentation because of end-products like ethanol and carbon dioxide, which give the kefir drink its unique taste (Kesenkaş et al., 2011b). The viable counts of yeasts remained in the range of 4.73-5.36 log cfu/mL in all samples until the end of a storage period (Table 7). Although insignificant differences were noted between samples at the first day of storage, significant differences were found during the rest ( $P < 0.05$ ). Thereat the control sample showed higher yeast counts (5.36 log cfu/mL) except at the 21<sup>st</sup> day. Thus, storage period apparently affected yeast counts only in samples B and C ( $P < 0.05$ ). Such data might indicate that the increased lactose amount positively affected the viable cell count of yeasts in kefir samples.

Table 7. Changes in the viable counts of *Lactococcus* spp., *Lactobacillus* spp. and yeasts in kefir samples during storage for 21 days (log cfu/mL, n=3,  $\pm$ SD)

	Samples	Day 1	Day 7	Day 14	Day 21
<i>Lactococcus</i> spp.	A	6.22 $\pm$ 0.04 <sup>c</sup>	6.31 $\pm$ 0.02 <sup>bc</sup>	6.05 $\pm$ 0.16	6.06 $\pm$ 0.21
	B	5.91 $\pm$ 0.04 <sup>aX</sup>	6.21 $\pm$ 0.08 <sup>abY</sup>	6.16 $\pm$ 0.08 <sup>Y</sup>	6.28 $\pm$ 0.06 <sup>Y</sup>
	C	6.62 $\pm$ 0.20 <sup>dZ</sup>	6.47 $\pm$ 0.00 <sup>cYZ</sup>	6.26 $\pm$ 0.02 <sup>X</sup>	6.35 $\pm$ 0.07 <sup>XY</sup>
	K	6.05 $\pm$ 0.01 <sup>b</sup>	6.03 $\pm$ 0.02 <sup>a</sup>	6.02 $\pm$ 0.03	6.04 $\pm$ 0.11
<i>Lactobacillus</i> spp.	A	6.23 $\pm$ 0.00 <sup>abXY</sup>	6.38 $\pm$ 0.12 <sup>bY</sup>	6.15 $\pm$ 0.02 <sup>aX</sup>	6.10 $\pm$ 0.03 <sup>X</sup>
	B	6.33 $\pm$ 0.06 <sup>b</sup>	6.10 $\pm$ 0.08 <sup>a</sup>	6.24 $\pm$ 0.17 <sup>a</sup>	6.17 $\pm$ 0.18
	C	6.74 $\pm$ 0.02 <sup>cW</sup>	6.43 $\pm$ 0.02 <sup>bY</sup>	6.53 $\pm$ 0.01 <sup>bZ</sup>	6.36 $\pm$ 0.01 <sup>X</sup>
	K	6.10 $\pm$ 0.13 <sup>a</sup>	6.03 $\pm$ 0.02 <sup>a</sup>	6.16 $\pm$ 0.01 <sup>a</sup>	6.19 $\pm$ 0.00
Yeasts	A	5.29 $\pm$ 0.10	5.31 $\pm$ 0.06 <sup>bc</sup>	5.18 $\pm$ 0.01 <sup>bc</sup>	5.20 $\pm$ 0.01 <sup>b</sup>
	B	5.35 $\pm$ 0.01 <sup>Y</sup>	5.18 $\pm$ 0.03 <sup>bY</sup>	5.07 $\pm$ 0.16 <sup>bXY</sup>	4.81 $\pm$ 0.15 <sup>aX</sup>
	C	5.16 $\pm$ 0.16 <sup>Y</sup>	4.92 $\pm$ 0.09 <sup>aXY</sup>	4.82 $\pm$ 0.01 <sup>aX</sup>	4.73 $\pm$ 0.01 <sup>aX</sup>
	K	5.25 $\pm$ 0.16	5.36 $\pm$ 0.05 <sup>c</sup>	5.36 $\pm$ 0.06 <sup>c</sup>	5.20 $\pm$ 0.04 <sup>b</sup>

<sup>a, b, c, d</sup>Means in the same column with different superscripts significantly differ (P<0.05)

<sup>X, Y, Z, W</sup>Means in the same row with different superscripts among kefir samples significantly differ (P<0.05)

SD: Standard deviation of means

A: 80 % cow milk - 20 % oat milk kefir; B: 60 % cow milk - 40 % oat milk kefir; C: 40 % cow milk - 60 % oat milk kefir; K: Control sample (100 % cow milk kefir)

## Conclusion

The importance of vegetal based fermented products as probiotic carriers increases day by day. At this point, oat based kefir products developed in this study was a product with an odour, taste and consistency that were found to be acceptable at maximum addition of 20 % oat milk. Thus it can be concluded that there was no need to mask eventually occurring off flavours below that ratio. Fermentation with kefir grains led to enough acid development and viable counts similar to the control sample. This study demonstrates a possibility to produce an acceptable and value added kefir based on oat milk that can be alternative to similar market products.

*Inovativan pristup: kefir na bazi mješavine kravljeg i zobenog mlijeka*

## Sažetak

Cilj ovog istraživanja bio je utvrditi mogućnost proizvodnje kefira iz mješavine kravljeg i zobenog mlijeka. Stoga su proizvedeni uzorci kefira s 20, 40 i

60 % zobenog mlijeka. Tijekom 21 dana skladištenja na hladnom ispitivan je utjecaj različitih omjera zobenog i kravljeg mlijeka na fizikalna, reološka, mikrobiološka i senzorska svojstva proizvedenih uzoraka kefira. Povećanje udjela zobenog mlijeka uzrokovalo je povećanje sinereze, smanjenje prividne viskoznosti te smanjenje proteolitičke aktivnosti u uzorcima kefira. Osim toga, povećanje udjela zobenog mlijeka rezultiralo je snižavanjem pH vrijednosti u uzorcima kefira. Statistička analiza pokazala je da se proizvedeni uzorci razlikuju u broju živih stanica laktokoka i laktobacila, s tim da je najveći broj utvrđen u uzorku s najvećim udjelom zobenog mlijeka. Kontrolni uzorak (bez zobenog mlijeka) sadržavao je povećan broj plijesni tijekom cijelog razdoblja čuvanja. Krajnji proizvod s udjelom od 20 % zobenog mlijeka i bez dodatka aroma pokazao se najprihvatljivijim prilikom senzorske ocjene od strane panela. Dobiveni rezultati mogu poslužiti u daljnjem razvoju proizvodnje kefira na bazi mješavine kravljeg i zobenog mlijeka.

*Ključne riječi:* kefir, zobeno mlijeko, senzorska analiza, reologija, mikrobiologija



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