

# The Effects of Temperature and Milk Fat Content on the Electrical Conductivity of Kefir during the Incubation

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**Abstract** In this study, the relationships between the electrical conductivity and temperature for different milk fat contents were investigated during the incubation of kefir. For kefir production, three milk samples at different fat contents (0.15, 1.7 and 3.0%) and two incubation temperatures (25 °C and 35 °C) were selected. The inoculum level was 2%. The kefir fermentation time was approximately 10 h and the pH of kefir samples were 4.4 in the last stage. The test results showed that electrical conductivity (EC) increased at both the temperatures. The average EC values of non fat milk kefir were in the range of 4.72 mS/cm – 6.4 mS/cm at 35 °C, and 4.72 mS/cm – 5.71 mS/cm at 25 °C. The average EC values of full fat milk kefir were in the range of 4.84 mS/cm – 6.41 mS/cm at 35 °C, and 4.84 mS/cm – 5.63 at 25 °C. The average EC values of low fat milk kefir were in the range of 4.54 mS/cm – 6.39 mS/cm at 35 °C, and 4.54mS/cm – 5.5 mS/cm at 25 °C. Electrical conductivity of samples showed little improvement at low incubation temperature and fat content. Statistically, there was a significant difference ( $p < 0.05$ ) in EC among samples.

**Keywords:** *electrical conductivity, kefir, fermentation*

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## 1. Introduction

Kefir is a fermented carbonated dairy product which contains approximately 1% lactic acid and 0.3–1% ethanol, and like yogurt, it is believed to originate from the Caucasus Mountains. Kefir is produced by the action of bacteria and yeasts contained in kefir grains [33]. Rheological properties of kefir have importance, since they affect the quality of the final product as well as its acceptance by the consumers. The major factors affecting rheological properties of kefir are the chemical composition of the milk used for production, the starter cultures, the incubation temperature, and the thermal processing of milk, etc [15]. The exotic aroma and flavor of kefir, a refreshing feature, and slightly acidic taste are the result of the coexistence of yeast and LAB in a symbiotic association, and depend on the diversity of the microbiota of each kefir grain [11,19,25,22].

Electrical conductivity measurements have been used in the food industry for many years to detect contaminants in water, to monitor microbial growth, metabolic activity and interference from inhibiting substances. [8,10,23,21] Electrical conductivity (EC) used to detect changes in milk composition associated with mastitis, is another proven measure which is now used with increasing frequency in the dairy industry. As is well-known, mastitis is associated with increased conductivity of udder tissue, as well as changes in milk's ionic composition. It is

likewise associated with decreased levels of certain mineral substances and increased levels of  $\text{Na}^+$  and  $\text{Cl}^-$ , all of which increase electrical conductivity [14,24]. For instance, detection of mastitis for quality control of milk, to analyze fermentation processes for production of cheese starters [27], to monitor the start-up and preheating phases of milk pasteurization processes [13].

Electrical conductivity is used as a simple method to monitor demineralization of food systems during conventional electro dialysis treatments. However, during bipolar membrane electroacidification, the  $\text{H}^+$  generated at the bipolar membrane, have a higher conductivity contribution in comparison with all other cations [31].

A simple device based on these properties could represent a useful and economic tool for the rapid quality evaluation of all fermented milk products during their preparation and be used subsequently by the food industry. Conductivity is considered a valid method for evaluating the growth and acidity of lactic acid bacteria in milk [5,16,27,30]. Since traditional methods for evaluating milk quality are lengthy, labor-intensive, and expensive [12]), several analytical and electrical methodologies have been developed to detect milk adulteration. Recently, electrical conductivity of milk has been studied as a means to detect freshness and adulteration of milk.

pH is a useful value for characterizing starter activity, but pH probes are often unstable, and their calibration and maintenance are difficult and pH measurements in high protein media such as milk are problematic. For this reason, electrical conductivity probes which are more

robust and inexpensive, can also be used to monitor acidification during lactic fermentation.

The aim of this research is to develop a model for process control during kefir fermentation depending on the relationship between electrical conductivity, incubation temperature and milk fat content.

## 2. Materials and Methods

### 2.1. Milk and Kefir Grains

In this research, pasteurized cow milk was obtained from commercial markets. Kefir grains were obtained from Ankara University Faculty of Agriculture, Dairy Technology Department in Turkey. These kefir grains were activated by transferring them into pasteurized full fat cow milk, without stirring, allowing them to grow for approximately at 25 °C for 24 h, filtering to remove the clotted milk, and rinsing with sterile water. The activation step was repeated 3 times.

### 2.2. Preparation of Kefir Samples

Six kefir samples were prepared from pasteurized milk with three different fat contents including full fat milk (3%), low-fat milk (1.7%) and non fat milk (0.15%) by adding 2% (w/v) kefir grains then incubated at 25 and 35°C, incubation temperatures. Incubation proceeded until pH value of 4.4 was reached at 25 and 35°C.

### 2.3. Fermentation Stage

At this stage, the kefir fermentation period was about 10 h. The fermentation of kefir was done by adding an inoculum consisting of 2% (w/v) kefir grains. The pH and electrical conductivity measurements of kefir samples were made at 60 minutes intervals until the desired pH was reached.

### 2.4. Composition Analysis

Cow milk samples were analyzed for fat and protein content according to AOAC standard method [3,4]. The lactose concentration in milk samples was determined according to the Lane–Eynon method based on the reduction of copper [6]. The calcium were determined using an atomic absorption spectrometric method [7]. The calorie values were calculated according to the energy conversion factors in the Turkish Food Codex [1]. The measurement of pH all Kefir samples were conducted at room temperature, using a microprocessor pH meter equipped with a glass electrode and a temperature probe (Hanna Instruments model 221, Ann Arbor, MI, USA). Certified buffers (pH 7.00 and pH 4.00) were used to calibrate the electrode.

### 2.5. Electrical Conductivity Measurement

Electrical conductivity values of fermented milk samples were determined by using a portable electrical conductivity meter (Martini Instruments Code MI806, Serial Number 767252 Romania) which has 0.01–20.00 mS cm<sup>-1</sup> measurement range and 0.1 mS/cm sensitivity. EC measurements were done at 60 min. interval during the kefir fermentation.

## 2.5. Statistical Analysis

This research was planned according to 2×3 factorial experimental designs. Determination of the correlative coefficients between the determined parameters and electrical conductivity were calculated with Microsoft Office Excel program. The SPSS ver.18.0 software version used the statistical analysis. Statistical significance for differences was tested at 5% probability level ( $p < 0.05$ ). The statistical indicator ( $R^2$ ) for regression goodness was obtained directly from the software.

## 3. Results and Discussion

The chemical composition of pasteurized cow milk used for production of kefir are presented in (Table 1).

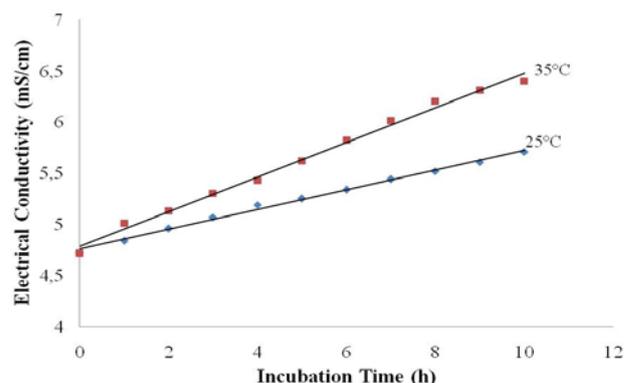
**Table 1. The average composition of milk used in the production of kefir (n=3)**

Component	Full fat milk (FF)	Low-fat milk (LW)	Non fat milk (NF)
Fat (%)	3.00	1.7	0.15
Protein (%)	3.1	3.2	3.1
Lactose (%)	4.5	4.7	4.5
Calcium(mg/100ml)	110	115	120
Energy (kcal/kJ)	57.4/240	46.9/196	31.7/132.5

The fat, protein, calcium and lactose contents of cow milk samples are given in Table 1. The fat contents of different fat milk samples ranged between 0.15 and 3.0%. The protein contents of different fat contents cow milk samples were detected between 3.1 and 3.2%. The Lactose contents of different fat milk samples ranged between 4.5 and 4.7 % (Table 1). The fat, protein, calcium and lactose content in milk samples were found appropriate of to Turkish Food Codex [2].

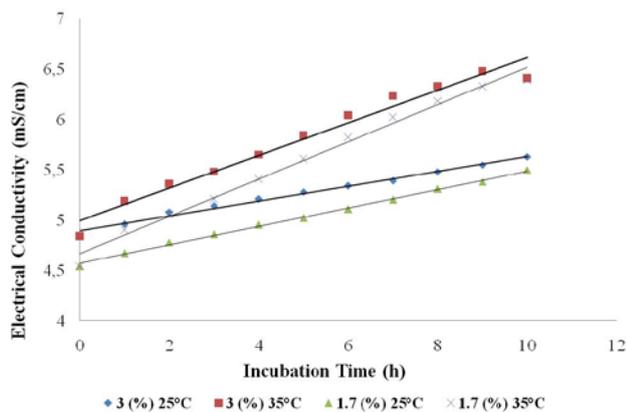
### 3.1. The Effects of Process Variables on EC of Kefir during Fermentation

The EC increased during the lactic acid fermentation in kefir samples. According to experiment results, the kefir samples which were fermented at 35 °C had a faster development of EC compared with those at 25 °C. As shown in Figure 1 the electrical conductivity increased in kefir samples with increasing incubation temperature. The average EC values of non fat milk kefir were in the ranges of 4.72 mS/cm – 6.4 mS/cm at 35 °C, and 4.72 mS/cm – 5.71 mS/cm at 25 °C.



**Figure 1.** The change of the EC as a function of time and temperature (25–35°C) for Kefir samples made with non fat milk during fermentation

The average EC values of full fat milk kefir were in the ranges of 4.84 mS/cm – 6.41 mS/cm at 35 °C, and 4.84 mS/cm – 5.63 at 25 °C. The average EC values of low fat milk kefir were in the ranges of 4.54 mS/cm – 6.39 mS/cm at 35 °C, and 4.54mS/cm – 5.5 mS/cm at 25 °C (Figure 2). The statistical analysis of the data showed that the incubation temperatures significantly influenced the electrical conductivity of kefir samples ( $P < 0.05$ ). Positive correlation was established between EC and incubation temperature in kefir samples  $r=0.483$  ( $P < 0.01$ ). Electrical conductivity increased linearly with incubation temperature. These results concur with the reports of Dejmek, Palaniappan and Sasty, Yongsawatdigul et al., Hennigsson et al. and Ruhlman et al [9,26,34,13,29]. Lactic acid fermentation is decreases the pH of the medium, and concomitantly increases its electrical conductance (EC), as a result of the accumulation of lactate ions during fermentation [17]. During milk fermentation lactic acid is excreted into the medium, which will decrease the pH of the medium and concomitantly increase the electrical conductance[21].



**Figure 2.** The variation of EC as a Function of time and temperature (25–35°C) for Kefir samples made with full fat and low fat milk during fermentation

Presence of fat has an influence on the electrical conductance of milk [18,28].

Ionic conduction due to the presence of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  is responsible for most of the electrical conductance of milk. However, the variation in of the fat globule size and the structure of the casein, which control the solubilisation of the colloidal salts, also contribute to the overall conductivity. The conductance of milk and fresh cream is attributed mainly to ions (particularly  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ). The electrical conductance of these dairy products is also influenced by the presence of fat [20,32]. The conductance of milk and cream decreases as the percentage of fat increases; for example, the conductance of skimmed milk is 7–10% higher than that of full fat milk and the conductance of a single cream (18% fat) is more than twice that of double cream (47.5% fat).

Fat is a nonconductor, which hinders the conduction of electricity by occupying volume and by impeding the mobility of ions [28]. As reported in the literature, the electrical conductivity was greatly influenced by the incubation time of the product [27]. Lactic acid fermentation decreases the pH of the medium, and concomitantly increases its electrical conductance, as a result of the accumulation of lactate ions during fermentation [17]. During milk fermentation lactic acid is

excreted into the medium, which will decrease the pH of the medium and concomitantly increase the electrical conductance [21]. In this study, electrical conductivity of kefir samples increased as the percentage of fat increased.

But, statistical analysis showed no significant difference in electrical conductivity changes between the kefir samples of different fat contents during the incubation time ( $P > 0.05$ ). Negative correlation was observed between EC and fat in kefir samples ( $r = -0.73$ ).

### 3.2. The Relationship between EC and Incubation Temperature

After fitting the experimental data into various equations using the least square method, a suitable model was created for the samples. This model with its  $R^2$  values is presented in Table 2. It was found that the effects of incubation time on the electrical conductivity of the kefir samples were significant, but it was also significant in the case of fat content.

**Table 2.** Regression equation of electrical conductivity ( $\sigma$ ) and temperature (T), and the coefficient of determination  $R^2$  values

Incubation Temperature (°C)	Fat Content (%)	Regression Equation	$R^2$
25	0.15	$\sigma = 4.759 + 0.096 T$	0.995**
	1.7	$\sigma = 4.571 + 0.092 T$	0.997**
	3	$\sigma = 4.894 + 0.074 T$	0.990**
35	0.15	$\sigma = 4.978 + 0.058 T$	0.969**
	1.7	$\sigma = 4.663 + 0.185 T$	0.989**
	3	$\sigma = 4.785 + 0.169 T$	0.994**

\* $R^2$ : coefficient of determination.

High coefficients of determination ( $R^2 > 0.95$ ) indicate the suitability of the linear model for predicting conductivity variation with temperature. The regression parameters are shown in Table 2. The  $R^2$  values of all the equations were greater than 0.95, thus indicating the suitability of the regression equation. (Table 2) gives the electrical conductivity–temperature relationships obtained for the entire experimental temperature (25–35°C), for three different fat concentrations of milk. The relationship between electrical conductivity and incubation temperature follows a linear trend. For different milk fat content of kefir samples, the EC values showed a linear regression at both incubation temperatures, until the end of fermentation (Table 2). The linear equation was found to be appropriate ( $R^2 = 0.955$ ) to successfully describe the temperature dependence of kefir's electrical conductivity increase after 10 h fermentation in the range of 25–35 °C. Both quality parameters can be estimated with linear models characterized by  $R^2$  values ranging from 0.969 to 0.997. The best model describing the electrical conductivity was obtained for the low fat (1.7%) kefir samples (25°C).

## 4. Conclusion

The results of this study showed that the electrical conductivity (EC) increased with increased incubation temperature. The rise of fermentation temperature caused an increase in EC values. The electrical conductivity of liquids including milk increases linearly with an increased temperature. Specific points may be characterized curves of conductivity changes using the main points of

inflection observed. The variation of the conductance over time was attributed to the breaking of chemical bonds between the casein micelles and the colloidal salts. On-line measurements of electrical conductivity data have been utilized to access time and rate feature points of lactic acid fermentations. Yoghurt, sour cream or cream fermentations for the manufacture of cottage cheese dressings or cultured butter, the end point of a fermentation is a critical factor in the quality of the product. Therefore, evolution of milk acidification is important and is generally followed by measuring pH with a glass electrode. However, pH determinations during fermentation of milk are not continuous because many government legislations do not authorize the use of glass electrodes in vats during the production of fermented dairy products. This restriction is aimed at preventing the occurrence of glass particles in the products in the advent of the shattering of a glass electrode. Samples must therefore be periodically taken and analyzed, which is labour intensive. Thus, there is a need to develop means to continuously follow acidification in production vats without the use of glass probes. An electrical conductivity system without a glass probe could be suitable for this purpose. Electrical conductivity is a measure of the resistance of a particular material to an electric current. In fermented milk, EC is determined by lactic acid production, and by the concentration of solubilized anions and cations.

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