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Mathematical Modeling of pH Variation as a Function of Temperature and Time in Kefir Production

Binnur Kaptan[1, *], Serap Kayısoglu[2], Omer Oksuz[1]

[1]Department of Food Science, Faculty of Agriculture, Namık Kemal Univetsity, Tekirdağ, Turkey
[2]Namık Kemal University, Vacational School, Tekirdağ, Turkey

Email address

bkaptan@nku.edu.tr (B. Kaptan), skayisoglu@nku.edu.tr (S. Kayısoglu), ooksuz@nku.edu.tr (O. Oksuz)

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Abstract

A mathematical model was developed to describe the effect of temperature and fermentation time on the kinetic parameters of pH change by kefir grains yeast population, using whole milk, semi-skimmed milk and skim milk of cows. Fermentation temperature (25–35 $^{\circ}$C), total fat level (3.0, 1.7, 0.15 %) and inoculum level (2%) w/v had simultaneous effects on the acidification process in kefir fermantation. The changes in pH of pasteurized cow milk inoculated with 2 % culture were investigated during fermentation at 25-35 $^{\circ}$C. Measurement of pH change was followed first order kinetics during kefir fermentation. The optimal kinetics model for pH change during fermentation of kefir was the linear mathematical model. Furthermore, statistical analysis indicated that fermentation temperature and time significantly affected pH change of kefir. pH reduction rate of kefir was maximum at semi-skimmed milk (1.7 %) at 35 $^{\circ}$C.

Keywords

Kefir, pH, Kinetic, Milk Fat, Fermentation, Time

1. Introduction

Kefir is a type of sour fermented milk in which kefir grains are employed as a starter culture (Bosch et al., 2006). The kefir grains have a specified structure and behave as biologically vital organisms. Various lactic acid bacteria (LAB) present in kefir grains or kefir products were isolated and identified by physiological and biochemical tests (Chen,Wang,& Chen, 2008; Witthuhn, Schoeman, & Britz, 2005). Besides LAB, yeasts and acetic acid bacteria have also been shown to be present in kefir grains.

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. (Güzel-Seydim et al.,2000; Leite et al., 2012; Magalhães et al., 2011;Öner et al., 2010). Fermented dairy products are charactericised by an acidic taste originating from the presence of lactic acid, a by-product of lactic fermentation. pH and titratable acidity are commonly used as measurements of acidity to determine the quality of milk before and during the production of fermented dairy products. An acidification process, which is highly dependent on temperature, is reflected by a decrease of kefir pH value. Because the acids concentration was not measured during the fermentation processes, the pH values were followed instead to check the influence of temperature on acidification. (Zajšek & Goršek,, 2010). The desired pH (4.4) values are obtained by changing the incubation temperature, the incubation time and ratio of culture in industrial production of kefir.

In conventional control systems, the value of pH is estimated by trial and error method which needs more time and personel experience. However, the estimation of pH is easier with modeling method than conventional control systems because computers are used in it. In this way, system errors can be corrected in a shorter time with this method. This may result in a better process control, enhanced food safety and quality, and a reduction of economic losses. There has been no information in literature on the evaluation of pH change kinetics during kefir fermantation.

The principal objective of this study was therefore to evaluate and compare the pH change kinetics during different treatments which had different fat content of milk and fermantation temperature of kefir

2. Materials and Methods

2.1. Milk and Kefir Grains

Milk was obtained from commercial markets. The chemical characteristics of pasteurised cow milk used for trial manufacturing of kefir are presented in Table 1. Kefir grains were obtained from Ankara University Faculty of Agriculture, Dairy Technology Department in Turkey. These kefir grains were activated by transferring them into pasteurised whole cow's milk, without stirring, allowing them to grow at approximately 25 °C for 24 h. filtered to remove the clotted milk, and rinsed with sterile water. The activation step was repeated 3 times.

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

	Whole milk (WM)	Semi-Skim Milk (SSM)	Skim milk (SM)
Fat (%)	3.00	1.7	0.15
Protein (%)	3.10	3.2	3.1
Lactose (%)	4.50	4.7	4.5
Calsiyum (mg/100ml)	110.00	115	120
Energy (kcal/kJ)	57.4/240	46.9/196	31.7/132.5

2.2. Preparation of Kefir Samples

Six kefir samples were prepared with cow's milk which had different fat contents: pasteurised whole milk (WM) (3 %), semi- skimmed milk (SSM) (1.7 %) and skim milk (SM) (0.15 %). Then they were incubated at 25-35 °C by adding 2 % (w/v) kefir grains. Incubation was continued until pH 4.4 at 25 °C -35 °C

2.3. Monitoring of pH

The pH values of the Kefir (measured after overnight maturation) and milk samples were measured with 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.

2.4. Fermentation Stage

At this stage the kefir fermentation period lasted about 10 h. The fermentation of Kefir were made by an inoculant consisting of 2% (w/v) kefir grains. Incubation was continued until pH 4.4 at 25-35 °C. The pH measurements of kefir samples were made by periodic measurements at 60 min intervals until the desired pH achieved.

2.5. Modeling of Changes in pH During Fermantation

The time that we need to reach an appropriate pH level at the end of the fermentation process depends on fat ratio of milk and fermentation temperature. The pH value was recorded 60 minutes intervals until the end of the fermantation process for every treatment. The number of data points was different for each treatments.

The first stages of the kefir fermantation involved a linear change in pH with time, however, linear regression was used to calculate the constant rates. In the final phase the drop in pH was more rapid and the behavior was nonlinear with time.

To model the kinetic changes during this stage, the pH data were converted to a logarithmic ratio form as follows (a fraction conversion model—Levenspiel, 1974):

$$Log\left(\frac{pH_t - pH_\infty}{pH_i - pH_\infty}\right)$$

where pH_t is the pH at given time, pH_i is the initial pH at kefir, which is 6.12, and pH_∞ is the pH after infinite time. It is noted that the pH value of all kefir samples are almost 4.4 which was used as the equilibrium pH (pH_∞).

2.6. Statistical Analysis

The general linear models (GLM) procedure was performed to see the significance of different treatments on pH change kinetics during kefir-making. Duncan grouping test was also performed to differentiate between the treatments. Simple regression analysis was performed to develop the models (log transformed for fraction conversion model) and determine the rate constants. 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

3.1. Changes of pH During Kefir Fermentation

Figure. 1 shows the values of the main pH change during the fermantation periods of kefir prepared by using different fat content milk and incubation temperature. During kefir fermentation the pH change was recorded by a pH meter in 60 min intervals.

The pH of the kefir decreased significantly faster at higher incubation temperature, and that indicates higher acids production, especially lactic acid. It is shown in the graphic that decrease in pH value was proportional to the temperature increase (Figure 1). Motaghi *et al.* (1997) reported that kefir manufactured by addition 5% Iranian kefir grains and incubation times of 12, 24, 36, 48, 60 and 72 hours had pH values in the range of 2.98 – 4.00.

Ismaiel *et al.* (2011) reported that in their study the final pH values were in the acidic side i.e. 2.91 – 4.04. Magalhaes et al. (2011) reported that before incubation the pH value of Brazilian kefir was 6.61±0.02 and after 24 hours of fermentation at 25 °C was 4.42±0.01.

Lowest pH was reached at 35 °C in low-fat milk kefir samples. At the end of the first hour of the beginning of fermentation the amount of pH in whole milk kefir samples were lower than the others (Figure. 1). The average pH value of kefir samples were in the range from.5.08 to 4.15.

The pH of the milk decreased significantly faster at higher incubation temperature, which indicates on higher acids production, especially lactic acid. 25 C fermentation temperature leads to increase in acidity especially lactic acid and,decrease in pH For example, pH changes during the fermentation was determined at 25 °C 6.09-5.08; 6.11-4.97; 6.12-4.98 and 35 °C 6.09-4.33; 6.11-4.15; 6.12-4.42, in whole milk , semi-skimmed milk, skim milk, respectively. (Fig. 1). It is shown that the decrease in pH value was proportional to the temperature increase. The lowest pH was reached at 35 °C in whole milk., and it takes 120 minutes to reach pH 5, 4.

During the fermentation and kefir manufacture process, pH values decreased progressively ($p < 0.05$), ranging from 6.11 to 4.15, as a consequence of organic acid production. According to Ozdestan and Uren (2010), pH values of kefir samples varied from 4.11 to 4.53.

A linear mathematical model was developed in order to follow the kefir pH decrease that depends on fermentation temperature after 10 h. fermentation. Furthermore, statistical analysis indicated that both fermentation temperatures (25-35)and fermentation time. also showed significant effect on the pH production

Modified fractional conversion model was well fitted to the experiment data and it could be regarded as sufficient to describe the pH production by mixed kefir grains yeast population in the milk medium as a function of fermentation times and temperatures. According to Zanatto and Basso (1992), a more homogenous coagulum are achieved with medium acidification rate.

3.2. Changes of pH Kinetics During Kefir Fermentation

The pH change kinetics during kefir fermentation for different treatments are shown in Figure. 2. Table 2 shown the Duncan grouping of mean value of rate constants, the associated R2 and coefficient of variance (%). The pH change acidification kinetics of the kefir gel were characterized by the maximum pH rate, fermentation time at which pH max was reached (pH_t) and it is also necessary to reach the end of the fermentation (4.4).

It is obvious from Figure. 1 that the pH change profile in fermentation stage is different from those in kefir fermentation time, with the curves showing a nonlinear behavior. It also appears that all samples would eventually reach an equilibrium pH. Due to nonlinear behavior of pH change in this stage, data were modeled using a fractional conversion model (semi-logarithmic model approaching an equilibrium value).

The equilibrium pH was close to 4.4 (reached after 480-600 min) and hence this level was employed in the model. The semi-logarithmic rate constants for pH change kinetics during the fermentation stage are shown in Figure. 2 for the different treatments. Rate of constants for pH change are summarized in Table 3 with Duncan's multiple range test indicating there was no significant difference between at WM-35 °C, SSM-25 °C, SM-25 °C samples, and at SSM-35 °C, SM-35 °C kefir samples.

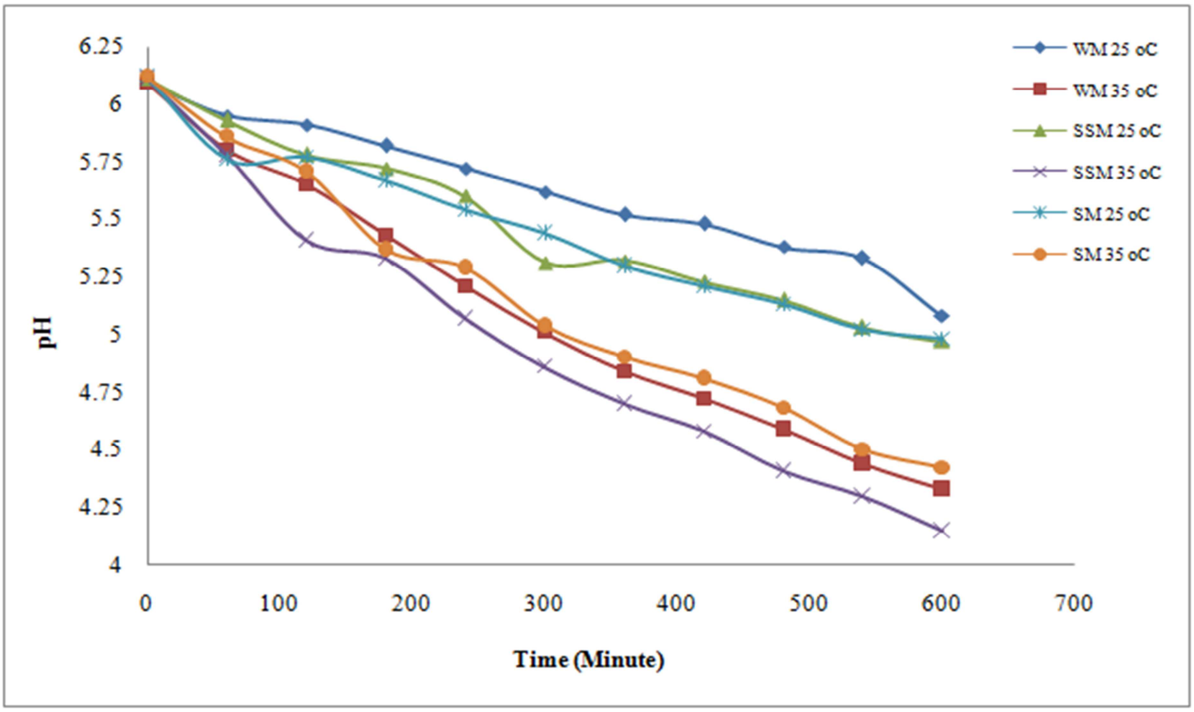

Figure 1. *Typical curve of pH change during fermantation for different treatments*

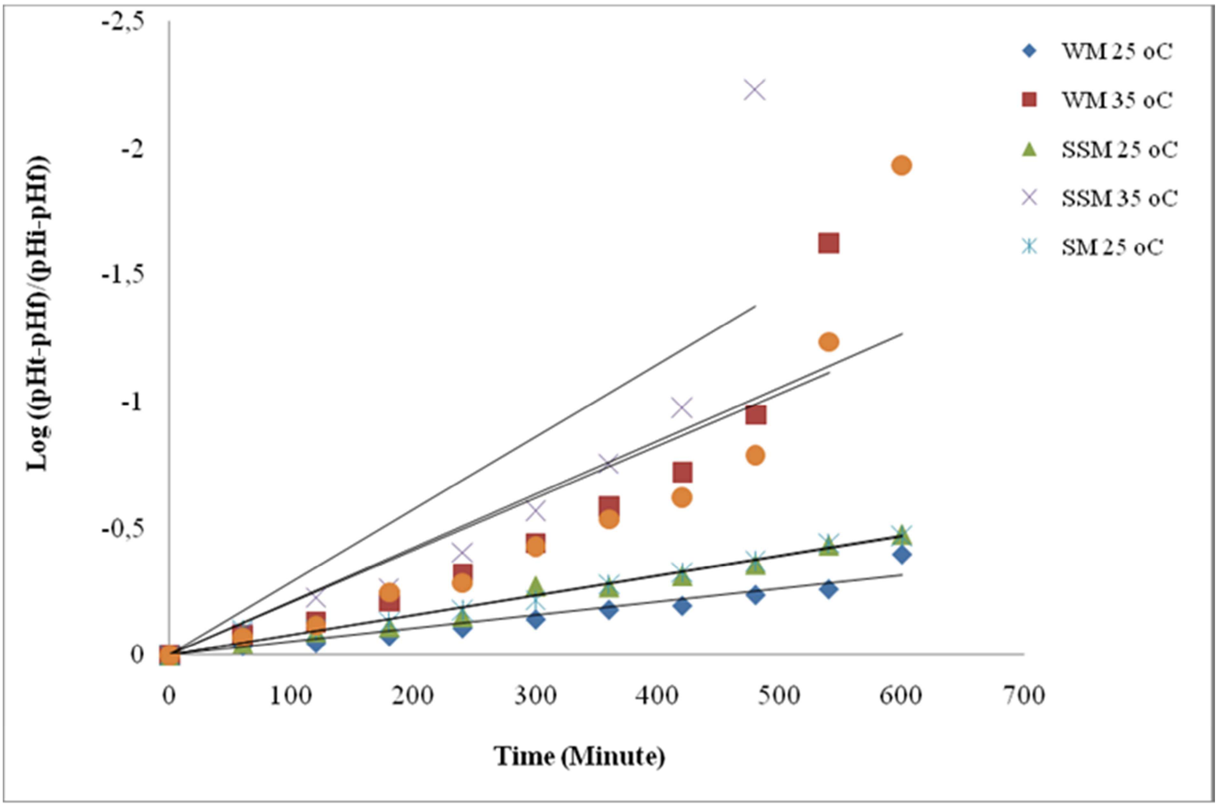

Figure 2. *The pH change kinetics during kefir fermentation for different treatments*

Table 2. *Duncan groupings of the treatments for rate of change of pH during kefir making*

Treatments	Rate Constant (min-1)	R2	F	Duncan Groupinga
WM 25°C	0,0006	0,939	137,773	B
WM 35 °C	0,0025	0,852	46,126	AB
SSM 25 °C	0,0008	0,984		AB
SSM 35 °C	0,0036	0,747	20,713	A
SM 25 °C	0,0008	0,985	574,355	AB
SM 35 °C	0,0026	0,819	40,674	A

[a]Duncan's multiple range test a means of groups with different letters are significantly different at $p<0.01$.

Statistically, however, the rate constants associated with fermentation temperature and milk fat content. Rate constant were lowest at WM-25 °C. The change in the pH rate during fermentation period may be due to the fact that most of the kefir samples, which is lactose fermentation, will turn the lactic acid lactose with it. The shortage of lactose could thus reduce the activity of lactic acid bacteria and the acid production in the kefir.

The time at which maximum acidification rate is observed (pH_t) was strongly influenced by fermentation temperature. Maximum acidification rate was attained earlier when fermentation took place at shorter fermentation time. Concerning fermentation time the positive effect of fermentation temperature is obvious from the regression coefficient. The time of kefir fermentation was significantly influenced by the fermentation temperatures.

In the case of microflora of kefir cultures the results can be explained that both fermentation temperature had a positive linear effect on the time required to reach maximum acidification.

The results showed that both fermentation temperature had a positive linear effect on the time required to reach maximum acidification (Figure 2). A higher rate of change in pH was observed for at 35 °C in WM (6.11-4.15). The whole milk at 25°C showed the least pH change rate(6.09-5.08). Fermentation time of kefir increased with the increase of the rate of milk fat at the same temperature. Torriani *et al.* (1996) studied the influence of the variation of parameters such as fat and solids content, inoculum size on the fermentation process. Whole milk maximum a cidification rate was attained earlier when fermentation took place at lower temperatures. So, in order to attain a medium acidification rate which favours the formation of a homogenous viscous gel, a higher fermentation temperature is preferred (Varghese and Mıshra, 2008).

Table 3. *Duncan's multiple range test for rate of change of pH during fermantation*

Treatment	Rate Costant	R2	CV %	F
WM-25 oC	0,0011	0,977	5,4	341.6**
WM-35 oC	0,0020	0,926	11.4	100.3**
SSM-25 oC	0,0025	0,890	7.0	67.9**
SSM-35 oC	0,0019	0,955	12.7	169.4**
SM-25 oC	0,0022	0,853	7.5	46.3**
SM- 35 oC	0,0021	0,892	11.0	66.0**

** significant at p<0.01 probability level

4. Conclusions

In the first part of this research, kefir samples were prepared by using three different fat content milks and two different fermentation temperatures. It is observed that the changes in pH values of the samples are prepared from the different fat content milks during different fermentataion temperatures. A linear mathematical model was formed the pH change kinetics during fermentation.

Furthermore, statistical analysis indicated that fermantation temperature affected significantly on pH change as a function of fermentation time and temparature. The maximum decrease of pH was at 35°C SSM. The increase in milk fat caused longer fermentation time at the same temperature. The results from this study were expected to be helpful for understanding the behaviour of pH change during the kefir fermentation process.

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