

Reliability of Time-Temperature Indicator From Corn and Red Palm Oil Blending For Monitoring Microbial Growth of Pasteurized Milk

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ABSTRACT

Time-Temperature Indicator made from corn oil and red palm oil had potential to be used in food cold chain. However, the evaluation of its reliability was required. The aim of this study was to evaluate the reliability of the indicator to monitor the changes of pasteurized milk quality based on microbial growth at several storage temperatures. This study was conducted in three stages. Stage 1 was making corn oil and red palm oil blending with 70:30 (%v/v) ratio. Stage 2 was measurement of diffusion length, coefficient, kinetics and activation energy at five storage temperatures (4, 29, 37, 44, and 51 °C). Stage 3 was counting the total microbes at three different storage temperatures (8, 29, and 40 °C). The result showed that the activation energy of corn oil and red palm oil Time-Temperature indicator was 36.796 kJ/mol. Meanwhile, the activation energy of pasteurized milk microbial growth kinetics was 44.021 kJ/mol. The reliability of the indicator was good, because the activation energy difference value between microbial growth and the indicator was lower than 25 kJ/mol.

Keywords: Corn oil, Pasteurization milk, Red palm oil, Time-temperature indicator

INTRODUCTION

Pasteurized milk is one of the products that requires cold chain distribution system. The product safety and quality might change as temperature changes during distribution. To monitor changes in temperature during distribution and storage, irreversible indicators such as TTI (Time-Temperature Indicator) are needed.

The TTI could be produced by using the principle of fluid diffusion. The fluid should have low melting point and stable viscosity (Khairunnisa, 2018). Also, good

commercial TTI should have the activation energy between 34-50 kJ/mol (Pocas et al., 2008).

Corn oil and red palm oil blending could be the best candidate to produce the fluid diffusion based TTI. Corn oil had -11 °C of melting point (Strayer, 2016). On other hand, red palm oil had 20.7 °C of melting point (Ulfah et al., 2016). Based on the previous research, corn oil and red palm oil blending resulted the best energy activation in 70:30 (%v/v) ratio (Widyasaputra et al.,

2022). Also, this blending had 2.47 °C of melting point (Widyasaputra et al., 2022).

The objective of this research was to evaluate the reliability of indicator to monitor the changes of pasteurized milk quality based on microbial growth at several storage temperatures. This evaluation was obtained based on checking the activation energy microbial growth kinetics of pasteurization milk against the activation energy of the TTI indicator label.

MATERIALS AND METHODS

Tools and Materials

The materials of this research were waterproof glossy photo paper 15 cm x 1.0 cm x 0.01 cm as the medium of diffusion (Printech), corn oil (Mazola, Moi Foods, Selangor) and red palm oil (Salmira, PT. Nutripalma Abadi, Bogor), pasteurization milk (Greenfields, PT. Greenfields Indonesia, Malang), PCA (*Plate Count Agar*).

The tools were refrigerator Samsung RT20 (Samsung Electronics Co., Ltd), incubator oven (Memmert, GmbH, Schwabach, DE), beaker glass 250 mL (Iwaki pyrex), beaker glass 500 mL (Iwaki pyrex), petri dish (Iwaki pyrex), infrared thermometer GM320 (Shenzen Capital Electronics Co., Ltd.), hotplate.

Methods

This study was performed in three stages. First, blending the corn oil and red palm oil with 70:30 (%v/v) ratio. The process was conducted with 40 °C of heating and stirring for 10 minutes (Widyasaputra et al., 2022). Second stage, measurement of diffusion length, coefficient, kinetics and activation energy at five storage temperature (4, 29, 37, 44, and 51 °C). The diffusion length measurement (cm) was conducted with soaking the medium (photo paper) in 2 mL of oil blend for 30 hours. The diffusion length measurement was performed after 30

hours. Diffusion coefficient (D) was calculated by equation:

$$D = \frac{x^2}{2t} \dots\dots\dots(1) \text{ (Khairunnisa et al., 2018)}$$

D was diffusion coefficient (cm²/ hour), x was diffusion length (cm), and t was time (hour).

Diffusion kinetics was calculated by using Arrhenius equation with modification:

$$\ln D = - \left(\frac{E_a}{R} \right) \frac{1}{T} + \ln D_0 \dots\dots\dots(2) \text{ (Li et al., 2008)}$$

D was diffusion coefficient (m²/s), E_a was activation energy (KJ/mol), R was gas constant (8.314 J mol⁻¹ K⁻¹) and T was temperature (K). The diffusion kinetics measurement was conducted with Arrhenius equation.

Third stage was measurement of total viable (microbial) count at three different storage temperatures (8, 29, and 40 °C). Total viable count was performed with plate count analysis at 0, 2, 4, 6, 24, 26, and 28 hours (modification from Khairunnisa et al., 2018). 10 mL pasteurization milk sample was diluted in 90 mL physiological solution (10⁻¹). The dilution was continued until 10⁻⁴ (for 0, 2, 4, 6 hours) and 10⁻⁷ (for 24, 26, 28 hours). 1 mL sample of each of the last four dilutions was poured into petri dish (duplicate). Then, plate count agar media was poured into the petri dish and slowly shaken to form a figure eight. The incubation was conducted at 30 °C for 48 hours. The analysis was repeated two times. Total viable count was calculated by equation:

$$N = \frac{\sum C}{(1 \times n_1) + (0.1 \times n_2) + d} \dots\dots\dots(3)$$

The kinetics of microbial growth was calculated by equation:

$$\ln \frac{N_0}{N} = k \cdot t \dots\dots\dots(4)$$

$$\ln k = - \left(\frac{E_a}{R} \right) \frac{1}{T} + \ln k_0 \dots\dots\dots(5)$$

N was total viable count (CFU/mL); ΣC was countable colony; n₁ was number of petri dish in dilution 1; n₂ was number of petri dish



in dilution 2; d was the lowest dilution, k was coefficient; R was gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$).

The statistical analysis was performed by Analysis of Variance with SPSS v.22. The kinetics calculation was performed by Microsoft Excel 2019.

RESULTS AND DISCUSSION

The good TTI should be sensitive to changes in temperature. The diffusion coefficient of corn oil and red palm oil based TTI was studied under five different temperatures. Figure 1 showed that increasing temperature could increase the diffusion coefficient. Diffusion coefficient was affected by time and temperature (Setiawan, 2012). The movement of molecules in oil was triggered by changing potential energy to kinetic energy during temperature increase (Khairunnisa et al., 2018).

The activation energy could be calculated by connecting $1/T$ (K^{-1}) and $\ln k$ (Figure 2). The regression equation used was $y = -4425.8x - 3.1187$ with R^2 value 0.9718. From regression equation and Arrhenius equation (2), the activation energy of 36.696 kJ/mol was obtained.

Total microbial growth of pasteurized milk (Figure 3.) followed first-order Arrhenius equation, so that the activation energy value was determined by connecting time (t, hours) and $\ln(N/N_0, \text{CFU/mL})$. The growth in 8°C storage temperature was the lowest than other. Pasteurization process could only kill 95% of microbes, therefore pasteurized milk need to be stored in low temperature (Fromm & Boor, 2004). In low temperatures, pasteurized milk had longer shelf life (Ziyaina et al., 2018).

Kinetics of total microbial growth in pasteurized milk showed in Figure 4. The Ea value could be determined by connecting $1/T$ (K^{-1}) and $\ln k$. An equation of straight line could be rendered into a kinetics equation (5).

The regression equation used was $y = -5294.9x + 16.176$ with $R^2 = 0.9195$. From the calculation, the Ea value of pasteurized milk (stored in 8, 29, and 40°C) was 44.021 kJ/mol.

The reliability of corn oil and red palm oil based TTI could be obtained by calculating the difference between Pasteurized milk Ea value and TTI Ea value. From table 1, the Ea difference was 7.225 kJ/mol. The reliability of indicator was good because the difference was lower than commercial TTI (25 kJ/mol) (Ellouze & Augustin, 2010; Khairunnisa et al., 2018; Park et al., 2013).

The Ea difference of the blending of corn oil and red palm oil based TTI was better than the blending of palm oil, canola oil and soybean oil. The Blending with 70:30 ratio of corn oil and red palm oil had 7.225 kJ/mol activation energy difference. The blending of palm oil, canola oil and soybean oil with 50:40:10, 50:25:25, and 50:10:40 had 23.816, 22.811, and 17.459 kJ/mol activation energy, respectively (Khairunnisa, 2018). Corn oil had -11°C of melting point, the melting point was lower than canola oil (-10°C), olive oil (-6°C), but higher than soybean oil (-16°C) (Strayer, 2016). The melting point of oil had close relation with diffusion rate which also affected the activation energy. The lower melting point could increase the diffusion rate (Widyasaputra, et al., 2022).

The blending of corn oil and red palm oil was easy to produce. But the blending ratio might be needing modification for cold chain food system with below 0°C storage temperature.

CONCLUSION

The TTI had 36.796 kJ/mol of activation energy. On other hand, pasteurized milk microbial growth kinetics had 44.021 kJ/mol of activation energy. The difference between the two was 7.225 kJ/mol (<25

kJ/mol), shown that indicator had good reliability.

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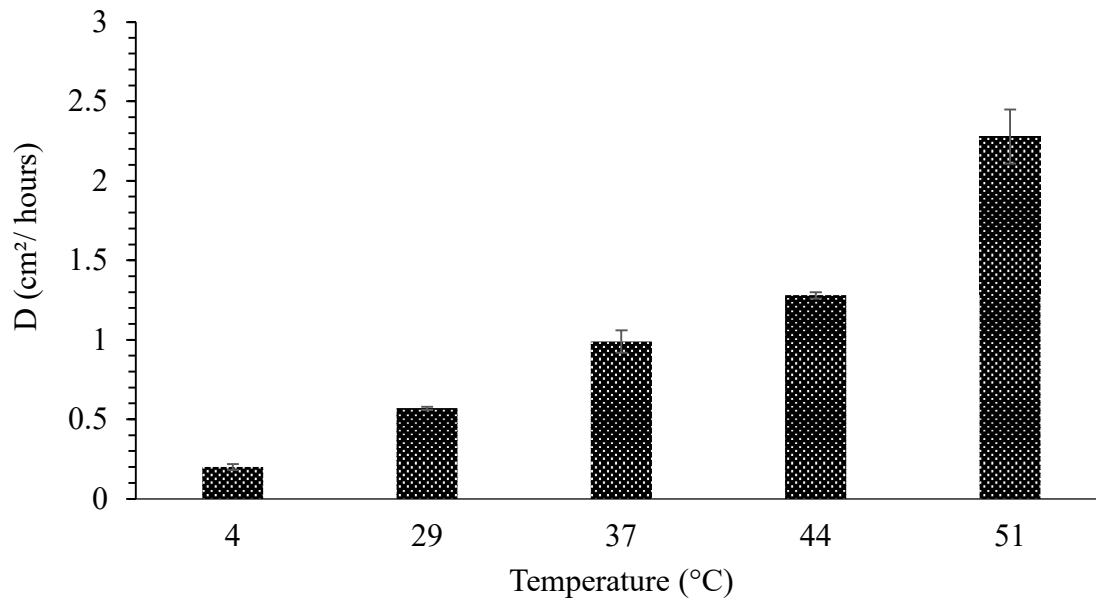


Figure 1. The diffusion coefficient of indicator (D) at some storage temperature

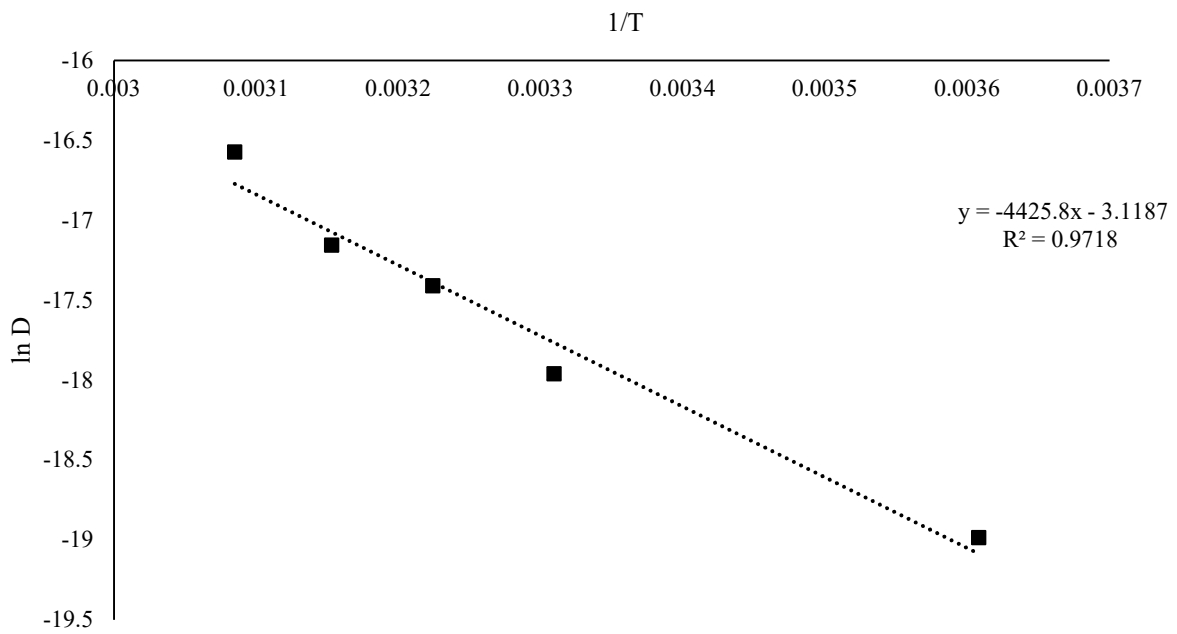


Figure 2. ln D and 1/T plot for corn oil and red palm oil TTI

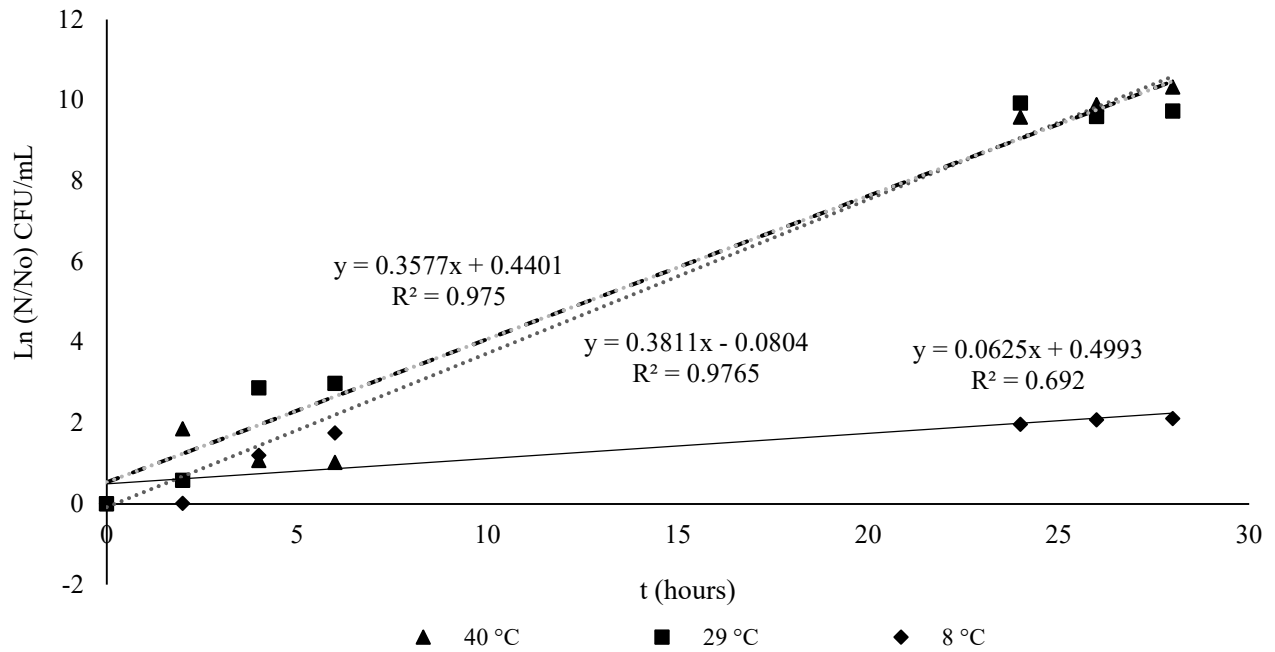


Figure 1. $\ln(N/N_0)$ and t plot for total microbial growth in pasteurized milk

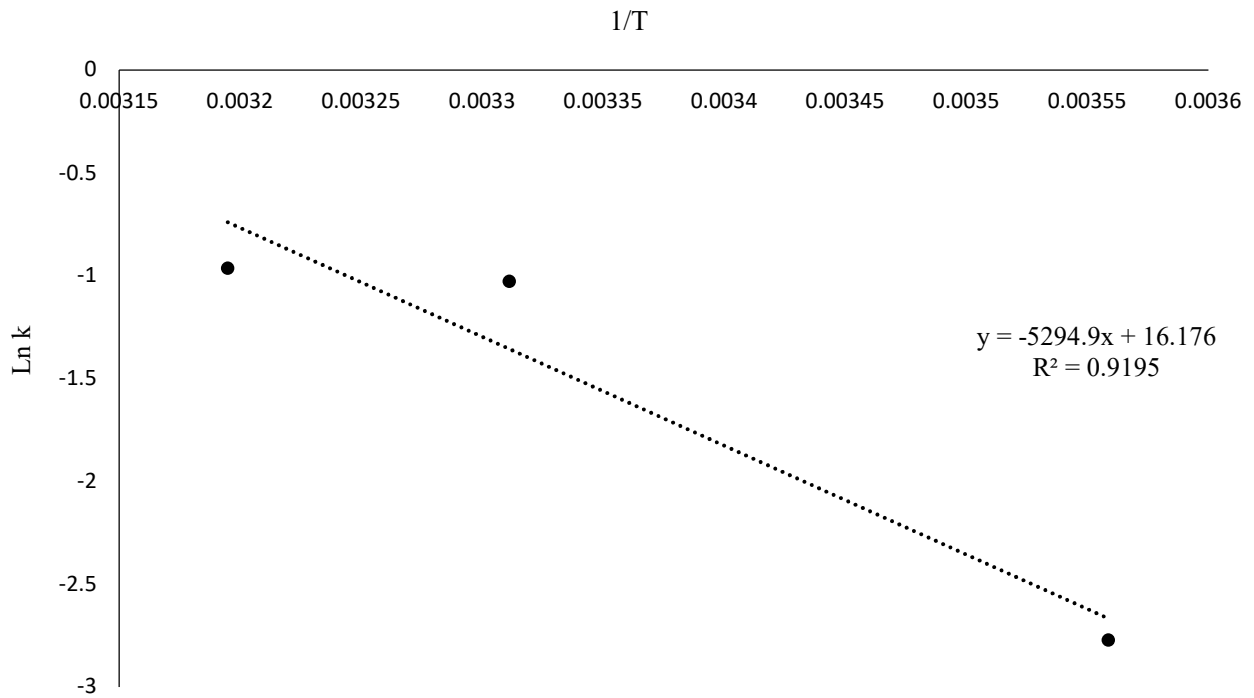


Figure 2. $1/T$ and $\ln K$ plot of total microbial growth in pasteurization milk

Table 1. Activation energy (Ea) of corn oil and red palm oil blend TTI and pasteurization milk

TTI Ea (KJ/mol)	Pasteurized milk Ea (KJ/mol)	Pasteurization milk Ea – TTI Ea (KJ/mol)
36.796	44.021	7.225