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Microbial growth kinetics models of raw milk in storage and transportation

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Abstract

The quality safety of raw milk largely determines the quality of dairy products. However, the amount of microorganisms in raw milk is easy to exceed in production which affects the quality of dairy products. The objective of this study was to establish microbial growth kinetics model of raw milk in storage and transportation as well as to predict the shelf life of raw milk in the cold chain. The results showed that TVC values increased with storage time and were modelled by Branayi and Roberts [9] model. The model was verified in a simulation experimental scheme for 15 hours in order to establish their applicability in the cold chain.

Key words: Raw milk, modelling, microorganism, temperature.

Introduction

In recent years, China dairy industry has been rapidly developed with the national dairy market's fast growth [1]. However, the results of dairy products selective examination show that the percent of pass is always at a low level in the last decade. That proves the existing dairy products safety control system is still imperfect and cannot fully guarantee the safety of dairy products [2-4].

Raw milk is a necessary raw material of dairy industry. The quality safety of dairy products largely depends on the raw milk. At present, the level of raw milk production is low in China (80% raw milk was produced by hand milking) [5]. The production condition is poor and raw milk is easily contaminated by bacteria. The microbial pollution in raw milk is the main factor to threaten dairy quality safety. Some microbes which are difficult to kill in production of dairy products will be gradually a large number of breeding, especially the long-term milk (shelf life more than 30 days) [6]. Also, some microbes, which can produce heat resistant enzymes, such as protease and lipase (after 140°C, 2 min treatment still has a 10% residual), will be activated in raw milk storage and transportation [7]. Those enzymes decompose protein and fat to lead bitter products, corruption of taste and clot.

Because the field of raw milk is generally far away from the factory of dairy products and the cold chain of raw milk is not perfect, the microorganisms are easy to be out of limits. Between the growth of microbes and the quality safety of raw milk exists a certain relationship, so it is possible to predict the fresh degree of raw milk based on the initial bacteria number and growth dynamics model [8].

This study monitored and analysed the change of microbial number on fresh raw milk stored at 0, 5, 10, and 20°C, and establish microbial growth dynamics model to demonstrate the applicability of the models in simulated transportation of raw milk.

Materials and Methods

Experimental material: Raw milk was got from Beijing Hualong milk factory and immediately refrigerated to laboratory at -5°C.

Temperature design: Constant temperatures of 0, 5, 10 and 20°C were selected for index changes measurements and a simulative raw milk storage and transportation was 20°C/1 h - 4°C/2 h -10°C/5 h - 20°C/0.5 h.

Total viable counts (TVC): The samples of 10 ml were transferred to a stomacher bag, 90 ml of 0.1% peptone water with salt (NaCl, 0.85%, w/v) was added and homogenized for 60 s with a stomacher. Total viable counts (TVC) were determined by counting the number of colony-forming units after incubation at 25°C for 48 h [10].

Model: The adjusted model of Baranyi and Roberts [9], combined with the growth model of Richard has been introduced for simulating the microbial growth. The model of Baranyi and Roberts [9] has been selected due to the following reasons: (i) it is easy to be applied; (ii) it is applicable under dynamic environmental conditions; (iii) the model parameters are biologically interpretable.

Under constant temperatures, TVC modelled with Baranyi and Roberts model as shown in Eq. 4 and the correlation coefficient (R^2) are shown in Table 2.

$$\begin{aligned} y(t) &= y_0 + \mu_{\max} A(t) - \frac{1}{m}\ln\left(1 + \frac{e^m \mu_{\max} \cdot A(t) - 1}{e^{m(y_{\max} - y_0)}}\right) \\ A(t) &= t - \lambda + \frac{\ln\left(1 - e^{-vt} + e^{-v(t-\lambda)}\right)}{v} \end{aligned} \tag{1}$$

where t is the time, y_0 is the natural logarithm of the inoculum (ln

x_0), x_0 is the initial cell number, m is characterizes the curvature before the stationary phase, y(t) = ln x(t) and μ_{max} is the maximum specific growth rate. λ is the lag phase of the microorganism, which is the time lapsed before the beginning of exponential growth.

Evaluation methods of growth models: The regression coefficient can reflect whether the model fits the growth of microbiology or not. It is used as an overall measure of the predict level. The higher its value ($0 < R^2 < 1$), the more accurate of the predict model.

Mean square error (MSE) is used to measure the change of numerical value. It is equal the square of the remaining value dividing degree of freedom. The lower its value (0 < MSE), the more accurate of the model.

$$MSE = \frac{RSS}{n} = \frac{\sum\left(\mu_{observed} - \mu_{predicted}\right)^2}{n} \tag{2}$$

N stands for the number of measurements; $\mu_{observed}$ is the measured value and $\mu_{predicted}$ is the predicted value. Bias factor indicates the distance between numerical value and the curve.

$$bias\,factor = 10^{\left(\frac{\sum \log\left(\frac{\mu_{observed}}{\mu_{predicted}}\right)}{n}\right)} \tag{3}$$

The higher its value (BF < 1), the more accurate of the model.

Accuracy factor means the distance between numerical value and the equivalent line. The lower its value (1 < AF), the more accurate of the model.

$$accuracy\,factor = 10^{\left(\frac{\sum \log\left|\frac{\mu_{predicted}}{\mu_{observed}}\right|}{n}\right)} \tag{4}$$

Results and Discussion

Effect of different temperatures on TVC: TVC was measured at 0, 5, 10 and 20°C as shown in Fig. 1. The initially total viable count was11.56 ln CFU/g. The value was lower than that reported by Li *et al.* [11] who found the initial total viable count of raw milk as high as 12.34 ln CFU/g. Differences of values could be explained the varieties of cow and the microorganisms of surrounding environment were different. Fig. 1 showed that the model could predict the growth of TVC well.

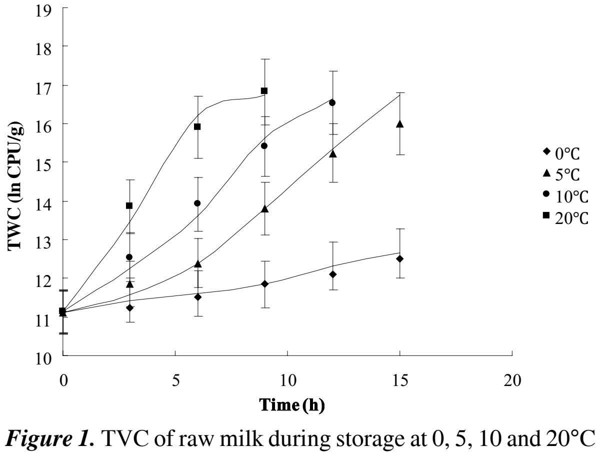

Figure 1. TVC of raw milk during storage at 0, 5, 10 and 20°C.

Table 1 shows that R^2, bias factor and accuracy factor were close to 1 and MSE's value close to 0, which proved the model could fit the actual circumstances of the microbial growth situation.

Table 1. Evaluation results of model at constant temperature.

Temperature	R^2	MSR	Bias factor	Accuracy factor
0°C	0.98087	0.255	0.922	1.085
5°C	0.87973	0.264	0.964	1.057
10°C	0.97769	0.483	0.987	1.033
20°C	0.99517	0.138	0.997	1.099

The maximum specific growth rate, μ_{max} was modelled with square model as shown in Eq. 5 ($R^2 = 0.954$):

$$\sqrt{\mu_{max}} = b\left(T - T_{min}\right) \tag{5}$$

where μ_{max} is the maximum specific growth rate, b is a constant and T_{min} is the minimum temperature of microbiology growth. Temperature dependence parameters b and T_{min} in Eq.5 were determined by fitting the equation to the μ_{max} values at different temperatures (Fig. 2).

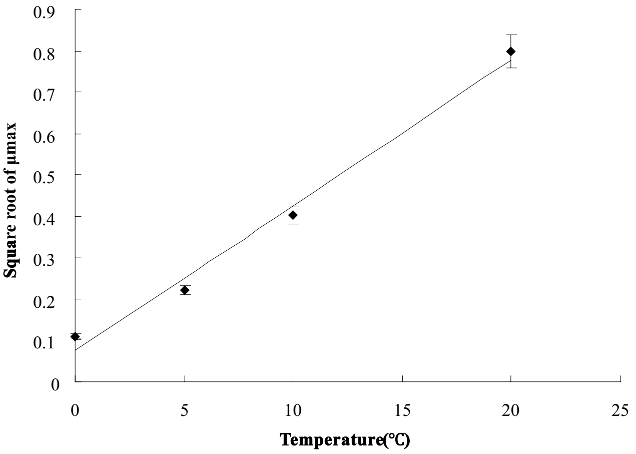

Figure 2. The effect of temperature on microbial growth rates.

The value of μ_{max} increased with temperature. Some bacteria strains, including psychrophilic bacteria, were active under freezing point. In this study, the minimum temperature of microbial growth was -2.2°C. The microbial growth rate was slow at freezing temperatures but growth rates increased at higher temperatures. Storage experimental results showed that square root model could describe the influence of temperature on growth rate under the condition of constant temperature

The validation of the model: A simulation experiment was put into effect in order to validate the application of model (Fig. 3). For further quantitative evaluation reliability of the growth kinetics model, the observed value and predicted value were compared (Table 2).

It was seen that the model could predict the growth of TVC under fluctuating temperature. Bias factor was 0.954 and accuracy factor was 1.468 (Table 2). The verification results showed the model which was established at constant temperature could predict the growth of TVC under fluctuating temperature rapidly and accurately (Fig. 3).

Table 2. The evaluation result of model at fluctuating temperature.

R^2	MSE	Bias factor	Accuracy factor
0.985	0.354	0.954	1.468

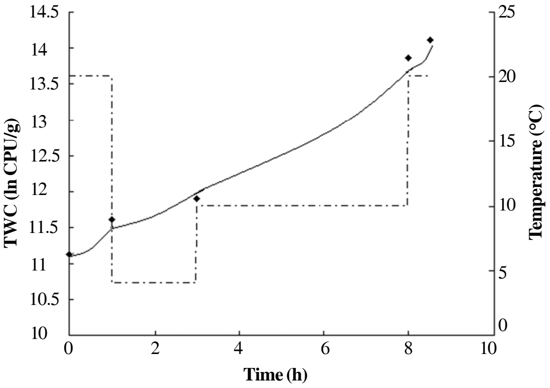

Figure 3. Predicted growth curve and observed values of TVC stored at a fluctuating temperature.

Conclusions

The number of TVC increases with temperature and time. The values of TVC can be predicted by Branayi and Roberts models accurately. Based on TVC of raw milk, a shelf-life of tilapia may be expected in an actual cold chain. TVC is considered as good quality index to predict the shelf life. If the temperature conditions of products could be always under observation, the quality status and the remaining shelf life of the products could be predicted by the models of quality indices.

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