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Kinetics models for the inactivation of *Alicyclobacillus acidiphilus* DSM14558^T and *Alicyclobacillus acidoterrestris* DSM 3922^T in apple juice by ultrasound

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ABSTRACT

In this paper, the ultrasonic inactivation efficacy of *Alicyclobacillus acidiphilus* DSM14558^T and *Alicyclobacillus acidoterrestris* DSM 3922^T inoculated into apple juice was investigated at power level from 200 to 600 W for treatment time from 1 to 30 min. The survival ratio of *A. acidiphilus* DSM14558^T and *A. acidoterrestris* DSM 3922^T decreased with the time of exposure to ultrasounds and with their power. Weibull distribution function, log-logistic model, modified Gompertz equation and biphasic linear model were used to describe the experimental data and the fitness of the models was assessed by the adjusted correlation coefficient (adj-R^2) and the root mean square error (*RMSE*). The results showed that, for *A. acidiphilus* DSM14558^T, the Weibull distribution function described well the characteristic of ultrasonic inactivation, while for *A. acidoterrestris* DSM 3922^T, the adequate one was the biphasic linear model. *Alicyclobacilli* had a much higher resistance to ultrasonic treatments in apple juice than in buffer, which indicated that the resistance of *alicyclobacilli* to ultrasound varied significantly depending on their environment; *A. acidoterrestris* DSM 3922^T, with the greatest microbial reduction of 4.56 log cycles at 600 W for 30 min, seemed more sensitive to ultrasonic treatments than *A. acidiphilus* DSM14558^T.

1. Introduction

Conventional thermal pasteurization and sterilization are widely used for inactivation of microorganism in food products. However, these thermal methods adversely affect the sensory qualities and nutritional values of the processed foods (Lee et al., 2009). Compared with the thermal treatments, non-thermal methods increase retention of flavors, colour and nutrient compositions. The increasing consumers demand for better quality food, leading to the development of non-thermal process technologies. Ultrasound, which refers to waves with a frequency of 20 kHz or more, is one of such technologies (Piyasena et al., 2003). The ultrasonic inactivation of some bacteria has been investigated, such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus subtilis* etc. (Pagan et al., 1999; Guerrero et al., 2001; Koda et al., 2009; Wrigley and Llorca, 1992; Manas et al., 2000; Raso et al., 1998; Limaye and Coakley, 1998). It has been known that the inactivation efficacy of microbe is subject to the type of bacterium, time of exposure to ultrasound and their power level, volume and composition of food being processed as well as the treatment temperature (Piyasena et al., 2003). And power level and treatment time are the major parameters of inactivation in ultrasound processing (Lee et al., 2009).

Some studies employed the first-order kinetic model to describe microbial survival characteristic curves (Lee et al., 2009). However, there has been growing evidence, showing that inactivation of microorganisms in some treatments may not follow the first-order kinetic model, especially in non-thermal process (Klotz et al., 2007). A number of models have been used to describe nonlinear inactivation kinetics, such as the log-logistic model, the Weibull distribution function and the modified Gompertz equation etc. (Smelt et al., 2002). For example, the Weibullian model has been satisfactorily used to model the thermosonication sensitivities of *Listeria innocua* in raw whole milk (Bermúdez-Aguirre et al., 2009). Lee et al. (2009) examined the responses of *E. coli* K12 to sonication, thermosonication, manosonication and manothermosonication treatments, and evaluated four models (Weibull, modified Gompertz equation and log-logistic models) in terms of their goodness of fit to the inactivation experimental data. Besides, Buzrul et al. (2005) obtained the survival curves of *A. acidoterrestris* treated under high hydrostatic pressure, used the Weibull model to describe the experimental data and investigated the adequacy of the model.

Initially, the interest in the genus *Alicyclobacillus* was purely academic until, however, in the recent years, the spoilage incidents concerning *alicyclobacilli* increased and more diverse types of products, e.g., shelf-stable iced tea, were contaminated (Walker and Phillips, 2005). At present, the genus *Alicyclobacillus* is more

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widespread than originally observed and they are becoming an industry-wide problem (Bevilacqua et al., 2008). Among the *alicyclobacilli*, *Alicyclobacillus acidoterrestris*, which can grow at pH values ranging from 2.5 to 6.0 at temperatures of 25 to 80 °C, was the species with the major impact on food processing, as it was widespread with a strong spoiling potential and had been detected in several spoiled commercial pasteurized fruit juices (Walker and Phillips, 2005). Except for *A. acidoterrestris*, *Alicyclobacillus acidiphilus*, another conventional species of *alicyclobacilli*, was isolated from acidic beverages, which is a thermoacidophilic, non-pathogenic and spore-forming bacterium with the odour of guaiacol (Matsubara et al., 2002). So far, many techniques, such as high hydrostatic pressure, high pressure of homogenization, and radiations etc., have been used to control the contamination of *Alicyclobacilli* (Bevilacqua et al., 2008). The physicochemical characteristics of food products have significant influence on ultrasonic inactivation of microbe (Piyasena et al., 2003). However, few papers were published on the inactivation of the bacteria exposed to ultrasound except the report by Yuan et al. (2009) who investigated the effect of ultrasonic treatments on *A. acidoterrestris* in apple juice and the changes of the juice quality. Not enough is known about kinetic models of ultrasonic inactivation of the microbe.

Therefore, in this study, *A. acidiphilus* DSM14558^T and *A. acidoterrestris* DSM3922^T in apple juice were taken as targeted microorganisms. The objectives of this study were (1) to obtain the inactivation curves of the targeted microorganisms inoculated into apple juice exposed to the ultrasound, (2) to fit these inactivation curves into four different models (Weibull, log-logistic, modified Gompertz equation and biphasic linear model), (3) to select the best model based on the adjusted correlation coefficient (adj-R^2) and the root mean square error (*RMSE*).

2. Materials and methods

2.1. Cultivation of microorganisms

A. acidiphilus DSM14558^T and *A. acidoterrestris* DSM3922^T (provided by Agricultural Culture Collection of China, Beijing, China) were used as the test microorganisms in this study. They were cultivated in the BAM broth, described by Yamazaki et al. (2000), at 45 °C for 20–24 h to obtain cells in the early stationary growth phase. Based on a preliminary experimentation, 50 mL of DSM14558^T or DSM3922^T subculture was inoculated into 450 mL of sterilized apple juice (the apple juice with pH 4.03 and 10.9 °Brix was prepared and autoclaved in our laboratory) and mixed evenly. The initial number of microorganisms ranged from 1.91×10^5 to 5.82×10^5 CFU/mL in the apple juice.

2.2. Ultrasound equipment and treatment process

The sonication treatments were conducted using an improved intermittent JY92-II ultrasonic cell pulverizer (Scienta Biotechnology Co., Ltd., Ningbo, Zhejiang Province, China) equipped with a diameter 6 mm of amplifier pole, operating at frequency of 25 kHz. The suspensions of microbial cells were sonicated in a cylindrical reactor, and the work time and intermittent time were all set as 3 s. Temperature control during ultrasound experiments was achieved by using a thermostatic circulator to maintain the temperature of cell suspension below 50 °C, which was not a sublethal temperature for the bacterium.

The microbial cells inoculated into apple juice were treated under ultrasound at different power levels (200, 400 and 600 W) for different duration of exposure (1, 5, 10, 20 and 30 min). After the ultrasonic treatments, samples were taken out, cooled down to room temperature and immediately assayed for the change of microbial number. The cell suspension without ultrasound treatment was regarded as control samples and all experiments were at least in triplicate.

2.3. Enumeration of viable cells

The number of viable cells before and after ultrasonic treatments was determined with the plate count method. Each sample (1.0 mL) before and after the ultrasonic treatments was serially diluted with 9 mL of aseptic 0.85% NaCl solution. The pH values of diluted samples were adjusted to 4.0. And then each of two aseptic BAM plates was evenly plated with 1.0 mL of diluted samples. Finally the plates were incubated at 45 °C for 48 h before enumeration.

2.4. Mathematical models and their assessment

Four commonly used mathematical models (Table 1) were adopted to analyze the ultrasonic inactivation data of *A. acidiphilus* DSM14558^T and *A. acidoterrestris* DSM3922^T by means of the Microcal Origin 7.5 (Microcal Software, Inc., Northampton, MA, USA). In general, the statistical correlation parameter, R^2, of a model can measure how well a linear or a nonlinear model fit the experimental data. However, this statistical parameter is susceptible to the model structure, the number of observations (n) and the number of parameters (p), accordingly, an adjusted R^2 (adj-R^2) was used to compare different nonlinear models, making allowance for the number of observations and parameters (Diels et al., 2003):

$$Adjusted\,R^2 = 1-\left(\frac{(n-i)(1-r^2)}{(n-p)}\right) \quad (1)$$

where, i is an indicator variable that is 1 if the model includes an intercept, and 0 otherwise.

In addition, the root mean square error (*RMSE*) measures the average deviation between the observed and the fitted values, which was used to evaluate the performance of model (Diels et al., 2003).

$$RMSE = \sqrt{\frac{(observed-predicted)^2}{n-1}} \quad (2)$$

2.5. Statistical analysis

The logarithmic survival ratio of microbe was evaluated with log N/N_0 (N is CFU after treatment, N_0 is initial CFU) and expressed as mean and standard deviations (SD). Analysis of variance (ANOVA) was carried out with the software MicroCal Origin 7.5 (Microcal Software, Inc., Northampton, USA). ANOVA test was based on a significance level of 95% ($p<0.05$).

Table 1
Mathematical models given by various authors for modeling the inactivation.

No.	Model name	Model	References
1	Weibull model	$\log\frac{N}{N_0} = -bt^n$	Peleg and Cole (1998)
2	Log-logistic model	$\log\frac{N}{N_0} = \frac{A}{1+e^{4\sigma(\tau-\log t)/A}} - \frac{A}{1+e^{4\sigma(\tau-\log t_0)/A}}$	Chen and Hoover (2003)
3	Modified Gompertz	$\log\frac{N}{N_0} = Ce^{-e^{BM}} - Ce^{-e^{-B(t-M)}}$	Xiong et al. (1999)
4	Biphasic linear model	$\log\frac{N}{N_0} = \log[ae^{-k_1 t} + (1-a)e^{-k_2 t}]$	Peleg (2006)