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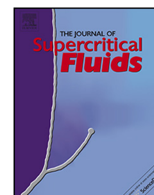

Validation of a mathematical model for predicting high pressure carbon dioxide inactivation kinetics of *Escherichia coli* spiked on fresh cut carrot

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ABSTRACT

Inactivation kinetics of *Escherichia coli* spiked on fresh cut carrot and exposed to high pressure carbon dioxide (HPCD) treatment at several conditions of pressure (6, 8, 10, and 12 MPa) and two conditions of temperature (26 and 35 °C) were obtained as a function of the treatment time (up to 30 min).

The Weibull model was applied to fit the inactivation kinetics and calculate δ and n model parameters for each pressure and temperature. The results demonstrated that the model was able to fit with good agreement the inactivation curves (high R^2 and low RMSE values). In a second attempt, the model parameters were correlated with CO_2 density resulting in a linear relationship. Validation of the proposed model was also performed at 6.6 and 10 MPa, 26 °C and at 8 MPa, 35 °C providing log reduction residual values (observed value–predicted value) lower than 0.50 and showing a good agreement between the experimental and the predicted inactivation data.

The model proved to be a powerful tool to fit and predict, in the proposed operative range, the inactivation kinetics of *E. coli* spiked on fresh cut carrot treated by HPCD. The results demonstrated the potential of a relative simple correlative model for the interpretation of the inactivation data and for HPCD process design and optimization.

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1. Introduction

High pressure carbon dioxide (HPCD) is receiving a high resonance as an innovative nonthermal food preservation technology. Since the 1980s it has been increasingly investigated as a promising technique to induce a pasteurizing/sterilizing effect when applied both to solid and liquid matrixes [1–3]. HPCD preservation method provides several advantages. Carbon dioxide (CO_2) used in this process is not only a powerful solvent for a wide range of compounds of interest in food processing, but is relatively inert, inexpensive, nontoxic, nonflammable, recyclable and readily available in high purity leaving no residues when removed after the process [4]. Furthermore, it is considered a Generally Recognized as Safe (GRAS) substance, which means it can be used in food products. Several papers demonstrated that HPCD is able to inactivate the natural microbial flora but also pathogens spiked on the products when the treatment is performed in batch, semi-batch or continuous equipments as showed by the inactivation kinetics described as a function of pressure, temperature and treatment time [5–7].

Traditionally, inactivation studies (above all thermal processing) employed first order kinetic parameters (D values and z values) to describe microbial survival count reduction [8]. However, there has been growing evidence that inactivation of microorganisms may not follow inactivation kinetics, especially for inactivation with nonthermal processing methods [9,10].

To describe non-linear inactivation kinetics, several models, such as Weibull, modified Gompertz, biphasic linear, and log-logistic models have been proposed and used to fit non-linear inactivation data of several microorganisms for inactivation by heat, high pressure processing or pulsed electric field [11,12]. Several theories have been proposed to explain the non-linearity of the inactivation kinetics. The most accredited considered the survival curve as the cumulative form of a temporal distribution of lethal events [13,14]. According to this concept, each individual microorganism is inactivated at a specific time. Because there is a spectrum of resistances to the treatment in the microbial population and some microorganisms are destroyed sooner or later than others, the shape of the survival curve is determined by a distribution of their sensitivities to the treatment. Thus, semi-logarithmic survival curves whether linear or with an upward or downward concavity are only reflections of treatment resistance distributions having a different mode, variance, and skewness, and not of mortality kinetics of different orders.

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In HPCD treatment, some studies applied mathematical models to describe microbial inactivation kinetics above all for liquid substrates: the modified Gompertz model was able to fit with good agreement the inactivation kinetics of *Escherichia coli* and *Saccharomyces cerevisiae* in liquid substrates [15,16]. A study of Erkmen [17] tested the reliability of several models (modified Whiting and Buchanan, Schnute, Richards, Stannard, Whiting and Buchanan, Gompertz, and Logistic) demonstrating that three-parameter models were statistically sufficient to describe the inactivation of *Listeria monocytogenes* in brain heart infusion broth under HPCD.

As regards solid substrates, accurate description and modeling studies of microbial inactivation kinetics for a better understanding of the process are still missing. This work was therefore undertaken to examine the response of *E. coli* spiked on fresh cut carrot treated by HPCD. The Weibull model was applied to describe the inactivation kinetics at different pressures (6, 8, 10, and 12 MPa), two temperatures (26 and 35 °C) and treatment times up to 30 min in order to obtain model parameters profiles as a function of CO_2 density. Treatment times longer than 30 were not taken into account for practical reason: in view of a possible exploitation of the technique at industrial scale, a pasteurization treatment longer than 30 min would not be feasible and most probably deleterious for the product.

2. Materials and methods

2.1. Bacterial strain and growth conditions

E. coli ATCC 25922 (DSMZ, Braunschweig, Germany) was grown on solid Luria–Bertani (LB) agar medium (Sigma–Aldrich Co., Milan, Italy) at 37 °C for 16 h. One colony was picked up and inoculated into 10 mL of LB medium. Bacterial culture was incubated at 37 °C with constant shaking (200 rpm) to stationary phase (16 h). Cells were collected by centrifugation at 6000 rpm for 10 min and re-suspended in 5 mL of phosphate buffered saline solution (PBS, Sigma–Aldrich Co., Milan, Italy) to reach a final concentration of 10^9 CFU/mL.

2.2. Fresh cut carrot contamination

Carrots (*Daucus carota L.*) were purchased from a local market, cut in 2 grams and spiked with 50 μL of *E. coli* with a concentration of 10^8 CFU/g. The samples were left 1 h in a sterile chamber at room temperature to let the microbial suspension absorb on the surface of fresh cut carrot and then were loaded in a SC–CO_2 multi-batch apparatus.

2.3. Experimental apparatus

SC–CO_2 treatment was performed in a multi-batch apparatus as described by Mantoan and Spilimbergo [18]. Briefly, the system consisted of 10 identical 15 mL-capacity reactors operating in parallel. All reactors were immersed in the same temperature-controlled water bath to maintain the desired temperature constant throughout the process and were connected to an on–off valve for independent depressurization at different treatment times. The solid samples spiked with *E. coli* were loaded into the reactors and pressurized with CO_2. The pump allowed the pressurization of the system with a rate of about 6 MPa/min. The operating parameters (temperature and pressure) were continuously recorded by a real time acquisition data system (NATIONAL INSTRUMENTS, field point FP-1000 RS 232/RS 485) and monitored by a specific software (LabVIEW™ 5.0). The process conditions tested were: 6–12 MPa at 26 and 35 °C with treatment times changing from 2 up to 30 min. Carbon dioxide physical state at temperatures and pressures investigated in the experimentation was shown in Fig. 1.

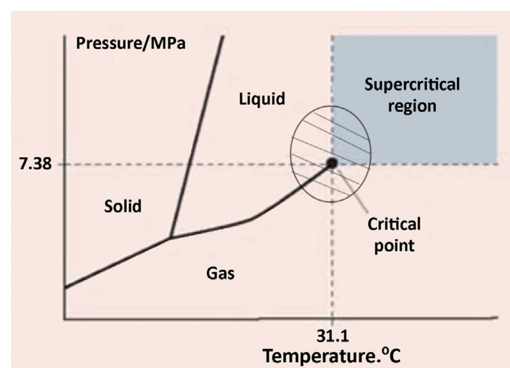

Fig. 1. Qualitative CO_2 *P–T* phase diagram: the temperatures and pressures investigated in the experimentation are indicated by the dashed area.

After the treatment slow depressurization of the reactors over approximately 1 min occurred.

2.4. Microbial analysis

The standard plate count technique was used to determine the initial microbial concentration and the efficiency of the treatment in reducing the number of the microorganisms spread on the surface of the samples. After HPCD, the samples were homogenized (1:2) with a phosphate buffer solution (PBS) in a Stomacher 400 (International P.B.I., Milano, Italy) at 230 rpm for 2 min. The homogenate was serially diluted in PBS and plated in duplicate on chromatic coli/coliform selective agar (Liofilchem, TE, Italy) at 37 °C for 24 h. The inactivation degree was determined by evaluating the log(*N*) versus time, where *N* (CFU/g, colony forming unit per gram) is the number of survivors after the treatment. Three independent experiments were carried out for each single experimental condition and the results were reported as mean values and standard deviations.

2.5. The inactivation model

In this paper, the GinaFIT® software [19] was used for carrying out a preliminary model discrimination among the models available in literature and once identified the one able to fit the inactivation kinetics the simulation and parameter estimation was performed. The microbial inactivation model used to fit with good agreement the inactivation data was the Weibull model [20], described by the equation:

$$\log(N) = \log(N|_{t=0}) - \left(\frac{t}{\delta}\right)^n \quad (1)$$

where n [−] indicates the concavity ($n < 0$) or convexity ($n > 0$) of the inactivation curve (if $n = 1$, the Weibull model reduces to the first-order model), δ is the time required to obtain the first bacteria log reduction [min]. Further, N represents the microbial concentration at treatment time equal to t and $N|_{t=0}$ corresponds to the number of colonies forming units per gram [CFU/g] measured in the untreated sample both expressed as (CFU/g). To identify the model equation, three parameters are required: n, δ and $N|_{t=0}$ as reported in GinaFIT tutorials. The calculation of R^2 and RMSE values was used to evaluate the goodness of model fitting [21,22].

3. Results and discussion

3.1. E. coli survival data

Inactivation kinetics of *E. coli* spiked on fresh cut carrot and treated by HPCD at 6, 8, and 12 MPa at 26 and 6, 10, 12 MPa at 35 °C

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