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Use of the Weibull model for lactococcal bacteriophage inactivation by high hydrostatic pressure

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Abstract

Four lactococcal bacteriophages (ϕLl6-2, ϕLl35-6, ϕLd66-36 and ϕLd67-42) in M17 broth were pressurized at 300 and 350 MPa at room temperature and their survival curves were determined at various time intervals. Tailing (monotonic upward concavity) was observed in all survival curves. The resulting non-linear semi-logarithmic survival curves were described by the Weibull model and goodness of fit of this model was investigated. Regression coefficients (R^2), root mean square error (RMSE), residual and correlation plots strongly suggested that Weibull model produced a better fit to the data than the traditional linear model. Hazard plots suggested that the Weibull model was fully appropriate for the data being analyzed. These results have confirmed that the Weibull model, which is mostly utilized to describe the inactivation of bacterial cells or spores by heat and pressure, could be successfully used in describing the lactococcal bacteriophage inactivation by high hydrostatic pressure.

Keywords: Lactococcal bacteriophages; High hydrostatic pressure; Weibull; Linear model

1. Introduction

Traditionally, microbial mortality has been treated as a process that follows first-order kinetics, that is,

$$\frac{dN(t)}{dt} = -k'N(t) \quad (1)$$

If Eq. (1) is integrated, we get

$$N(t) = N_0 \exp(-k't) \quad (2)$$

or

$$\ln\left(\frac{N(t)}{N_0}\right) = -k'(t) \quad (3)$$

where $N(t)$ and N_0 are the number of survivors after an exposure time t and initial number of microorganisms (microbial cells or viruses) and spores (cfu/ml or in case of viruses pfu/ml), respectively, and k' is a temperature-dependent rate constant. Since microbial inactivation usually results in several orders of magnitude reduction in the population size, it has been customarily described in terms of base-10 logarithms (Peleg, 2003):

$$\log_{10} S(t) = -kt \quad (4)$$

or

$$\log_{10} S(t) = -\frac{t}{D} \quad (t \geq 0) \quad (5)$$

where $S(t)$ is the survival ratio, i.e., $S(t)=N(t)/N_0$, $k=k'/\ln 10$ and D is the D-value or the decimal reduction time in minutes (time required for one log reduction in the number of microorganisms) (Peleg, 2000; Buzrul and Alpas, 2004).

The traditional first-order model assumes that all the organisms in a population have independent time and same (equal) probability of mortality. The question is now whether or not that is a reasonable assumption. A single organism is either alive or dead because of a lethal cause such as heat or high pressure. Such a lethal event is considered to be a probabilistic event, in other words, it is unlikely that all the organisms behave in the same way and that the death of a single cell is due to one single event, as is assumed in the traditional approach. Rather, inactivation time varies to some extent for each

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microorganism in a population, even if the population is pure. Thus, there is biological variation or heterogeneity between the cells of a population of the same microorganism. Consequently, the survival curve can be seen as a cumulative form of underlying distribution of individual inactivation times (van Boekel, 2002; Buzrul et al., 2005a).

Although there are organisms and bacterial spores that have linear or approximately linear semi-logarithmic survival curves under certain conditions, there is growing evidence that microbial semi-logarithmic survival curves need not necessarily be linear and in most cases they are not. An alternative survival model is the one based on the assumption that the organism's or spore's resistances (to lethal treatments like heat or pressure) have a Weibull distribution (Peleg et al., 2003):

$$\log_{10} S(t) = -b(T,P)t^{n(T,P)} \quad (6)$$

where $b(T,P)$ and $n(T,P)$ are temperature- (T) and pressure (P)-dependent coefficients, namely, scale (b) and shape (n) factors, respectively.

Such a model presents the main advantage of remaining very simple and being sufficiently robust to describe both monotonic downward concave (shoulder) survival curves ($n>1$) and monotonic upward concave (tailing) survival curves ($n<1$). Obviously, the model includes the traditional case where the survival curve is linear ($n=1$) (Mafart et al., 2002; Buzrul et al., 2005a).

High hydrostatic pressure (HHP) has been proposed as an alternative technique to thermal processing to destroy foodborne organisms in order to enhance safety and shelf life of at least some foods (Alpas et al., 2000; Alpas et al., 2003; Buzrul et al., 2005b,c). HHP is a three-variable process consisting of pressure, time and temperature. For effective use of this method in food preservation, it is necessary to study the interaction of these factors and determine the minimum conditions to obtain desirable levels of microbial destruction while maintaining a maximum degree of sensory and nutritional quality (Alpas et al., 1998; Buzrul and Alpas, 2004; Buzrul et al., 2005a).

Bacteriophages are the most serious threat for dairy fermentations among which are the lactococcal bacteriophages (Daly et al., 1996). Technological and biochemical functions of the starter cultures are severely affected when the fermentation environment is contaminated by phages. By the inhibition of the starter culture activity, reduction (slow-vats) or total failure (dead-vats) of acid production occurs (Josephsen and Neve, 1998). Therefore, bacteriophages must be removed from the processing environment. For this purpose, there are several attempts, which include improved fermentation process technology and the use of molecular biology tools (Konings et al., 2000).

HHP has been used for the inactivation of viruses and bacteriophages. Kingsley et al. (2002) studied the inactivation of Hepatitis A virus (HAV), poliovirus and Norwalk-like virus (NLV). After 5 min application, while HAV and NLV were inactivated at pressures of 450 and 275 MPa, respectively, poliovirus was unaffected at pressures up to 600 MPa. The inactivation of non-foodborne human pathogenic virus, human immunodeficiency virus (HIV) has also been studied at a pressure range of 400–600 MPa for 10 min and a reduction of 4–5 $\log_{10}$ cycles was reported (Otake et al., 1997). It has also been suggested that the studies on HHP processing of dairy products, especially on bacteriophage inactivation, could provide important information to the dairy industry (Ray et al., 2001).

Although there exist some modeling studies related with viruses (Noël et al., 2001; Tuckwell and Wan, 2004), there is only one study related with phage inactivation by HHP and non-linear models in literature (Chen et al., 2004). Therefore, the objective of this study is to present an example of how such a method (Weibull model) can be implemented in the analysis of lactococcal bacteriophage inactivation by HHP, where inactivation does not follow first-order kinetics, as has been generally assumed.

2. Materials and methods

2.1. Bacterial strains, bacteriophages and media

Bacterial strains and their lytic phages were obtained from Ankara University Culture Collection (Ankara, Turkey). Four strains of *Lactococcus lactis* subsp. *lactis* were grown and maintained in M17 broth (Oxoid Ltd., England) at 30 °C (Terzaghi and Sandine, 1975). Bacterial strains were kept in M17 broth containing 40% glycerol at 4 °C as stocks. The phages (ϕLl6-2, ϕLl35-6, ϕLd66-36 and ϕLd67-42) differing in their host specificities were propagated in M17 broth with host organisms at 30 °C with the addition of 1 M $CaCl_2$. Phage stocks were prepared from single plaques and kept in M17 broth containing 40% glycerol at 4°C.

2.2. HHP treatment of bacteriophages

Prior to pressure treatment, high titer (10^6–10^7 pfu/ml) bacteriophage suspensions in M17 broth were centrifuged at 6000 rpm for 15 min in a centrifuge (Beckman, USA) to separate the bacterial lyzates in the suspension and filtered through 0.45-μm pore size syringe filters (Corning, Germany). The phage suspensions were dispensed in 2-ml portions in sterile plastic cryovials (Sterilin, UK) in duplicate. HHP treatments of bacteriophage suspensions were performed by using a designed and constructed lab-scale unit (capacity: 30 cm^3, maximum P: 500 MPa). The time of pressure increase and pressure release was approximately 5–10 s for the designed system. A mixture of deionized water and glycol was used as the pressure-transmitting medium. The equipment consists of a pressure chamber of cylindrical design, two end closures, a means for restraining the end closures, a pressure pump and a hydraulic unit to generate high pressure for system compression and also a temperature control device. The pressure vessel was made of hot galvanized carbon steel and piston was hard chrome plated and polished to mirror finish (steel-type heat-treated special K) which was processed into the required sizes at Electrical and Electronic Engineering Department of Middle East Technical University, Ankara, Turkey. The liquid was heated prior to pressurization to the desired temperature by an electrical heating system surrounding the chamber. Pressuriza-