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# Evaluation of the Gauss-Eyring model to predict thermal inactivation of micro-organisms at short holding times

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ABSTRACT

Application of mild (non)-thermal processing technologies have received considerable interest as alternative to thermal pasteurisation, because of its shorter holding time and lower temperature aiming for an improved product quality. To understand and develop these alternative technologies, like pulsed electric fields, a proper comparison between the conventional thermal and alternative process is necessary. Up to recent, no suitable models were available to predict the inactivation of micro-organisms by a thermal process at a chosen short holding time, due to non-linearity. The recently developed Gauss-Eyring model with two variables temperature and time has the properties to be a suitable model to apply for short holding times, and was tested for this purpose.

Therefore, this study aims to validate if the Gauss-Eyring model can be used to describe non-linear isothermal (a fixed temperature with varying holding time) and isotime (a fixed holding time with varying temperature) thermal inactivation data, and if it is a suitable model to predict the thermal inactivation as a function of temperature for short holding times.

Inactivation data of *Escherichia coli*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Salmonella* Senftenberg and *Saccharomyces cerevisiae* in orange juice were collected via isothermal and isotime inactivation kinetics. Survival of the tested micro-organisms was modelled with the Gauss-Eyring model, which contains three parameters $\sigma$, $Tr$ and $Z$. The transition of 'no inactivation' to 'inactivation' (i.e. the 'shoulder' in inactivation curves) can be characterised as the temperature-time ($T$,$t$) combination where $T = Tr - Z \cdot log_{10}(t)$, with $Tr$ as the reference temperature defined for 1 s treatment, $Z$ as the temperature needed for a 10-fold increase of decrease of the holding time $t$, and $\sigma$ as the temperature width of the distribution.

The Gauss-Eyring model fitted well to the experimental data, and revealed different sensitivity for the tested micro-organisms. Based on the parameter estimations, survival curves for the desired short holding times were predicted.

## 1. Introduction

Thermal pasteurisation of food products is widely used to eliminate a desired number of micro-organisms and to inactivate enzymes. In order to achieve a sufficient level of inactivation, the process has to be carried out at sufficient high temperatures and for a sufficient amount of time. Although inactivation of micro-organisms and enzymes increases with temperature and duration of the heating process, the use of high temperatures for a longer time, will also (partly) destroy compounds responsible for fresh flavour and nutrients in the product. Therefore, alternative milder preservation processes have received considerable interest over the last decades, as they allow processing at lower temperatures and/or shorter holding times compared to conventional thermal processes, aiming for an improved, yet safe, product quality. Alternative methods can be either optimised thermal processes, such as ohmic heating and microwave heating, or non-thermal processes, such as pulsed electric fields (PEF) and high hydrostatic pressure. These non-thermal techniques can both be used as single technology, or in a combination with mild heat, to optimise for microbial inactivation and quality retention. In an industrial setting, PEF processing is usually combined with mild heat to enhance inactivation, while impact on product attributes is limited (Raso et al., 2014; Timmermans et al., 2014).

To better understand the electroporation and/or thermal effect on the microbial inactivation in a combined process of PEF and mild heat, a comparison to a conventional thermal process is important. However,

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comparison of the two processes is difficult, or not feasible, as different set-ups and time scales are used to measure microbial inactivation kinetics. The most common method to measure thermal inactivation of a microbial population is based on isothermal kinetics. This method of isothermal kinetics is based on fast heating of a microbial sample to a desired temperature, followed by sampling at regular (holding) times, to enumerate to the number of survivors.

Contrary, in emerging technologies like continuous PEF processes, the microbial inactivation kinetics cannot be characterised by a holding time series, as the temperature holding section is absent. The PEF treated product is immediately cooled after leaving the treatment zone where electric pulses are received, at the same point where the highest temperature is reached. By the design of a continuous flow PEF process, kinetic inactivation series can be made by variations of electric field strength, duration of the pulse and/or number of pulses applied to the product, resulting in different maximum temperatures (Raso et al., 2014). As the residence time in the treatment chambers is fixed and no holding time is used, inactivation kinetics of a PEF treatment can be characterised as series with different maximum temperatures and a fixed 'holding' time. Often the intensity of the PEF treatment is expressed in electrical energy applied to the system, but this can be converted to maximum temperatures using the specific heat capacity of the matrix (Siemer et al., 2014; Timmermans et al., 2014).

For a proper comparison between the conventional thermal process and a PEF treatment combined with mild heat, temperature-time conditions should be equal for both processes. Since the temperature-time exposure in a PEF process involves a short time (~1 s) to leave the treatment chamber and enter the cooling section, an accurate estimation of the thermal inactivation at this temperature-time combination is essential.

It is therefore important to define a method and model to describe 'isotime' inactivation: a single holding time with varying temperatures. As a preparatory step we need to consider a two variable (temperature and time) thermodynamic model for inactivation to compare data that is obtained either as constant temperature or at fixed holding time.

Thermal inactivation kinetics is often described by first-order kinetic models, with parameters $D_T$ and $Z$. $D_T$, the decimal reduction time, is defined as the time needed to reduce the number of viable micro-organisms in suspension with a factor 10, at temperature $T$. The $Z$-value is defined as the change in temperature required to change $D_T$ by a factor of 10. The parameter $D_T$ has been widely used in the calculation of the efficacy of pasteurisation and sterilisation processes, conceivably because it is so simple (Peleg and Normand, 2004; Van Boekel, 2008). Although there are micro-organisms that show linear or approximately linear semi-logarithmic survival curves, most microbial survival curves are not linear in practice (Van Boekel, 2002).

In order to account for the usually observed non-linearity, (empirical) models have been proposed to describe curves with a shoulder, curves with a tail (or biphasic curves) or curves including both a shoulder and a tail (sigmoid curves). Examples of these models include the Weibull model (Peleg and Cole, 1998; Van Boekel, 2002), biphasic linear model (Cerf, 1977), biphasic logistic model (Whiting, 1993), log-normal distribution (Aragao et al., 2007), reparameterized Gompertz model (Den Besten et al., 2006) and Geeraerd model (Geeraerd et al., 2000). Although these non-linear models fit very well to the survival data, most of these kinetic models are kinetic models with time as a single variable, and not both temperature and time as variables. The temperature dependence of the $D_T$-value is analysed using secondary models (Arrhenius-like) to obtain a $Z$-value. Secondary models based on polynomials accounting for additional variables ($T$, pH, water activity) have been studied (Gil et al., 2016), but it is questionable if and how these models can be used to describe isotime inactivation, which requires extrapolation to very short holding times.

Recently, a thermodynamic model (Gauss-Eyring) for enzyme inactivation was presented, with model parameters reference temperature $Tr$ and $Z$-value that are directly linked to standard activation enthalpy and entropy values of proteins (Mastwijk et al., 2017). This model was also explored as global model for the microbial inactivation of *Listeria monocytogenes* exposed to combined heat and pH stress. Interestingly, this primary model expresses the inactivation kinetics as a function of both temperature and time. The characteristics of this model allow to handle data sets where inactivation is observed as a single holding time with temperature as a variable, i.e. to handle datasets where treatment time cannot be varied, and to make predictions for processes with very short holding times.

The objective of this study was to validate the Gauss-Eyring model to describe non-linear isothermal as well as 'isotime' thermal inactivation data of *Escherichia coli*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Salmonella* Senftenberg and *Saccharomyces cerevisiae*, and to predict the thermal inactivation of these five micro-organisms as a function of temperature at short, fixed holding times.

## 2. Material and methods

### 2.1. Micro-organisms and culture conditions

Fresh cultures of *Escherichia coli* ATCC 35218 were prepared by plating from frozen stock culture on TSB (Oxoid) agar plates. Plates were incubated overnight (o/n) at 37 °C. A single colony isolate was used to inoculate a 100 mL flask with 10 mL TSB broth and cultivated for 24 h at 20 °C in an Innova shaking incubator (180 rpm) (2nd o/n). From this culture, 200 μL was used to inoculate 19.8 mL fresh TSB broth supplemented with 1% glucose (Sigma-Aldrich) (100 mL flask) and incubated for 24 h at 20 °C and 180 rpm (3rd o/n). The cultivation of *Salmonella* Senftenberg ATCC 43845 was similar to the protocol above, for the other micro-organisms specific agar composition and different incubation temperatures for the first o/n cultivation were used: *Listeria monocytogenes* NV8 was incubated at 30 °C using BHI agar (Oxoid), *Lactobacillus plantarum* ATCC 14917 was incubated at 30 °C using MRS broth (Merck) and *Saccharomyces cerevisiae* CBS 1544 was incubated at 25 °C using glucose-peptone-yeast agar, containing 40 g glucose, 5 g peptone (Fluka) and 5 g yeast extract (Oxoid) per 1 L distilled water. 1% glucose was added to the medium during the third o/n cultivation to mimic sugar content in fruit juice, resulting in a reduction of the pH for *E. coli*, *S.* Senftenberg and *L. monocytogenes*, when compared to the pH of the medium without extra glucose (Table 1). After the 3rd o/n incubation, microbial cells were pelleted by centrifugation (4000 rpm, 5 min) at 20 °C. Pellet was resuspended in 20 mL sterile peptone physiological salt diluent (PSDF, Tritium) and centrifuged again at same conditions. This step was repeated once, and after the third centrifugation step, pellet was dissolved in 20 mL pasteurised (30 min kept at 98 °C) orange juice (Minute Maid, original) to a cell density of $10^7$–$10^8$ cfu/mL (yeast) or $10^8$–$10^9$ cfu/mL (bacteria).

### 2.2. Heat treatment

For the thermal treatment, 130 μL of the inoculated orange juice was transferred into a 200 μL glass capillary (1.6 mm diameter, 125 mm length, Blaubrand® Intramark, Wertheim, Germany). Both ends were

**Table 1**
Effect of 1% glucose addition on the pH of the medium during the third overnight incubation. $t = 0$ is the pH measured at the start of the overnight cultivation, when the glucose is added. $t = 24$ h is at the end of the third overnight cultivation.

| Species/strains used | pH at $t = 0$ h | pH at $t = 24$ h without 1% glucose | pH at $t = 24$ h with 1% glucose |
|---|---|---|---|
| *Escherichia coli* | 7.1 | 8.1 | 5.1 |
| *Lactobacillus plantarum* | 5.5 | 4.5 | 4.5 |
| *Listeria monocytogenes* | 7.3 | 5.9 | 5.1 |
| *Saccharomyces cerevisiae* | 5.0 | 4.5 | 4.5 |
| *Salmonella* Senftenberg | 7.1 | 8.2 | 5.1 |