XXI Congreso de Ecuaciones Diferenciales y Aplicaciones
XI Congreso de Matemática Aplicada
Ciudad Real, 21-25 septiembre 2009
(pp. 1–8)

Mathematical Models in Food Engineering

Ángel Manuel Ramos[1], Nadia Smith[2]

[1] *Dpto. Matemática Aplicada, Universidad Complutense de Madrid. E-mail:* angel@mat.ucm.es.
[2] *Dpto. Matemática Aplicada, Universidad Complutense de Madrid. E-mail:* nas.smith@mat.ucm.es.

Keywords: Modelling, Simulation, High pressure, Bacterial inactivation, Enzymatic inactivation

Abstract

Nowadays, in industrialized countries, food products that are frequently consumed, are processed in order to prolong their shelf life, to avoid as much as possible their decomposition, and to maintain or even improve their natural qualities such as flavor and color. Decomposition of food is mainly due to microorganisms and enzymes, since they are involved in the physical and chemical processes of transformation of food substances. At present, consumers look for minimally processed, additive-free food products that maintain their organoleptic properties. This has promoted the development of new technologies for food processing. One of these new emerging technologies is high hydrostatic pressure, as it has turned out to be very effective in prolonging the shelf life of foods without losing its properties. This work deals with the modelling and simulation of the effect of the combination of Thermal and High Pressure Processes, focussing on the inactivation that occurs during the process of certain enzymes and microorganisms that are harmful to food. We propose various mathematical models that study the behavior of these enzymes and microorganisms during and after the process.

1 Introduction

Many methods of food conservation are based on heat application. For classical thermal treatments the temperature is in a range from 60 to 120°C, and the processing time can vary from a few seconds to several minutes. The main aim of these processes is to inactivate microorganisms and enzymes that are harmful to food, in order to prolong its shelf life, to maintain or even to improve its natural qualities, and mainly to provide consumers with products in good conditions. The problem of processing food via thermal treatments is that it may loose a significant part of its nutritional and organoleptic properties. At present, consumers look for minimally processed, additive-free food products that maintain

their organoleptic properties. Therefore the development of new technologies with lower processing temperatures has increased notoriously in the past years.

One of the new emerging technologies in this field is the combination of thermal treatments (at moderate temperatures) with high hydrostatic pressure, thereby reducing the problems described above. Recent studies ([2],[8]) have proven that high pressure causes inactivation of enzymes and microorganisms in food, while leaving small molecules (such as flavor and vitamins) intact, and therefore not modifying significantly the organoleptic properties of the food.

Two principles underlie the effect of high pressure. Firstly, the principle of le Chatelier, according to which any phenomenon (phase transition, chemical reaction, chemical reactivity, change in molecular configuration) accompanied by a decrease in volume will be enhanced by pressure. Secondly, pressure is instantaneously and uniformly transmitted independent of the size and the geometry of the food. This is known as isostatic pressure.

Microorganisms include bacteria, funghi, archea and protists. Food spoilage and food-borne illnesses caused by microorganisms are mainly due to bacteria and funghi (yeasts and molds). Foods may be contaminated by microorganisms at any time during harvest, storage, processing, distribution, handling, or preparation. The effects of high pressure on microorganisms in food are determined by several factors such as the temperature during the pressure treatment, the food constituents, the physiological state of the microorganism and the dispersion medium. However, they mainly depend on the pressure level and the microorganism being analyzed ([1],[8]).

Enzymes are substances that act as catalysts in living organisms, regulating the rate at which chemical reactions proceed without themselves being altered in the process. Most chemical reactions that occur within all living organisms are regulated by enzymes. For this work we are going to focus on protein enzymes. A large protein enzyme molecule is composed of one or more amino acid chains. The amino acid sequence determines the characteristic folding patterns of the protein's structure, which is essential to enzyme specificity. If the enzyme is subjected to changes, such as fluctuations in temperature or pressure, the protein structure may lose its integrity (this is known as denaturation) and its enzymatic ability. The effects of high pressure on enzymes also depend strongly on the enzyme: some enzymes can be inactivated at room temperature by a few hundred MPa while others can withstand 1000 MPa. Because of the extreme pressure stability of some food quality enzymes, combined processes (e.g. pressure and temperature) might be necessary for enzyme inactivation at industrially relevant pressures ([4]).

2 Mathematical modelling of Microbial and Enzymatic Inactivation

Kinetic parameters and models are used for the development of food preservation processes to ensure safety. They also provide tools to compare the impact of different process technologies on reduction of microbial populations or enzymatic activity. In this section we present mathematical models and the parameters that describe Microbial and Enzymatical Inactivation due to the combination of thermal and high pressure treatments.

Notation

$A(t;T,P)$	Enzymatic activity at time t, for a process at constant pressure P and temperature T	
A_0	Enzymatic activity at time 0	
D	Decimal reduction time	[min]
$D_{T_{\text{ref}}},\ D_{P_{\text{ref}}}$	Decimal reduction time at reference temperature / pressure	[min]
E_a	Activation energy	[kJ/mol]
k	Inactivation rate	[min^{-1}]
$k_{T_{\text{ref}},P_{\text{ref}}}$	Inactivation rate at reference temperature and pressure	[min^{-1}]
$N(t;T,P)$	Microbial population at time t, for a process at constant pressure P and temperature T	[cfu/g]
N_0	Initial microbial population	[cfu/g]
$P,\ P_{\text{ref}}$	Pressure / Reference pressure	[MPa]
t	Time	[min]
$T,\ T_{\text{ref}}$	Temperature / Reference temperature	[K]
ΔV^*	Volume of activation	[cm^3/mol]
z_T	Temperature resistant coefficient	[K]
z_P	Pressure resistant coefficient	[MPa]

The traditional approach to describing changes in microbial populations as a function of time has used the first-order kinetic model[1]:

$$\begin{cases} \dfrac{\mathrm{d}N(t)}{\mathrm{d}t} = -kN(t), & t \geq 0, \\ N(0) = N_0, & \\ [\textbf{Solution} : N(t) = N_0 \exp(-kt)] & \end{cases} \tag{1}$$

where $N(t)$ is the microbial population at time t, N_0 is the initial microbial population and k is the *inactivation rate constant* [min^{-1}], also called *death velocity constant* in the case of microorganisms. The same model can be used to estimate the changes in the enzymatic activity as a function of time by changing $N(t)$ for $A(t)$, and N_0 for A_0.

We have found in the literature (e.g. [9]) another equation used very often to calculate changes of microbial population as a function of time:

$$\log\left(\frac{N(t)}{N_0}\right) = \frac{-t}{D}, \tag{2}$$

where D is the *decimal reduction time* [min], or time required for a 1-log-cycle[2] reduction in the microbial population.

[1] We have found in the literature higher-order models that describe changes in microbial populations as a function of time ([10]).

[2] A 1-log-cycle reduction is equivalent to reducing the population dividing it by ten. In the same way, a n log-cycle is equivalent to reducing the population dividing it by 10^n.

The kinetic parameters that have to be determined for each model are k and D (the relationship between k and D is $k = \frac{\ln(10)}{D}$, thereby it is possible to move from one model to the other), respectively. With these parameters we can describe the microbial population reduction (or the enzymatic activity) at constant pressure and temperature.

The following step is to take into account temperature and pressure dependence (to start we will study each dependence separately), completing the models in order to calculate the microbial population $N(t;T,P)$ (or the enzymatic activity $A(t;T,P)$) for time instant t, in a process at arbitrary constant temperature T and pressure P (in adequate ranges).

We will start by finding equations for $k(T)$ and $k(P)$, i.e. the effects of temperature and pressure on the inactivation rate, if we are following model (1). The temperature dependence is given by Arrhenius' equation:

$$k(T) = k_{T_{\text{ref}}} \exp\left(\left(\frac{-E_a}{R}\right)\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right), \tag{3}$$

where $k(T)$ [min^{-1}] is the inactivation rate for an arbitrary temperature T [K], T_{ref} [K] is a reference temperature, $k_{T_{\text{ref}}}$ [min^{-1}] is the inactivation rate at reference temperature, E_a is the activation energy[3] [J/mol] and $R = 8,314$ [J/(mol K)] is the universal gas constant. And the pressure dependence is given by the following equation (based on Eyring's equation):

$$k(P) = k_{P_{\text{ref}}} \exp\left(\frac{-\Delta V^*(P - P_{\text{ref}})}{RT}\right), \tag{4}$$

where $k(P)$ [min^{-1}] is the inactivation rate for an arbitrary pressure P [MPa], P_{ref} [K] is a reference pressure, $k_{P_{\text{ref}}}$ [min^{-1}] is the inactivation rate at reference pressure and ΔV^* is the volume of activation[4]. With equations (3) and (4) we can now construct models for $N(t;T)$ and $N(t;P)$, respectively, by coupling each equation with model (1).

The next step is to find an equation for $k(T,P)$, i.e. the influence of pressure and temperature (at the same time) on the inactivation rate. In [6] the authors propose the following equation (other possibilities may be found in the literature):

$$k(T,P) = k_{T_{\text{ref}},P_{\text{ref}}} \exp\left(-B\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right) \exp(-C(P - P_{\text{ref}})), \tag{5}$$

where $k(T,P)$ [min^{-1}] is the inactivation rate for temperature T [K] and pressure P [MPa], and $k_{T_{\text{ref}},P_{\text{ref}}} = k(T_{\text{ref}},P_{\text{ref}})[\text{min}^{-1}]$, B [K] and C [MPa] are kinetic constants that express the dependence of $k(T,P)$ on temperature and pressure. Therefore coupling model (1)

[3]Activation energy (chemistry): the minimum amount of energy that is required to activate atoms or molecules to a condition in which they can undergo chemical transformation or physical transport.

[4]The volume of activation is interpreted, according to transition state theory, as the difference between the partial molar volumes of the transition state (V) and the sums of the partial volumes of the reactants at the same temperature and pressure.

with equation (5) we get the complete model, as follows:

$$\begin{cases} k(T,P) = k_{T_{\text{ref}},P_{\text{ref}}} \exp\left(-B\left(\dfrac{1}{T}-\dfrac{1}{T_{\text{ref}}}\right) - C(P-P_{\text{ref}})\right), & T,P \text{ in adequate ranges,} \\ \dfrac{\mathrm{d}N(t;T,P)}{\mathrm{d}t} = -k(T,P)\ N(t;T,P), & t \geq 0, \\ N(0;T,P) = N_0, & \\ [\textbf{Solution} : N(t;T,P) = N_0\ \exp(-k(T,P)\ t)] & \end{cases} \tag{6}$$

for the microbial population, and by changing $N(t;T,P)$ for $A(t;T,P)$ and N_0 for A_0, we have the complete model for the enzymatic activity.

On the other hand, if we are following equation (2) (we remind that we have only seen this model for microbial inactivation, not for enzymatic inactivation but, as explained before, both models are equivalent), we will need to find equations for $D(T)$ and $D(P)$, which go as follows ([9]):

$$\log\left(\frac{D(T)}{D_{T_{\text{ref}}}}\right) = -\frac{T-T_{\text{ref}}}{z_T} \tag{7}$$

$$\log\left(\frac{D(P)}{D_{P_{\text{ref}}}}\right) = -\frac{P-P_{\text{ref}}}{z_P} \tag{8}$$

where z_T [K] (resp., z_P [MPa]) is the thermal (resp., pressure) resistance constant that can be defined as the temperature (resp., pressure) increase needed to accomplish a 1-log-cycle reduction in the decimal reduction time value D [min]; $D_{T_{\text{ref}}}$ (resp., $D_{P_{\text{ref}}}$) [min] is the reference decimal reduction time at reference temperature T_{ref} [K] (resp., reference pressure P_{ref} [MPa]) within the range of temperatures (resp., pressures) used to generate experimental data. Using equations (7) and (8), and coupling them with model (2), we can again construct models to estimate $N(t;T)$ and $N(t;P)$, respectively. In this case, we do not have a model for $N(t;T,P)$, as we have not found in the literature any equation for $D(T,P)$. We show the model for $N(t;P)$:

$$\begin{cases} D(P) = D_{P_{\text{ref}}} 10^{-\frac{P-P_{\text{ref}}}{z_P}}, & P \text{ in an adequate range,} \\ \dfrac{\mathrm{d}N(t;P)}{\mathrm{d}t} = -\dfrac{\ln(10)}{D(P)}\ N(t;P), & t \geq 0, \\ N(0;P) = N_0. & \\ [\textbf{Solution} : N(t;P) = N_0\ 10^{-\frac{t}{D_{P_{\text{ref}}}} 10^{\frac{P-P_{\text{ref}}}{z_P}}}] & \end{cases} \tag{9}$$

For this model, the kinetic parameters we have to determine are P_{ref}, $D_{P_{\text{ref}}}$ and z_P.

When model (1) coupled with equation (3) (or (4)), and model (2) coupled with equation (7) (or (8)) are applied to microbial population reduction rate data over the same temperature (resp., pressure) range, a relationship between the two coefficients E_a and z_T (resp., ΔV^* and z_P) can be obtained (we remind that at constant temperature and pressure, we have the following relationship between k and D: $k = \frac{\ln(10)}{D}$). The following relationships are satisfied:

$$E_a = \frac{\ln(10)RT^2}{z_T} \qquad \text{and} \qquad \Delta V^* = -\frac{\ln(10)RT}{z_P} \tag{10}$$

All the models studied until now predict microbial and enzymatic inactivation for isothermal and isobaric treatments, i.e. at the same temperature or pressure during the whole treatment. But this is not very realistic if we want to use these models for industrial applications. For example, in practical industrial processes, a pressure increase produces a temperature increase due to the generation of adiabatic heat. Our next aim is to present a mathematical model that allows to predict microbial inactivation (or enzymatic activity) after a dynamic process with pressure and temperature that change with time. This means that $k(T)$, $k(P)$, $k(T,P)$, $D(P)$ or $D(T)$ will not be constant anymore with respect to time, as they depend on temperature and pressure, which now depend on time. If $T(\cdot), P(\cdot) : [0, t_{\mathrm{f}}] \to \mathbb{R}$ are known functions, we can construct the complete model. For example, if we are considering model (6), we just have to change the constants T and P for the functions $T(t)$ and $P(t)$. Then, the solution is the following:

$$N(t; T(\cdot), P(\cdot)) = N_0 \ \exp\left[-\int_0^{t_{\mathrm{f}}} k(T(\tau), P(\tau)) \ \mathrm{d}\tau\right] \tag{11}$$

This model is useful to study the behavior of an enzyme or a microorganism after a dynamic treatment with known pressure and temperature profiles. The problem is that we do not always know a priori such profiles. A typical example is when we know approximately the pressure profile before the process but we do not know the temperature profile. Therefore, our last step is to create a model that can predict (under certain initial conditions) the temporal/spatial distribution of temperatures of the food sample under treatment. In order to do this, we can use a heat transfer model (see [5] and [7]) that is well adapted to the particular case under study. For instance, we can use the heat equation for a stationary, homogeneous and isotropic solid body with heat generation inside (in our case this heat is generated due to the pressure increase), that goes as follows:

$$\rho C_p \frac{\partial T}{\partial t} - \nabla \cdot (k \nabla T) = \beta \frac{\partial P}{\partial t} T, \qquad \text{in } \Omega \times (0, t_{\mathrm{f}}) \tag{12}$$

where Ω is a spatial domain that contains the food, ρ is the density [kg/m^3], C_p the specific heat [J/kg K], k the thermal conductivity [W/mK], β the thermal expansion coefficient [K^{-1}] and $P(t)$ describes the pressure [MPa] as a function of time. This equation has to be completed with suitable initial and boundary conditions (see [5]). Other heat transfer models can be used, which could be more complicated (e.g. adding Navier-Stokes equations) or simpler (e.g. changing the PDE system (12) for an ordinary differential equation). We can then write the complete coupled model as follows:

$$\begin{cases} \rho C_p \dfrac{\partial T}{\partial t} - \nabla \cdot (k \nabla T) = \beta \dfrac{\partial P}{\partial t} T, & \text{in } \Omega \times (0, t_{\mathrm{f}}) \\ \text{Boundary + Initial conditions} \\ k(T(\cdot), P(\cdot)) = k_{T_{\mathrm{ref}}, P_{\mathrm{ref}}} \exp\left(-B\left(\dfrac{1}{T(\cdot)} - \dfrac{1}{T_{\mathrm{ref}}}\right) - C(P(\cdot) - P_{\mathrm{ref}})\right), \\ \dfrac{\mathrm{d}N(t; T(\cdot), P(\cdot))}{\mathrm{d}t} = -k(T(\cdot), P(\cdot)) \ N(t; T(\cdot), P(\cdot)), & t \in [0, t_{\mathrm{f}}], \\ N(0; T(\cdot), P(\cdot)) = N_0, \\ T(0) = T_0. \end{cases} \tag{13}$$

3 Example of the modelling procedure

We are going to explain, using an example, the modelling procedure we follow when applying the theory to the practice. The procedure is the following:

- Experimental observation: We have obtained our data from [3]. The aim is to study the reduction of strain Lm.17 of *Listeria monocytogenes*[5] isolated from raw ham and inoculated in minced raw ham. The concentration of *listeria monocytogenes* is measured in [cfu/g][6]. All the experiments were done at the same temperature $T = 25°\text{C}$ (data of changes in temperature due to an increase of pressure were not recorded in the experiments, and therefore we suppose pressure and temperature are constante during the process), whence we will not be able to study the temperature dependence; however we do have experimental data at different pressures (500 and 600 MPa). With the experimental results we can build a table of values $(t_i, N(t_i))$ for certain processing times t_i [min].

- Mathematical modelling: This part has been explained all along Section 2. Specifically, we are going to use model (1) coupled with equation (4) and model (9).

- Kinetic parameter identification: Firstly, we consider the measurements done at the same pressure (and at the same temperature), therefore we follow model (1) and model (2). Using linear regression we identify the kinetic parameters k and D. Secondly, as we have data measured at different pressure values, we follow equation (4) and model (9) in order to find a formula to express the pressure dependence of $k(P)$ and $D(P)$. The parameters we identify are $\Delta V^* = -28.0389$ cm^3/mol, $P_{\text{ref}} = 550$ MPa, and $k_{P_{\text{ref}}} = 0.8818$ min^{-1} for $k(P)$; $z_P = 203.4609$ MPa, $P_{\text{ref}} = 550$ MPa and $D_{P_{\text{ref}}} = 2.6113$ min for $D(P)$. We do this again using linear regression.

- Model validation with experimental data: Once we have identified the kinetic parameters, we have to validate our model with experimental data. Ideally we should have enough data to be able to validate the model with values that have not been used to identify the parameters. However, this is not always the case, and for this specific example we had to validate the model with the same data values.

- Conclusions: With the experimental measurements of the concentration of strain Lm.17 of *listeria monocytogenes* at different pressures and different time instants, we can identify the kinetic parameters described above, which allows us to estimate the bacterial concentration $N(t;P)$ after t processed minutes at any pressure P (in an adequate range). In particular, the formulas we have determined for $N(t;P)$ are

$$N(t;P) = N_0 \ \exp^{-k_{P_{\text{ref}}} \exp\left(\frac{-\Delta V^*(P-P_{\text{ref}})}{RT}\right) t}, \tag{14}$$

$$N(t;P) = N_0 \ 10^{-\frac{t}{D_{P_{\text{ref}}}} \ 10^{\left(\frac{P-P_{\text{ref}}}{z_P}\right)}}, \tag{15}$$

[5] *Listeria monocytogenes* is a gram-positive, rod shaped bacterium commonly found in soil, water, sewage, plants and food. It is incredibly hardy and able to grow in a very broad range of temperatures. It is the bacterium responsable for listeriosis, a lethal food-borne infection.

[6] cfu: Colony forming unit

where $N_0 = 10^{6.8}$ [cfu/g], $R = 8.314$ [J/mol K] and $T = 298.15$ [K] (the rest of the values are given above). Clearly, the higher the pressure, the higher the inactivation rate $k(P)$ and the lower the decimal reduction time $D(P)$. We have also obtained a formula to calculate the required time to achieve a n-log-cycle reduction, that is

$$t(P, n) = n\ D_{P_{\text{ref}}}\ 10^{-\frac{P - P_{\text{ref}}}{z_P}}. \tag{16}$$

In http://www.mat.ucm.es/momat/software the most recent version of a program that performs the simulations of the models described in this work can be found.

Acknowledgements

This work has been done in the framework of the project MTM2008-04621/MTM of the Ministry of Science and Innovation (National Plan of I+D+i 2008-2011) and the Consolidation Project of Research Groups funded by the Banco Santander and the Universidad Complutense de Madrid (Ref. 910480). We also thank Mònica Gassiot Cordomí and Esteban Espuña, S.A. for the experimental data [3] and their collaboration.

Bibliography

[1] G.Arroyo, P.D.Sanz, G.Préstamo, Effect of high pressure on the reduction of microbial population in vegetables. *Journal of Applied Microbiology* 82. 1997. pp. 735-742.

[2] J.C.Cheftel, Review: high-pressure, microbial inactivation and food preservation. *Food Science Technology International* 1. 1995 pp. 75-90.

[3] Dr Secondo Gola, Resultados microbilógicos encargados por Esteban Espuña, S.A, a la Stazione Sperimentale per l'industria delle conserve alimentari in Parma. Parma, 5 Marzo 2004.

[4] M. Hendrickx, L. Ludikhuyze, I. Van den Broeck and C. Weemaes, Effects of high pressure on enzymes related to food quality. *Trends in Food Science and Technology* 9, 5. Elsevier, 1998 pp. 197-203.

[5] J.A. Infante, B. Ivorra, Á.M. Ramos and J.M. Rey: On the Modelling and Simulation of High Pressure Processes and Inactivation of Enzymes in Food Engineering. Article accepted for publication in *Mathematical Models and Methods in Applied Sciences (M3AS)*, ISSN: 0218-2025 (paper), ISSN: 1793-6314 (online).

[6] L.Ludikhuyze, I.Van den Broeck, C.Weemaes, C.Herremans, J.F. Van Impe, M.Hendrickx and P.Tobback, Kinetics for Isobaric-Isothermal Ianctivation od Bacillus subtilis α-Amylase. *Biotechnol. Prog.* , 1997, 13, pp. 523-538.

[7] L.Otero, Á.M.Ramos, C.de Elvira and P.D.Sanz, A Model to Design High-Pressure Processes Towards an Uniform Temperature Distribution. *Journal of Food Engineering*, ISSN: $0260 - 8774$, Vol 78 (2007), $1463 - 1470$. DOI link: http://dx.doi.org/10.1016/j.jfoodeng.2006.01.020.

[8] J.P.P.M.Smelt, Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology* 9, Elsevier, 1998 pp. 152-158.

[9] U.S. Food and Drug Administration. Center for Food Safety and Apllied Nutrition, *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies.* 2000.
(Web: http://www.cfsan.fda.gov/∼comm/ift-over-html)

[10] R.Xiong, G.Xie, A.E.Edmondson, M.A.Sheard, A mathematical model for bacterial inactivation. *International Journal of Food Microbiology* 46 (1999) pp. 45-55. Elsevier.