Challenge tests: Validation of a Thermal Treatment

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e-SafeFood
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Objectives

You will learn:

- How to employ the microbial kinetic parameters (obtained from a challenge test or from the literature) to validate a mild thermal treatment

- How to estimate the evolution of microbial concentration under a given temperature profile (i.e., dynamic conditions)

Materials needed:

- This presentation

- Accompanying spreadsheet “Validation – sausage smoking.xls”

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Contents

- Aspects of process validation
- Aspects of challenge testing
- Models for thermal treatment validation
    - Primary model: The loglinear microbial death model
    - Secondary model: The Arrhenius model
    - Tertiary model: the small time-interval approach
- Assessing the effectiveness of a mild thermal treatment (smoking)
    - An example for assessing the effectiveness of smoking to inactive *Salmonella* in raw sausages
    - Scenario 1: Lower initial contamination in sausages
    - Scenario 2: Higher temperature in the smoking chamber
- Conclusions

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Introduction

Process Validation

Documentation and Traceability

Provides a documented history of the microbiological processes, facilitating traceability

Consistency and Reproducibility

Produce repeatably and reliably the desired product within its specified design parameters.

Product Quality Assurance

Allows microbial risks to be controlled throughout the production process in a systematic manner.

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Challenge tests

- A microbial challenge test, is used to assess whether a product's preservation or antimicrobial systems are effective

- This test is commonly used to determine a product's ability to resist microbial contamination; and to assess the effectiveness of a thermal or non-thermal treatment to attain certain microbial reduction in the food

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Introduction

Aspects of a challenge test

Simulation

Simulates real-life conditions including factors such as temperature, pH, and other environmental conditions that may inhibit microorganisms growth

Methodology

Involves introducing a known quantity of microorganisms into the product and then monitoring the microbial population at regular intervals

Evaluation Criteria

Challenge tests are based on regulatory requirements or industry standards, and compared to predetermined acceptance criteria for microbial load samples in the product under test.

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Introduction

The challenge test can be used to evaluate the resistance of a microorganism to a thermal or non-thermal treatment

Results from a challenge test must be first analysed to extract microbial kinetic parameters, which can be later used for prediction and validation of thermal/non-thermal processes

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Thermal processes

Raw materials

Preparation

Thermal treatment

In the production of ready-to-eat (RTE) heat-treated food products, the thermal treatment stage is the most common critical control point (CCP) for controlling foodborne pathogens.

Is this step designed to eliminate the hazard or to reduce its occurrence to an acceptable level?

Yes

CCP

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Designing a challenge test for thermal treatment

Objective: Validating that the process effectively eliminates target microorganisms at a specified temperature and duration, or at given temperature profile.

Identify target Microorganism

Establish Inoculation Level

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Designing a challenge test for thermal treatment

Establish Inoculation Levels: Determine the initial microbial levels for the target microorganism. It is essential to maintain a balance between a high enough load to challenge the thermal treatment process but still within a reasonable and allowable range.

Identify Critical Process Parameters: Identify the critical process parameters (CPPs) that significantly influence the effectiveness of the thermal treatment.

Implement Monitoring and Measurement: Incorporate a robust monitoring system to measure critical process parameters during the challenge test.

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Calculating death rates based on microbial counts

Fit proper primary and secondary predictive microbiology models: The objective is to characterise how the death rate varies as a function of the temperature (and other relevant intrinsic or extrinsic factors)

Primary model for death:

The loglinear death model is the most common primary model for microbial death used when assessing thermal treatments

Secondary model for death:

The Bigelow model and the Arrhenius model are the most common secondary models used to describe the effect of temperature on microbial death rate

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Primary model for death

Loglinear death model: is the time required at certain temperature to reduce the microbial population in one log. It is a measure of thermal resistance.

$$log_{10} N(t) = log_{10} N(0) - kt$$

Decimal reduction time (D-Value): is the time required at certain temperature to reduce the microbial population in one log. It is a measure of thermal resistance, and is calculated as the reciprocal of the parameter k

$$\mathrm{D} = \frac{1}{K}$$

D values at different storage temperatures are needed to fit the secondary model for death.

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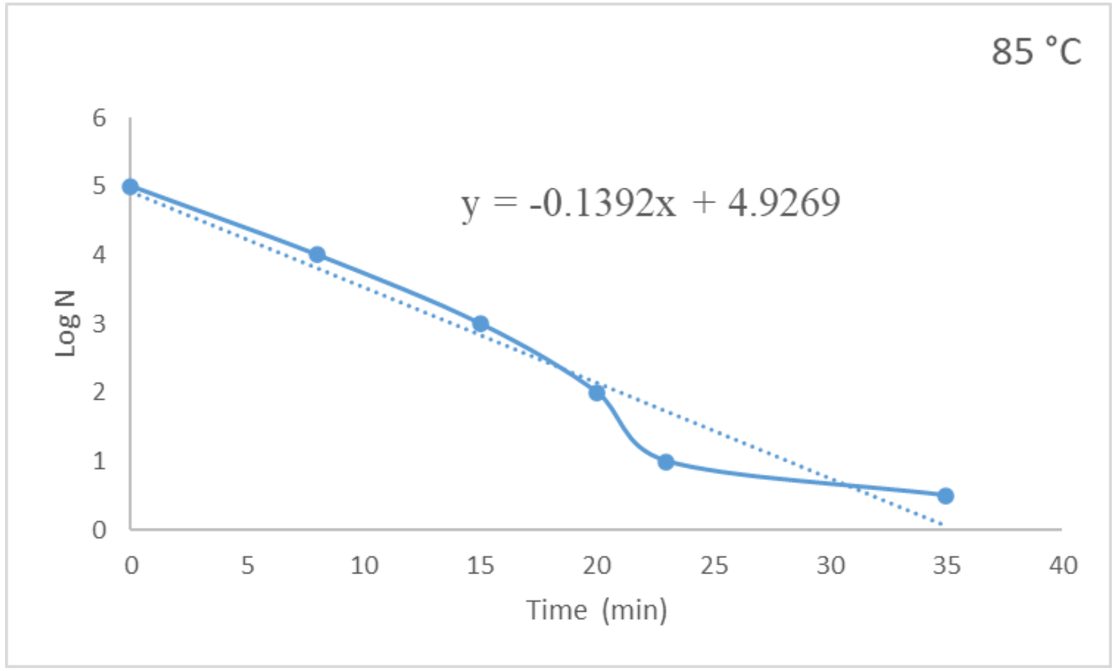
Primary model for death

The D-value of bacteria at each temperature can be calculated from the linear regression model for log10 of the surviving bacterial cells and heating time. The D-value is the negative inverse slope of the plot



$$D = \frac{1}{0.1392} = 7.18\ min$$

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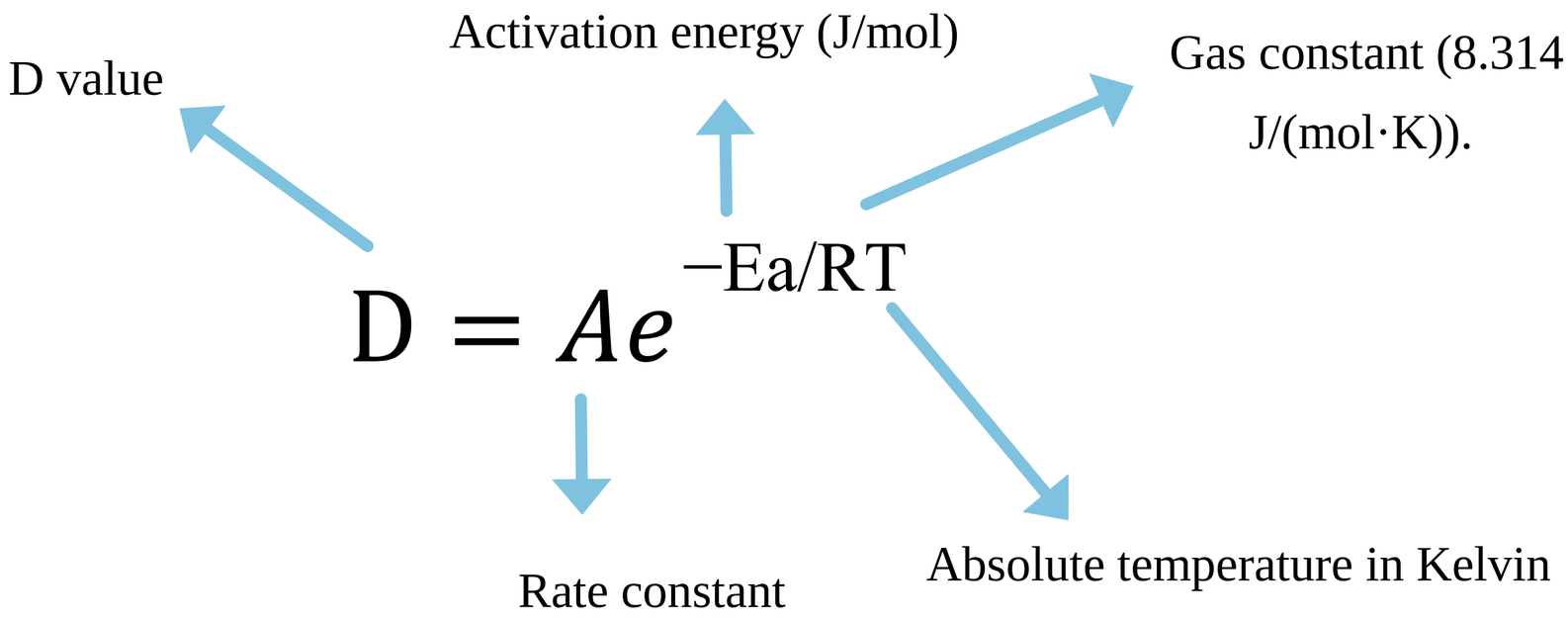
Secondary model for death

The Arrhenius equation is a mathematical formula that describes the temperature dependence of reaction rates. It can be applied to biological processes such as how heat affects the inactivation of bacteria. The Arrhenius equation is expressed as follows:



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Tertiary model

To predict the change in microbial concentration, as temperature increases (i.e., changes in time), the tertiary model must be solved. This can be done either by a differential equation or by the small time-interval technique

The solution of Y(t) evaluated at a temperature profile, T(t), could be approached as:

Microbial population at the previous time step (one step before n)

Time step: t_n- t_{n-1}

$$Y(t_n) = Y(t_{n-1}) - \frac{\Delta t}{D_{average}}$$

Predicted microbial population after time interval t_n

Average D over the time interval t_{n-1} and t_{n+1}

$$D_{average} = \frac{D(t_{n-1}) + D(t_{n+1})}{2}$$

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Example: Assessing the effectiveness of a mild thermal treatment (smoking) to inactivate *Salmonella* in raw sausages.

Sausages are smoked during 50 minutes in a chamber at air temperature of 65 ºC. We wish to know if the time is sufficient to reach a 7.0 log reduction of *Salmonella*.

Open the file “Validation – sausage smoking.xls”

Data on thermal inactivation of *Salmonella* in breaded pork patties was taken from Osaili et al (2007) assuming similar composition to raw sausages

- Osaili, T. M., Griffis C. L., Martin, E. M., Beard, B. L., Keener, A. E & Marcy, J. A. (2007). Thermal Inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in Breaded Pork Patties. *Journal of Food Science*, 72, 2.

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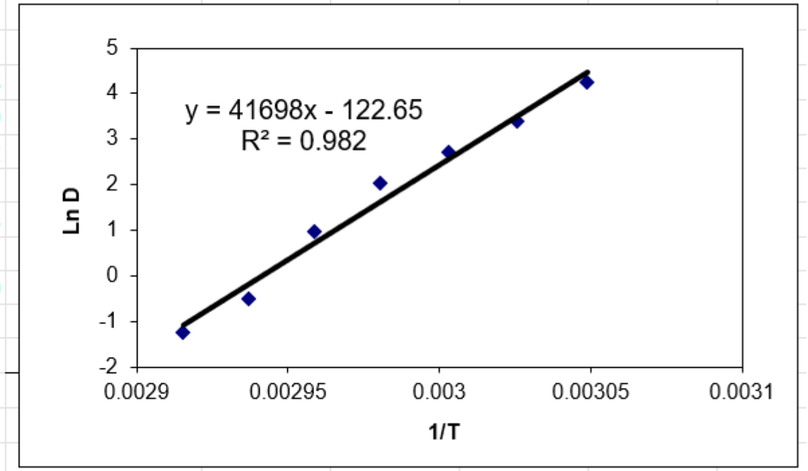
Initial Data:

Linearising Arrhenius equation: $\mathrm{Ln}\, D = \ln A - \frac{E_a}{R}\left(\frac{1}{T}\right)$

Temperature (ºC)	D (min)
55	69.48
57.5	29.99
60	15.2
62.5	7.71
65	2.64
67.5	0.61
70	0.29



Ln A and Ea/R are parameters extracted from the fitted line, now to be used in prediction mode for solving the tertiary model

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Step 1

In the excel sheet, the initial parameters **Y0** and **Air temperature** must be set. In addition, set the death kinetic parameters **Ln A** and **Ea/R**, previously estimated

Concentration pre-smoking Y0 (log CFU/g)	Air temperature in smoking chamber (ºC)	Ln A (1/min)	Ea/R
7	65 °C	-122.6	41698.09

The table below in the excel sheet is made to provide the predictions of Y(t) for the temperature profile (first and second column)

Microbial death to be solved in small time intervals				
Time (min)	Temperature (ºC)	D (min)	Y(t) (log10 CFU/g)	C(t) (log10 CFU/g)
0.000	25			
0.017	25.02			
0.100	25.16			
0.183	25.32			

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Step 2

Implement the two equations (1) and (2) in Excel to calculate death rate and microbial population

$$D = \exp\left(LnA - \frac{Ea}{R}\left(\frac{1}{T}\right)\right) \quad (1)$$

$$Y(t_n) = Y(t_{n-1}) - \frac{\Delta t}{D_{average}} \quad (2)$$

Implement the equation (1) if T > 55 °C

We can assume death rate=0 when T < 55 °C (assuming growth below this temperature is negligible)

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Step 2

$$D = \exp(LnA - \frac{Ea}{R}\left(\frac{1}{T}\right)) \qquad Y(t_n) = Y(t_{n-1}) - \frac{\Delta t}{D_{average}}$$

Microbial death to be solved in small time intervals				
Time (min)	Temperature (ºC)	D (min)	Y(t) (log10 CFU/g)	C(t) (log10 CFU/g)
7.6	62.96859442	8.273486556	6.868969668	7395536.22
7.6833333	63.09213091	7.904530738	6.858450083	7218551.902
7.7666667	63.20932289	7.56997756	6.847463967	7038238.292
7.85	63.32041199	7.266131311	6.83601687	6855148.546
7.9333333	63.42563644	6.9897553	6.824115627	6669843.238
8.0166667	63.52523077	6.738007818	6.811768249	6482883.981
8.1	63.61942538	6.508387856	6.798983825	6294827.377
8.1833333	63.70844627	6.298688982	6.785772404	6106219.395

Drag theses formula down for the subsequent rows

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Step 3

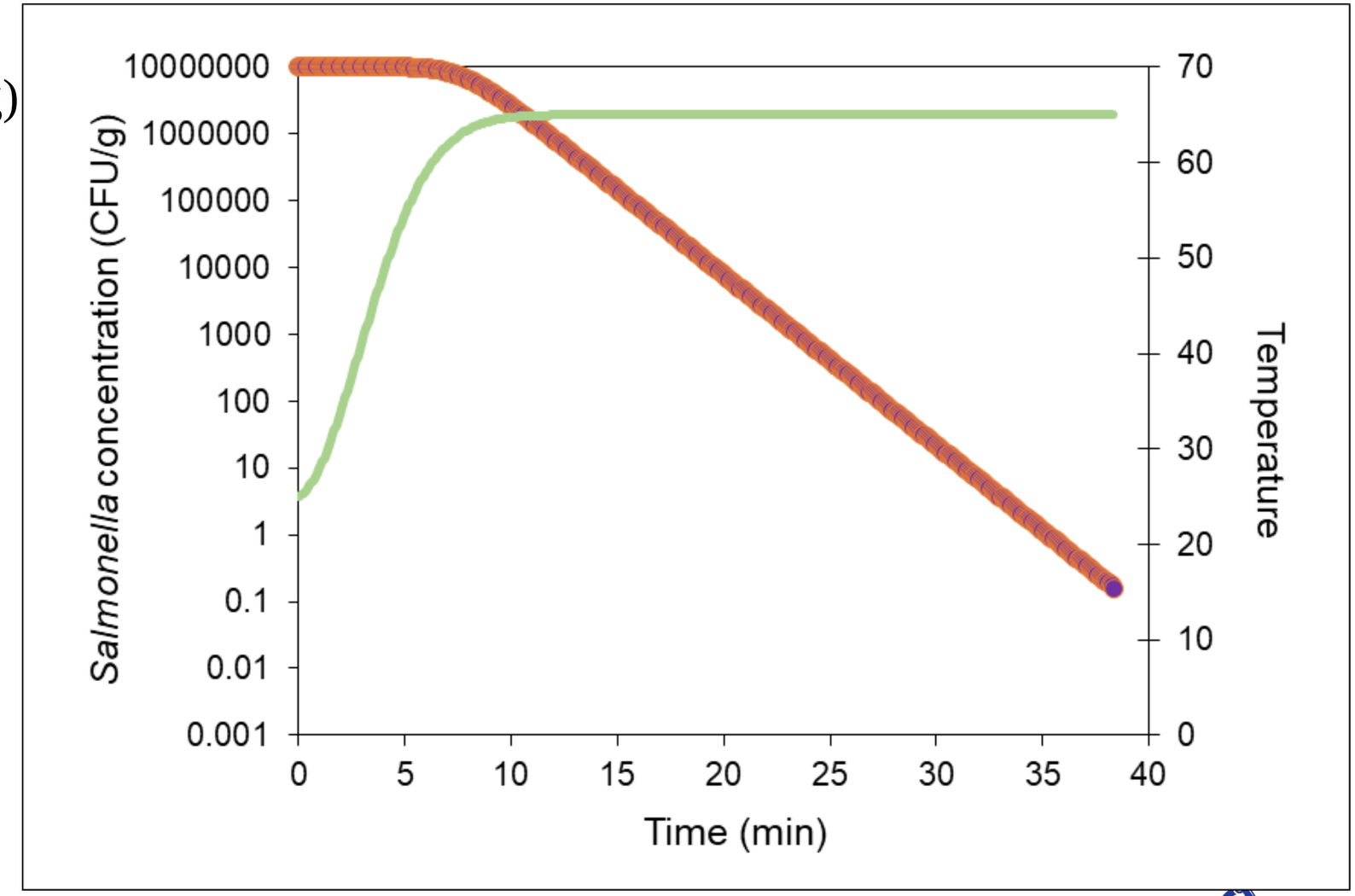
Results

In this scenario, *Salmonella* concentration pre-smoking
Y0 was assumed high (7.0 log CFU/g)

It produces a slower decline in population, assuming other factors remain constant.

Notice that a 7-log reduction is attained in ~35 min, therefore lower than 50 min.

The smoking process has been validated



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Other scenarios

•Let us now compare different scenarios by adjusting parameters such as the microbial concentration pre-smoking and the temperature of the smoking chamber

1) Using the same spreadsheet, simulate *Salmonella* inactivation over time until sausages reach a temperature of **65 °C,** now with an initial concentration of **2 log CFU/g**.

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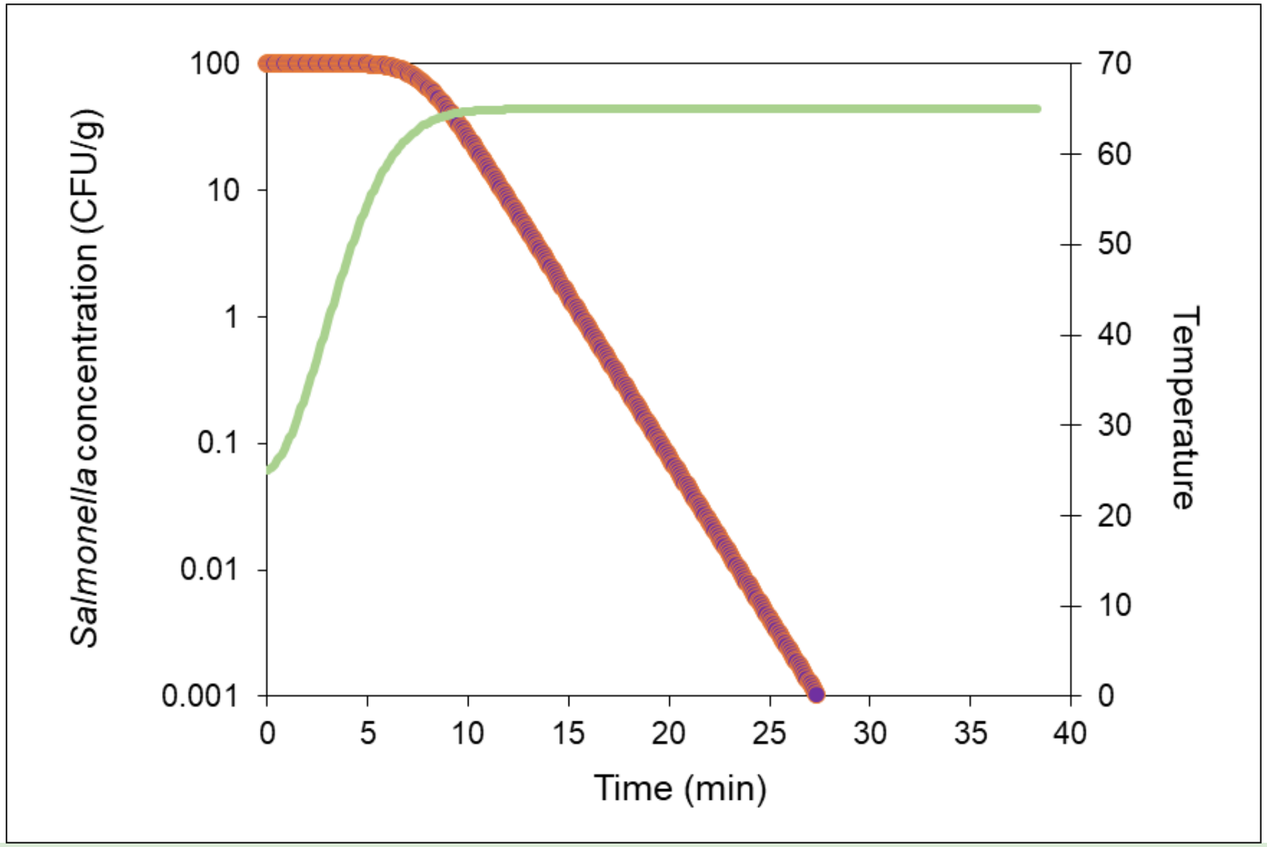
Other scenarios

Parameters used to solve the tertiary model for smoking

Y0 (log CFU/g)	LnA (1/min)	Ea (J/mol)
2	-122	41698.08898

Results



The initial inoculum represents the starting population of microorganisms before smoking. Now *Salmonella* population will die-off well before the end of smoking

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Other scenarios

2) Now imagine the smoking chamber temperature has changed to 75 ºC.

Simulate *Salmonella* inactivation in smoking sausages over time with an initial concentration of **2 log CFU/g**

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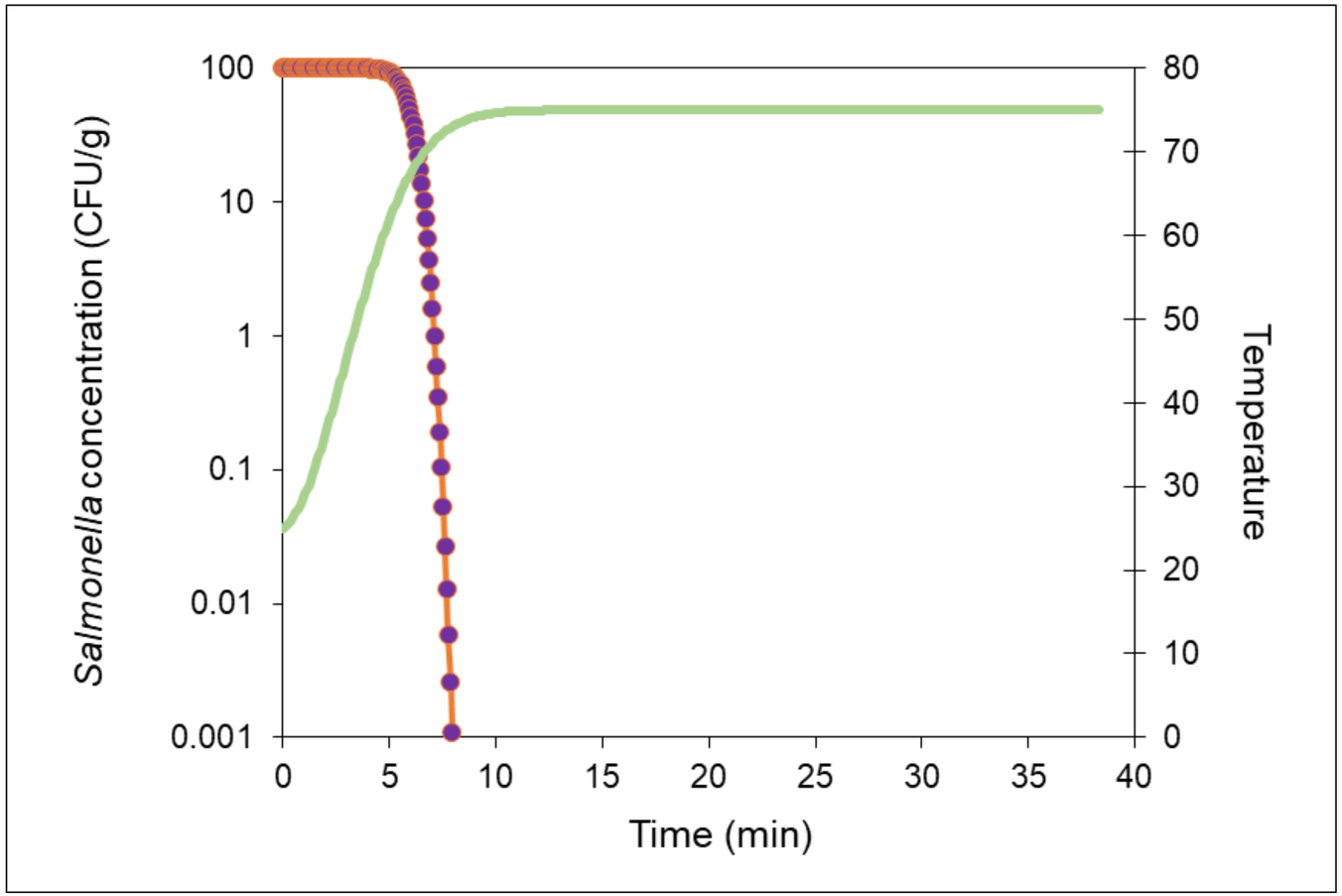
Other scenarios

Parameters fixed	
a=	-2.55E-04
b=	-5.66E-06
Air temperature (ºC)	75
Initial temperature (ºC)	25

Results



Increasing the smoking temperature accelerates the death of *Salmonella* population. From an initial contamination of 100 CFU/g, the concentration dropped to 0.001 CFU/g (1 cell in 1000 kg sausages) in about 8 minutes

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Conclusion

- ✓ To validate a thermal (or non-thermal) treatment, microbial kinetic parameters must be obtained from challenge test studies; yet they can be available from the literature or from databases.

- ✓ Many product-related factors (proximate composition, physicochemical properties, packaging) affect the death kinetics of the target microorganism in the food. The microbial kinetic parameters chosen must describe the target microorganism in that particular food.

- ✓ The thermal treatment regime to validate implies that the heating temperature and holding time must be known. Such temperature profile will drive the solution of the microbial concentration in time.

- ✓ The estimation of the microbial concentration in time at a given temperature profile requires the solution of a tertiary model (primary plus secondary), which can be approached by solving the equation for small time intervals.

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Further reading

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