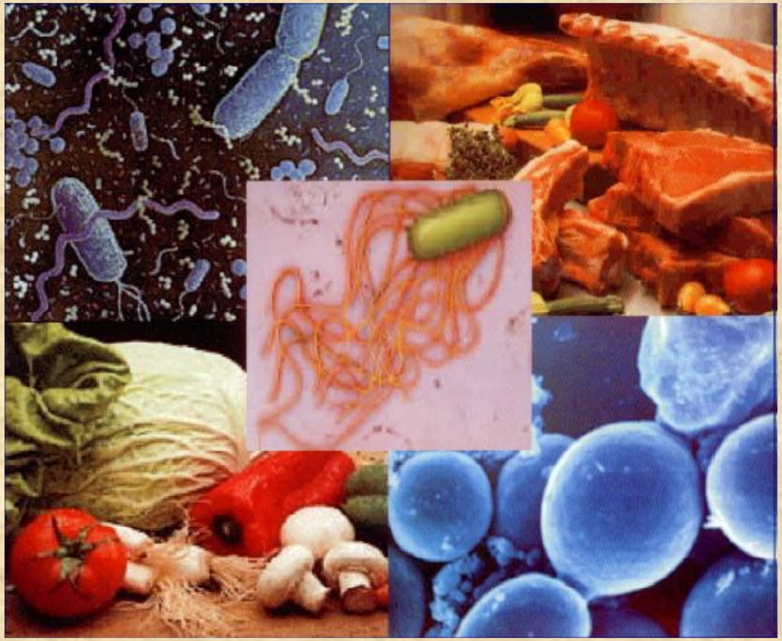


Predictive Microbiology

A tool to support food safety decisions

Models of microbial inactivation

- aplication in foods

Teresa Brandão and Cristina Silva

UNIVERSIDADE CATÓLICA PORTUGUESA
Escola Superior de Biotecnologia

7th December 2007

Mathematical modelling

some concepts …

$$y = f\,(x,\, q) + e$$

Parameters estimation

minimization of the residuals

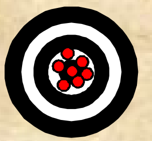
between **experimental** values

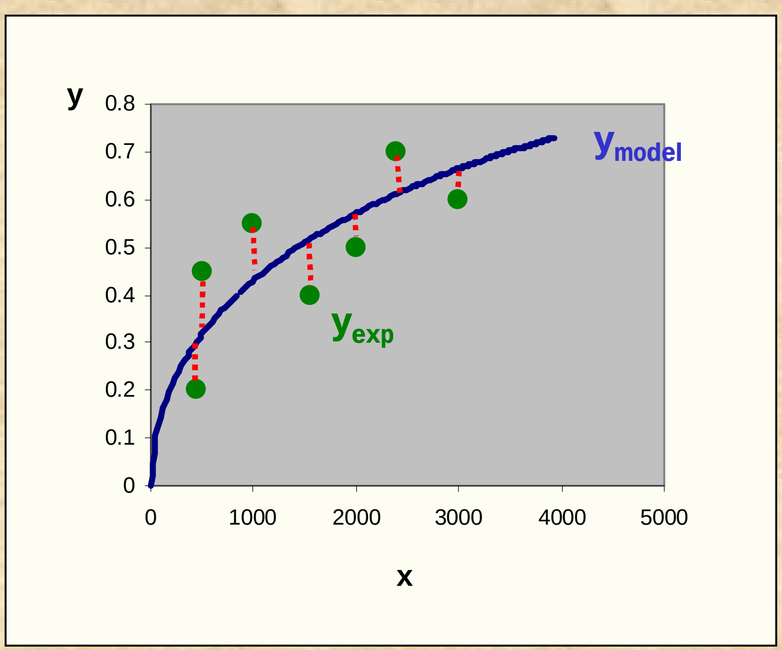
and the ones predicted by the **model**

q^*

Precise ?

Accurate ?





Mathematical modelling

objective

precise and accurate description of data

model adequacy

quality of the parameters

Mathematical modelling

mechanistic models

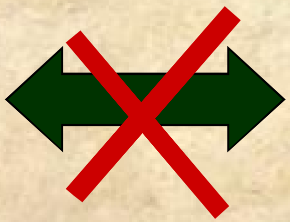
- fundamental description of the processes involved
- more complex

empirical models

- *black boxe*
- more simple (or not!)
- practical application

advantages and **disadvantages** should be considered
decision depending on the **final goal**

Mathematical modelling

mathematical complexity  **model adequacy**

Mathematical modelling

advantages

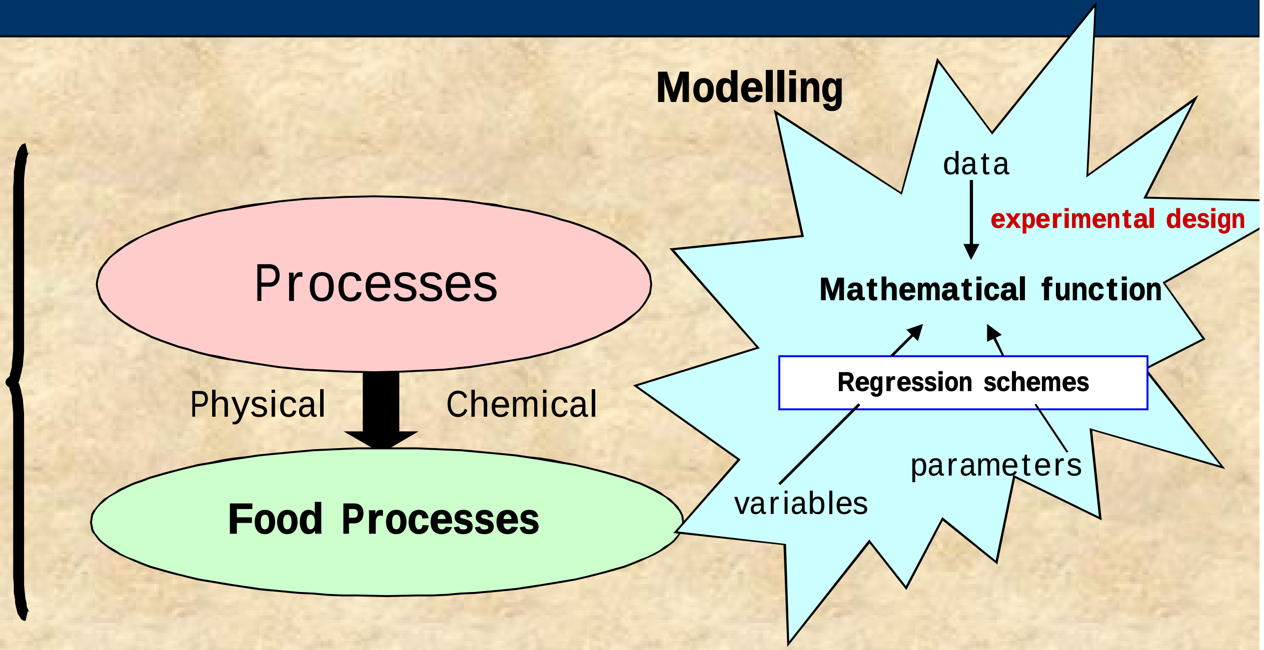
- knowledge of the process
- process effect in the product
- control of the variables involved

Mathematical modelling

Transport Phenomena
• heat
• mass
• *momentum*

Reaction kinetics

Properties

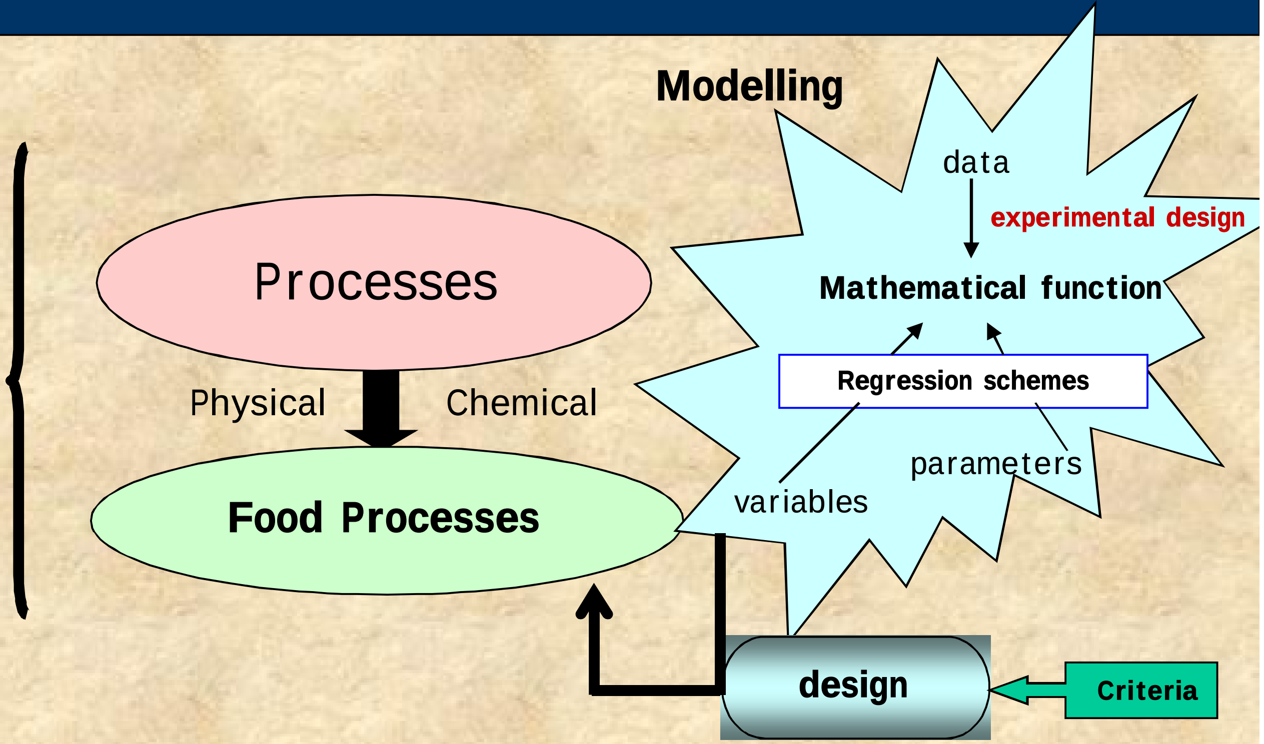


Mathematical modelling

Transport Phenomena
- heat
- mass
- *momentum*

Reaction kinetics

Properties



Mathematical modelling

Transport Phenomena
- heat
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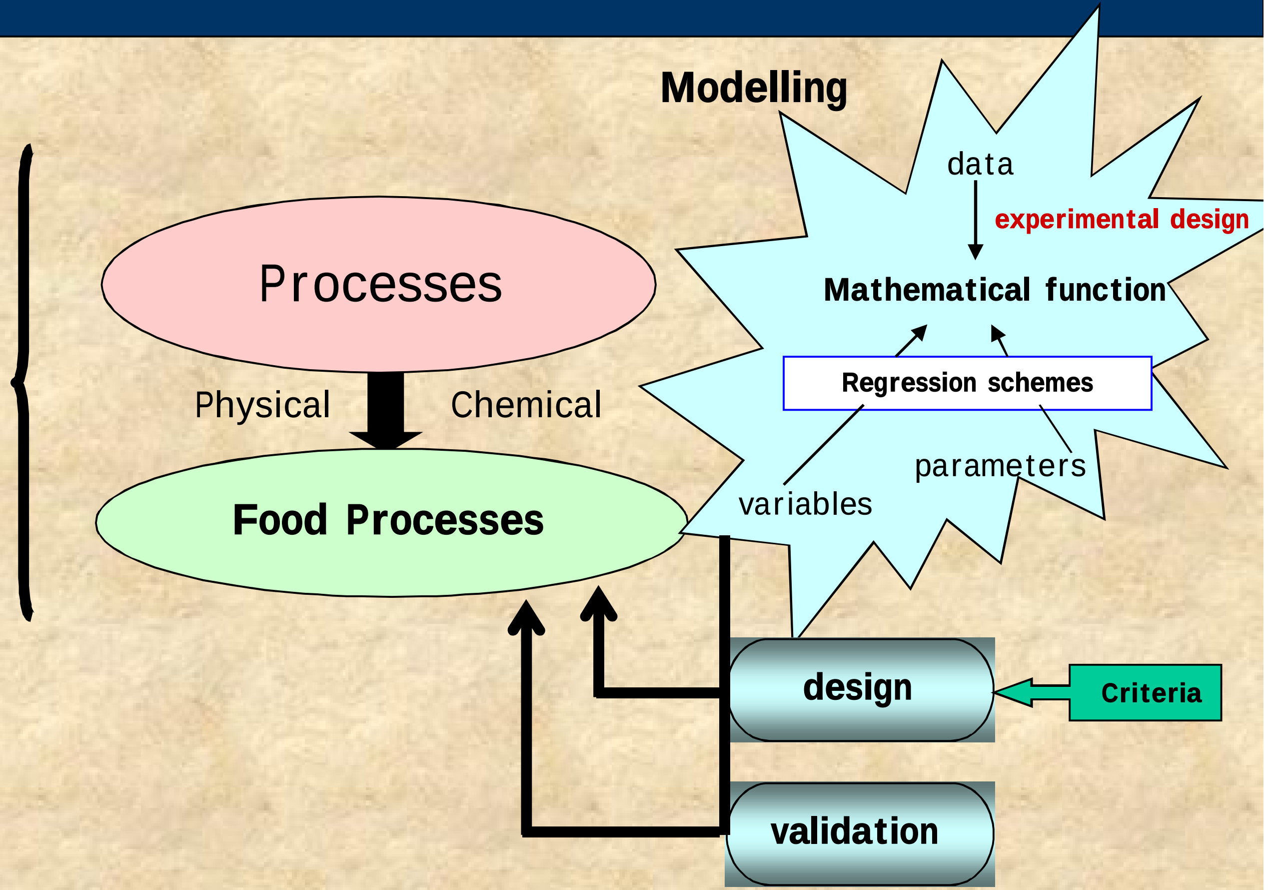
Reaction kinetics

Properties



Mathematical modelling

Transport Phenomena
• heat
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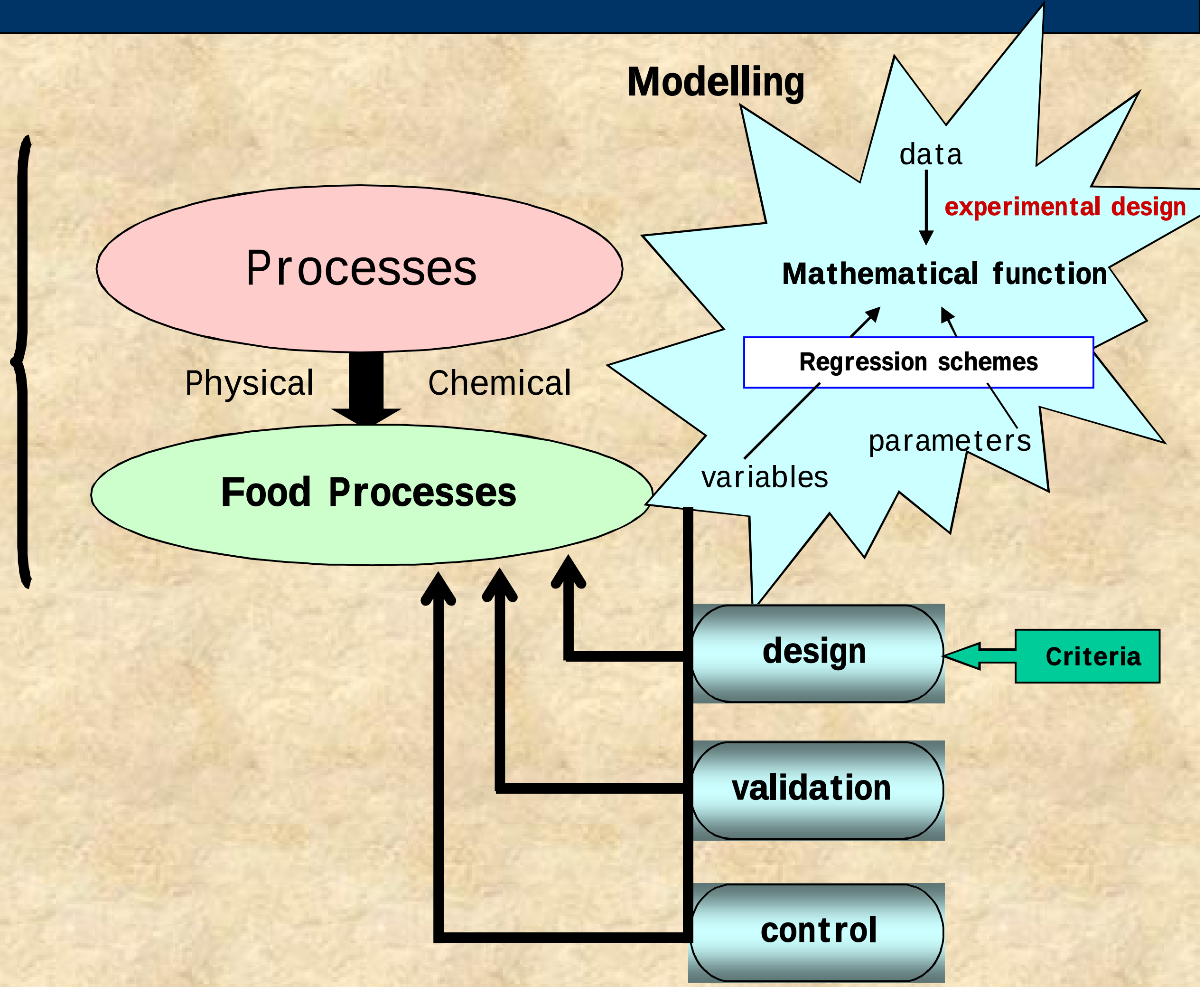
Reaction kinetics

Properties

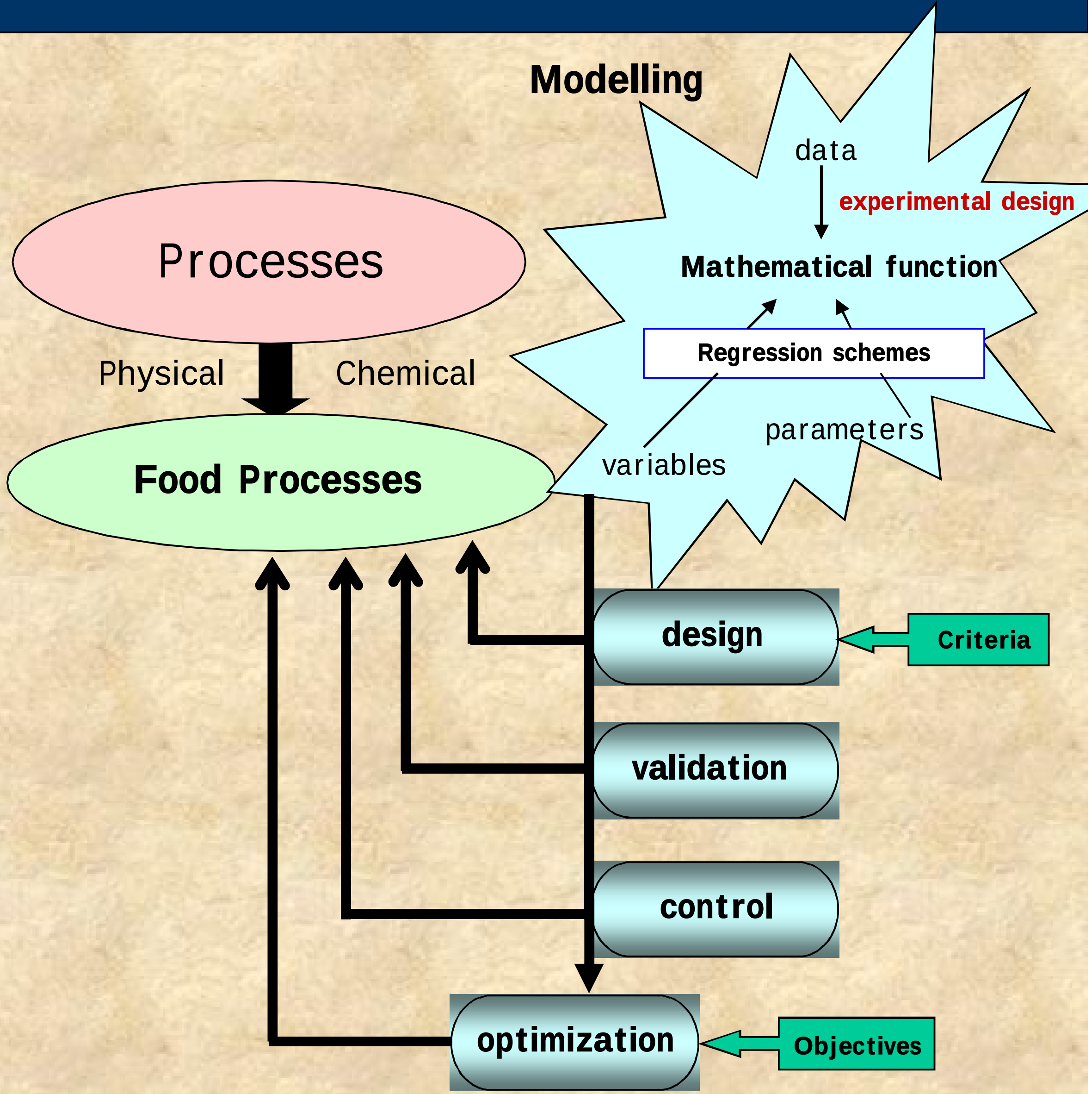


Mathematical modelling

Transport Phenomena
- heat
- mass
- *momentum*

Reaction kinetics

Properties

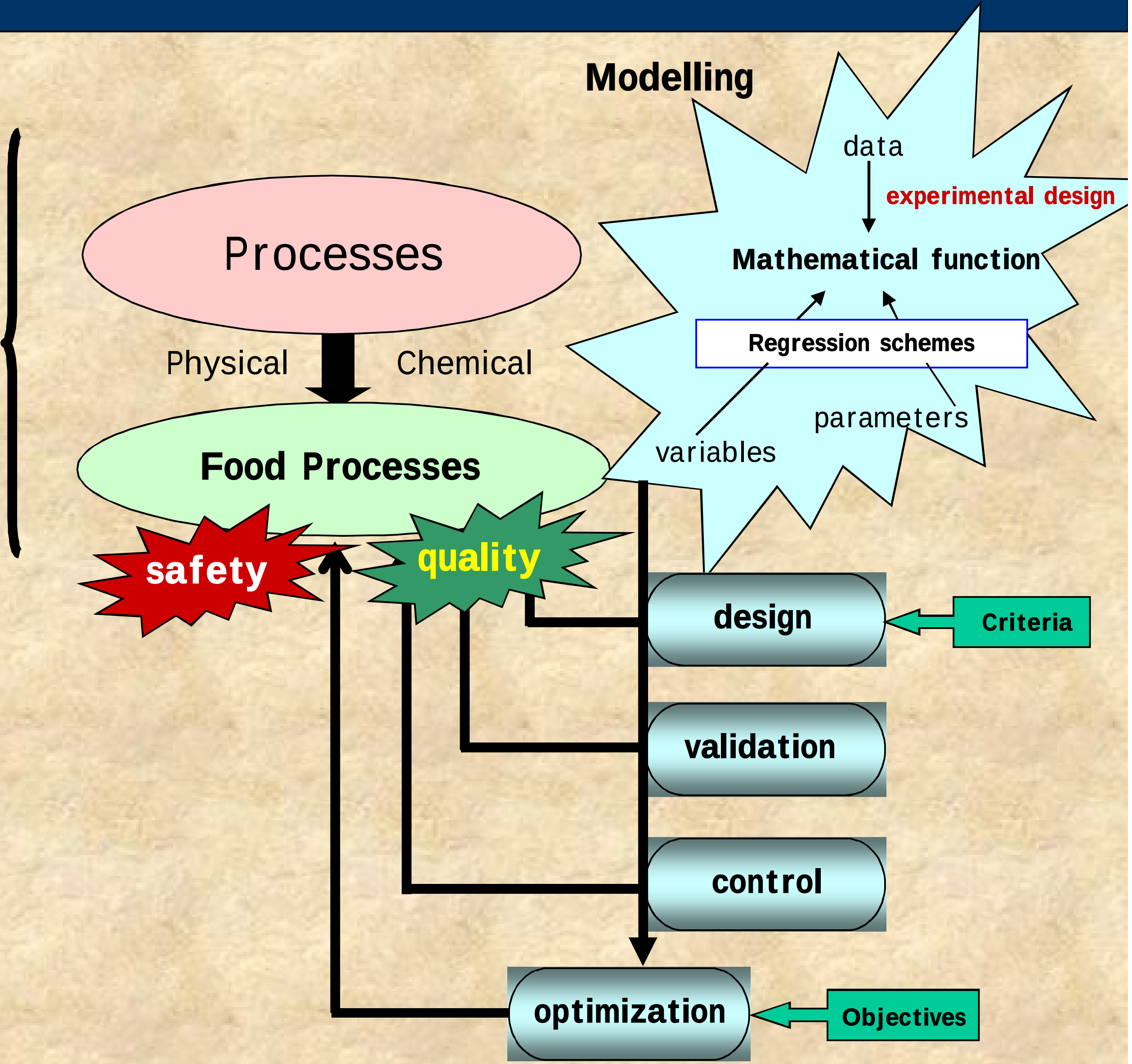


Mathematical modelling

Transport Phenomena
- heat
- mass
- *momentum*

Reaction kinetics

Properties



Mathematical modelling

safety

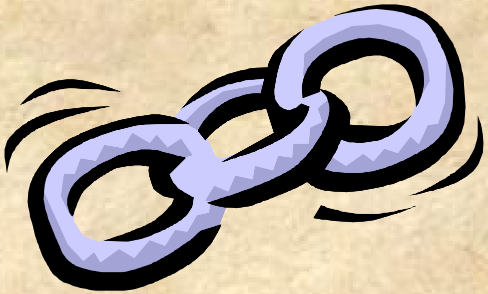
Mathematical modelling

predictive microbiology

Mathematical modelling

predictive microbiology

microbiology



statistics

mathematics

aplication

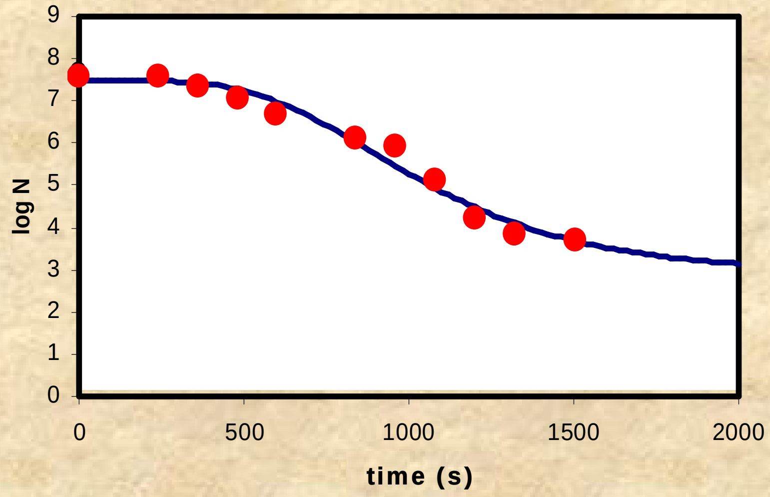
- prediction / simulation
- development of efficient inactivation processes

↓

contribution to safety

inactivation

Sigmoidal behaviour

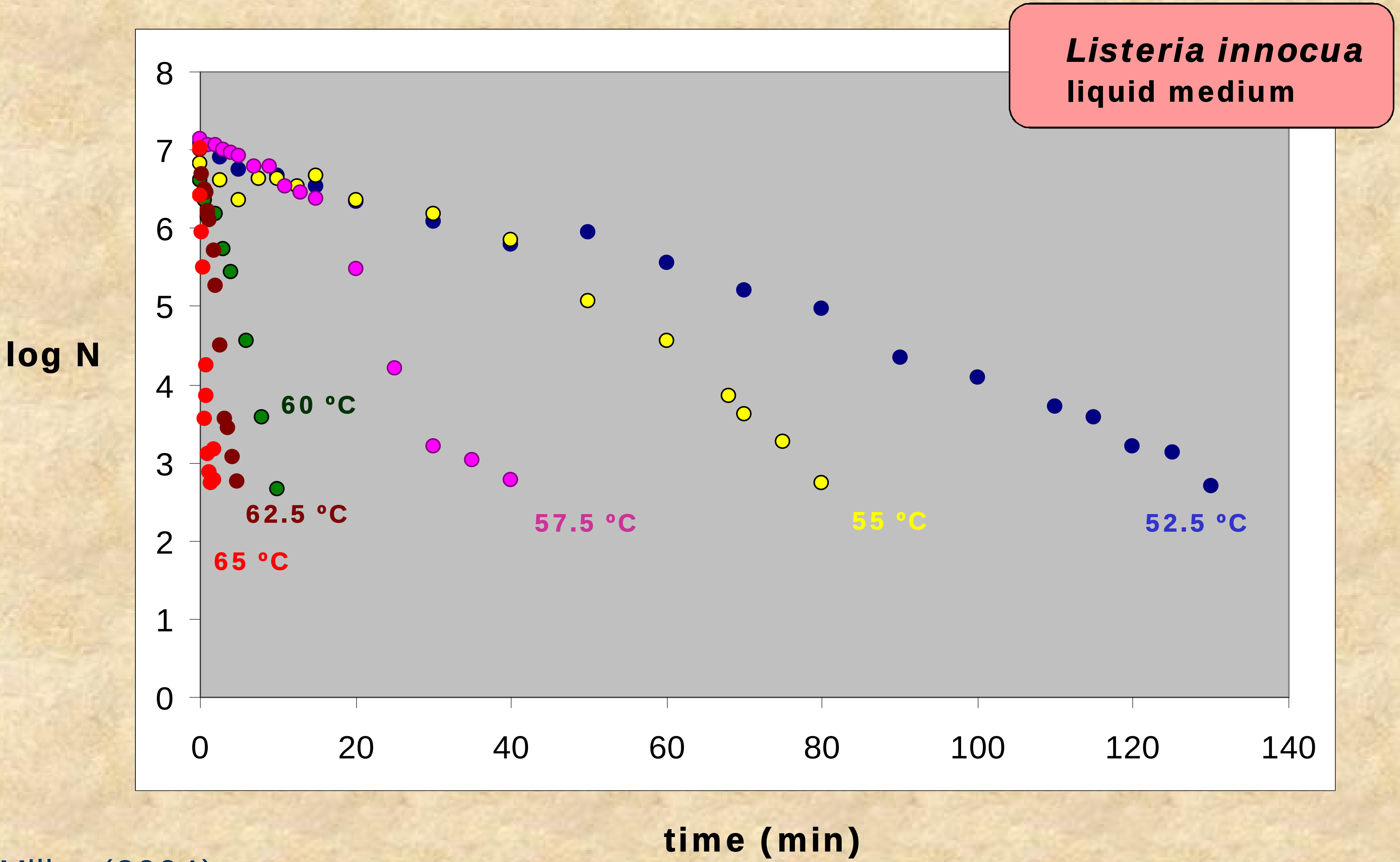


Reflects the presence of microbial sub-populations

more **temperature resistent**

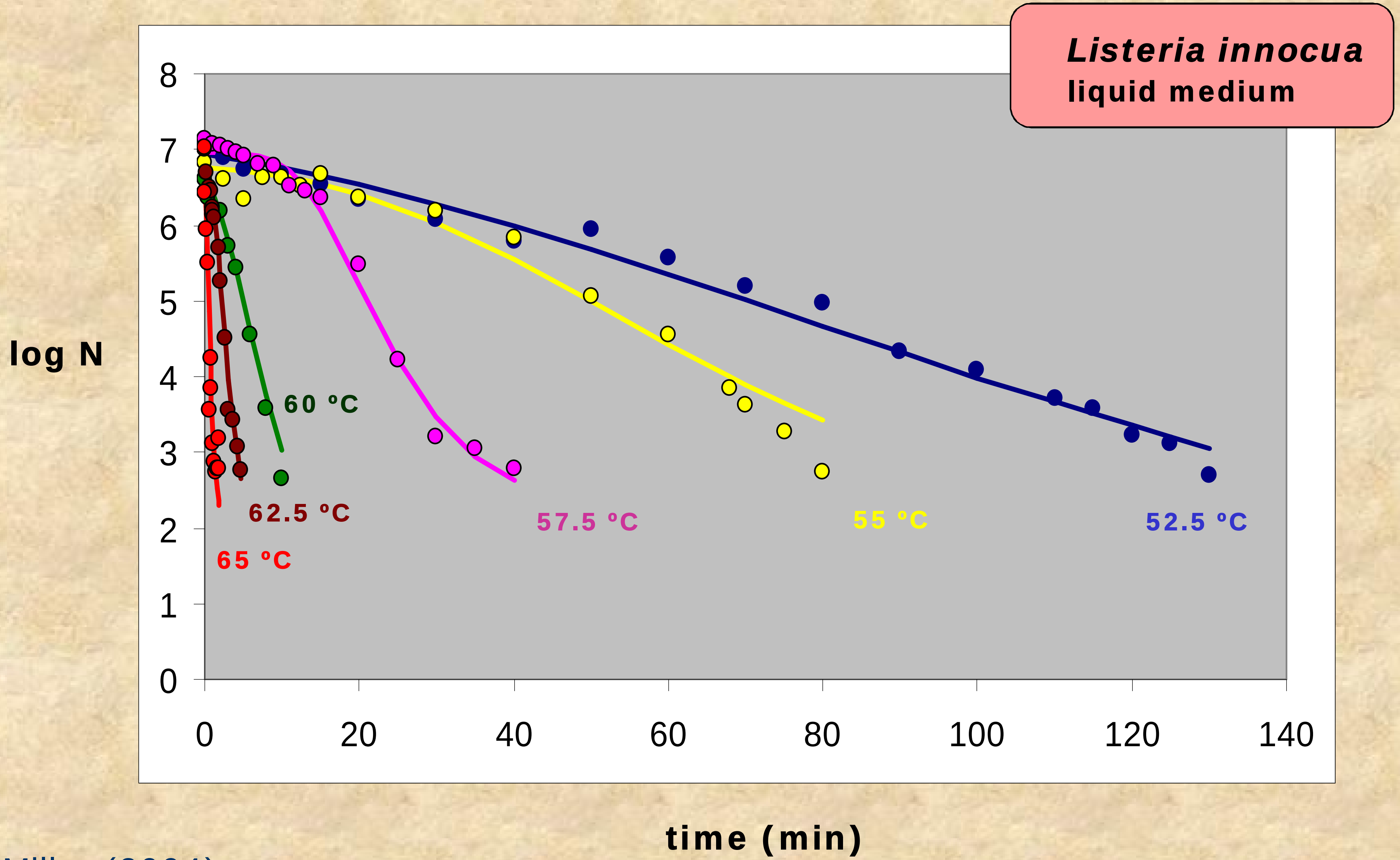
(or other environmental **stressing factor**)

Example



Miller (2004)

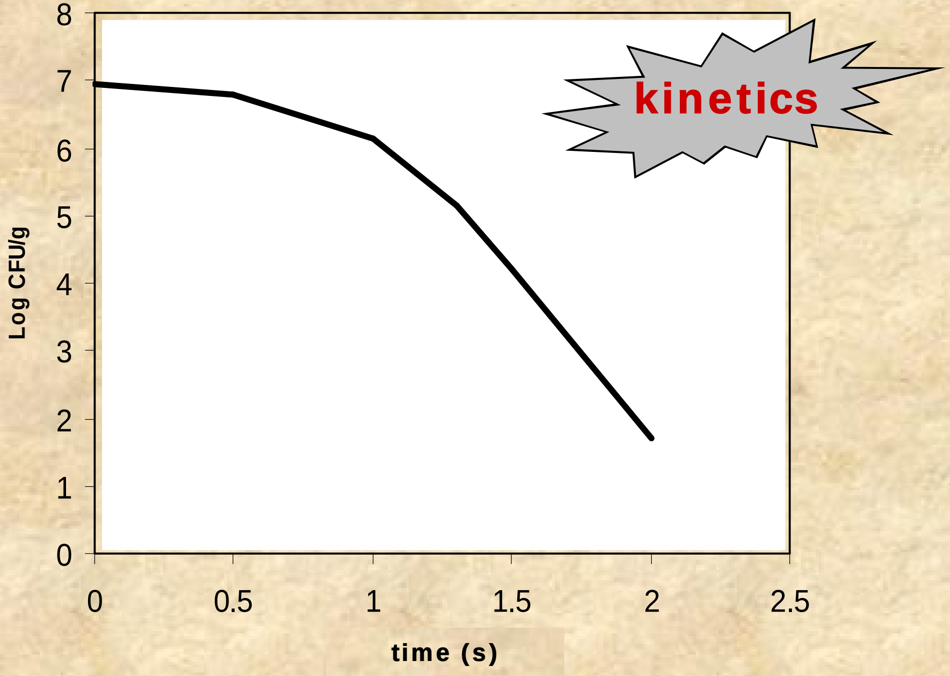
Example



Miller (2004)

Mathematical models

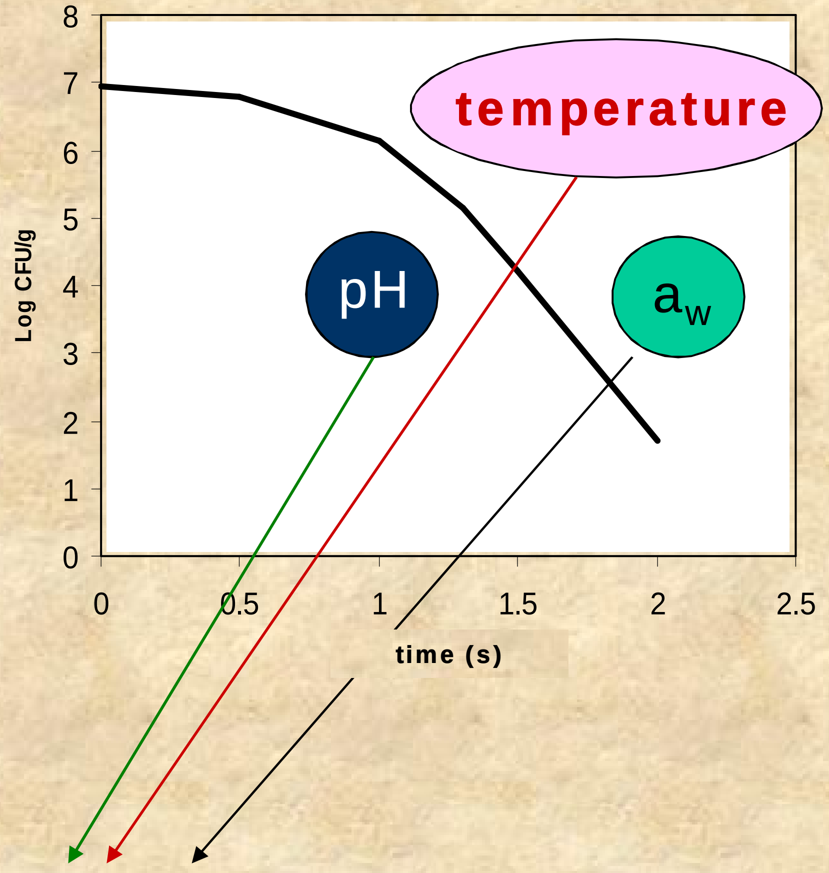
❖ **primary**



parameters

Mathematical models

❖ **primary**

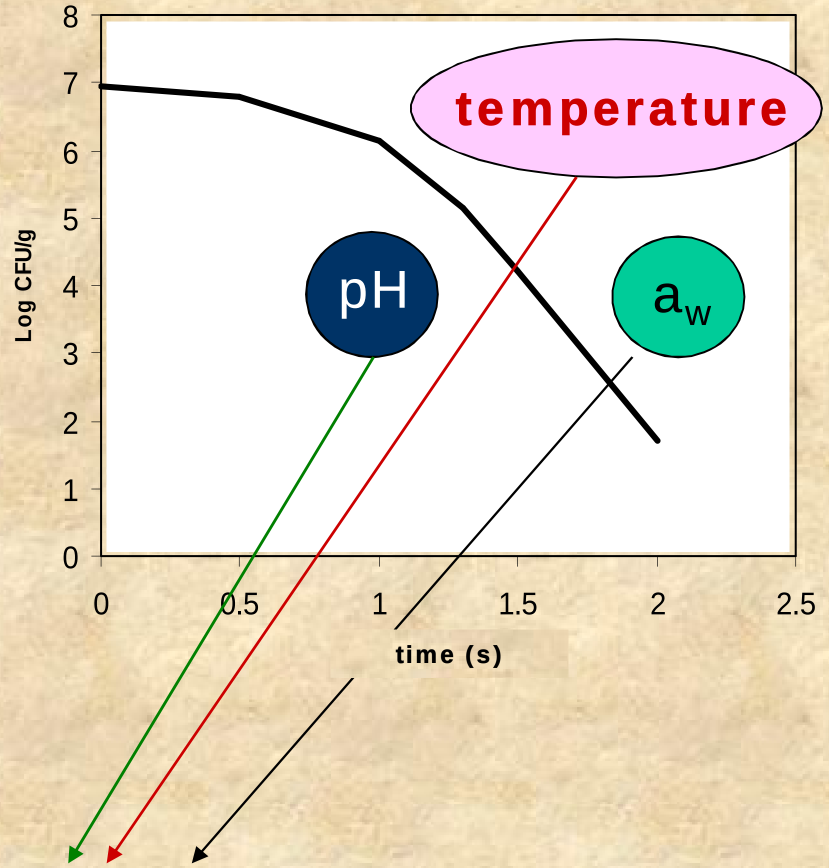


❖ **secundary**

parameters

Mathematical models

- **primary**

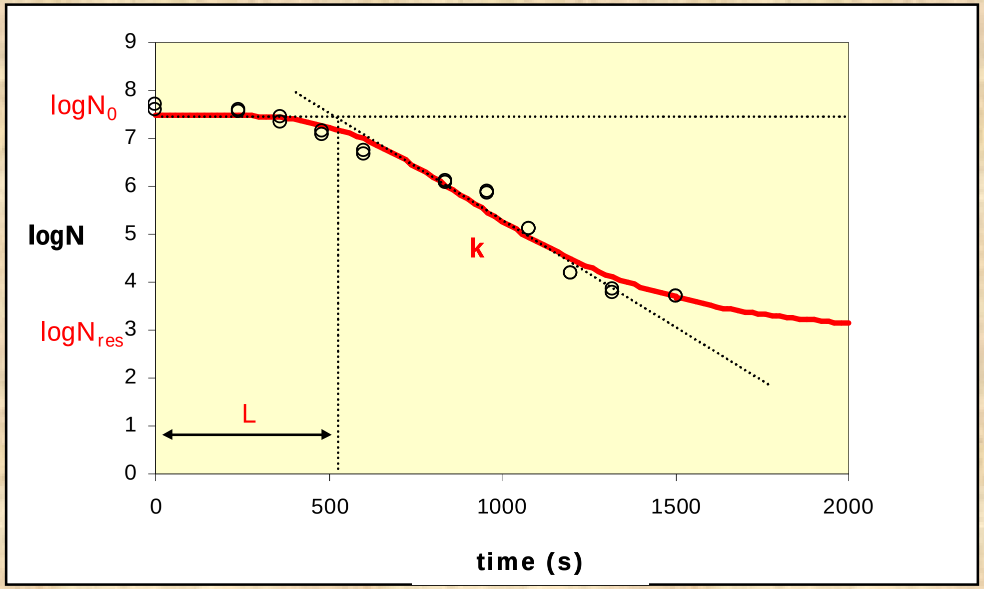


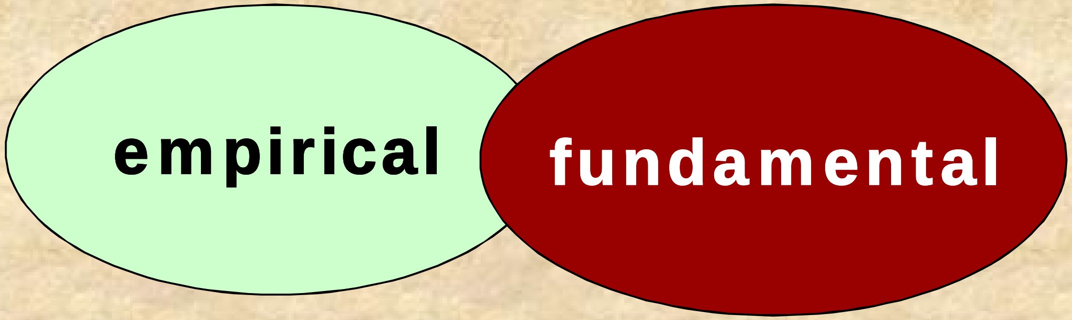
- **secundary**
  parameters

- **tertiary -** all models integration - **software**

Models for inactivation

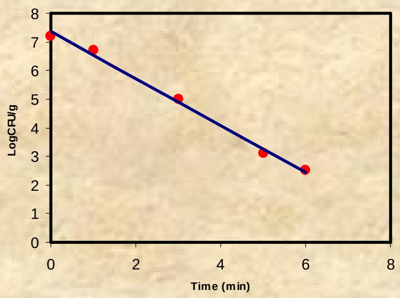
- primary





Models for inactivation

- **primary**



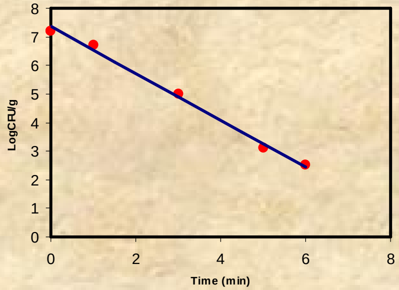
$$N = N_0 \exp(-kt) \Leftrightarrow \log N = \log N_0 - \frac{t}{D}$$

1st order

D – decimal reduction time

Models for inactivation

❖ **primary**



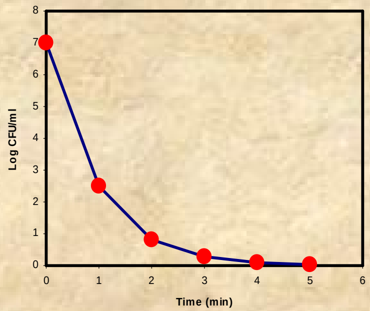
$$N = N_0 \exp(-kt) \Leftrightarrow \log N = \log N_0 - \frac{t}{D}$$

1st order

D – decimal reduction time

$$\frac{N}{N_0} = F_1 \exp(-k_1 t) + (1 - F_1)\exp(-k_2 t)$$

Cerf (1977)



biphasic

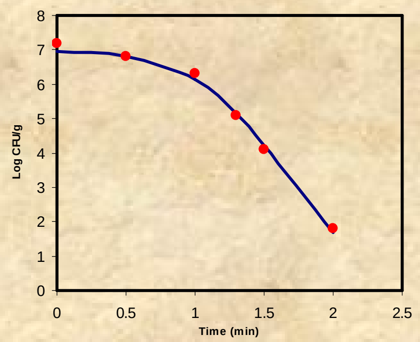
F_1 – fraction of inactivated microorganisms
k_1 e k_2 – kinetic constants

$$\log \frac{N}{N_0} = \log\left(\frac{2F_1}{1 + \exp(k_1 t)} + \frac{2(1 - F_1)}{1 + \exp(k_2 t)}\right)$$

Kamau et al. (1990)

Models for inactivation

- **primary**



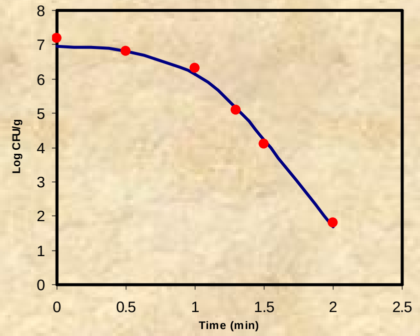
Whiting & Buchanan (1992)

$$\log \frac{N}{N_0} = \log\left(\frac{F_1(1 + \exp(-k_1 L))}{1 + \exp(k_1(t - L))} + \frac{(1 - F_1)(1 + \exp(-k_2 L))}{1 + \exp(k_2(t - L))} \right)$$

L – lag or shoulder

Models for inactivation

❖ primary



Whiting & Buchanan (1992)

$$\log \frac{N}{N_0} = \log\left(\frac{F_1(1+\exp(-k_1L))}{1+\exp(k_1(t-L))} + \frac{(1-F_1)(1+\exp(-k_2L))}{1+\exp(k_2(t-L))}\right)$$

L – lag or shoulder

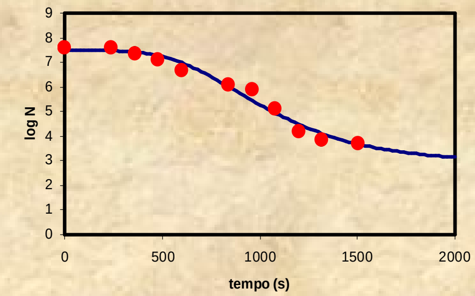
Cole et al. (1993)

$$\log N = \alpha + \frac{w-\alpha}{1+\exp\left(\frac{4\sigma(\lambda-\log t)}{w-\sigma}\right)}$$

Distribution of microbial population heat sensitiveness

Models for inactivation

- **primary**



Baranyi et al. (1993)

$$\frac{dN}{dt} = -k\,\alpha(t)\,\beta(t)\,N$$

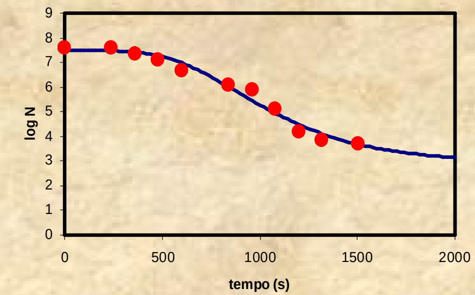
$$N(t = 0) = N_0$$

function 'tail'

function 'lag'

Models for inactivation

❖ primary



Baranyi et al. (1993)

$$\frac{dN}{dt} = -k\,\alpha(t)\,\beta(t)\,N$$

$$N(t = 0) = N_0$$

function 'tail'

function 'lag'

Geeraerd et al. (2000)

$$\frac{dN}{dt} = -k_{max}\; k_Q(Q)\; N$$

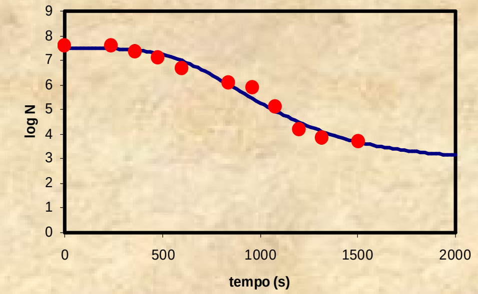
$$\frac{dQ}{dt} = -k_{max}\; Q$$

$$\log\left(\frac{N}{N_0}\right) = \log(\exp(-k_{max}t))\frac{1 + Q(0)}{1 + Q(0)\exp(-k_{max}t)}$$

Q – variable related to the physiological state of microorganisms

Models for inactivation

❖ **primary**



Gompertz

Bhaduri et al (1991)
Linton et al. (1995, 1996)
Xiong et al. (1999)

↓

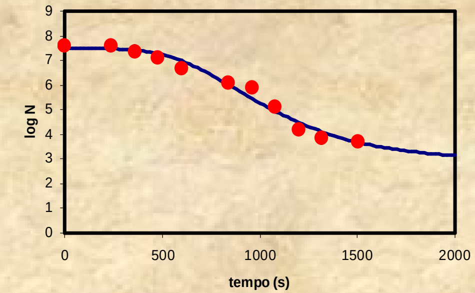
Listeria monocytogenes

$$\log N = \log N_0 + \log\left(\frac{N_{res}}{N_0}\right)\exp\left(-\exp\left(-\frac{k\ e}{\log\left(\frac{N_{res}}{N_0}\right)}(L - t) + 1\right)\right)$$

Reparameterization for inactivation based on Zwitering et al. (1990)

Models for inactivation

❖ **primary**



Gompertz

Bhaduri et al (1991)
Linton et al. (1995, 1996)
Xiong et al. (1999)

↓

Listeria monocytogenes

$$\log N = \log N_0 + \log\left(\frac{N_{res}}{N_0}\right)\exp\left(-\exp\left(-\frac{k\ e}{\log\left(\frac{N_{res}}{N_0}\right)}(L-t)+1\right)\right)$$

Reparameterization for inactivation based on Zwitering et al. (1990)

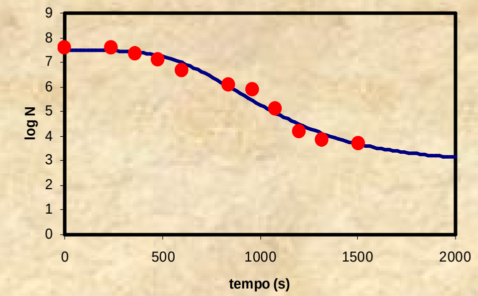
Logistic

$$\log N = \frac{c}{1+\exp(k(t - L))}$$

c – constant

Models for inactivation

❖ **Gompertz**



elected in our research

$$\log N = \log N_0 + \log\left(\frac{N_{res}}{N_0}\right) \exp\left(-\exp\left(-\frac{k\ e}{\log\left(\frac{N_{res}}{N_0}\right)}(L - t) + 1\right)\right)$$

or

$$\log\frac{N}{N_0} = \log\left(\frac{N_{res}}{N_0}\right) \exp\left(-\exp\left(-\frac{k\ e}{\log\left(\frac{N_{res}}{N_0}\right)}(L - t) + 1\right)\right)$$

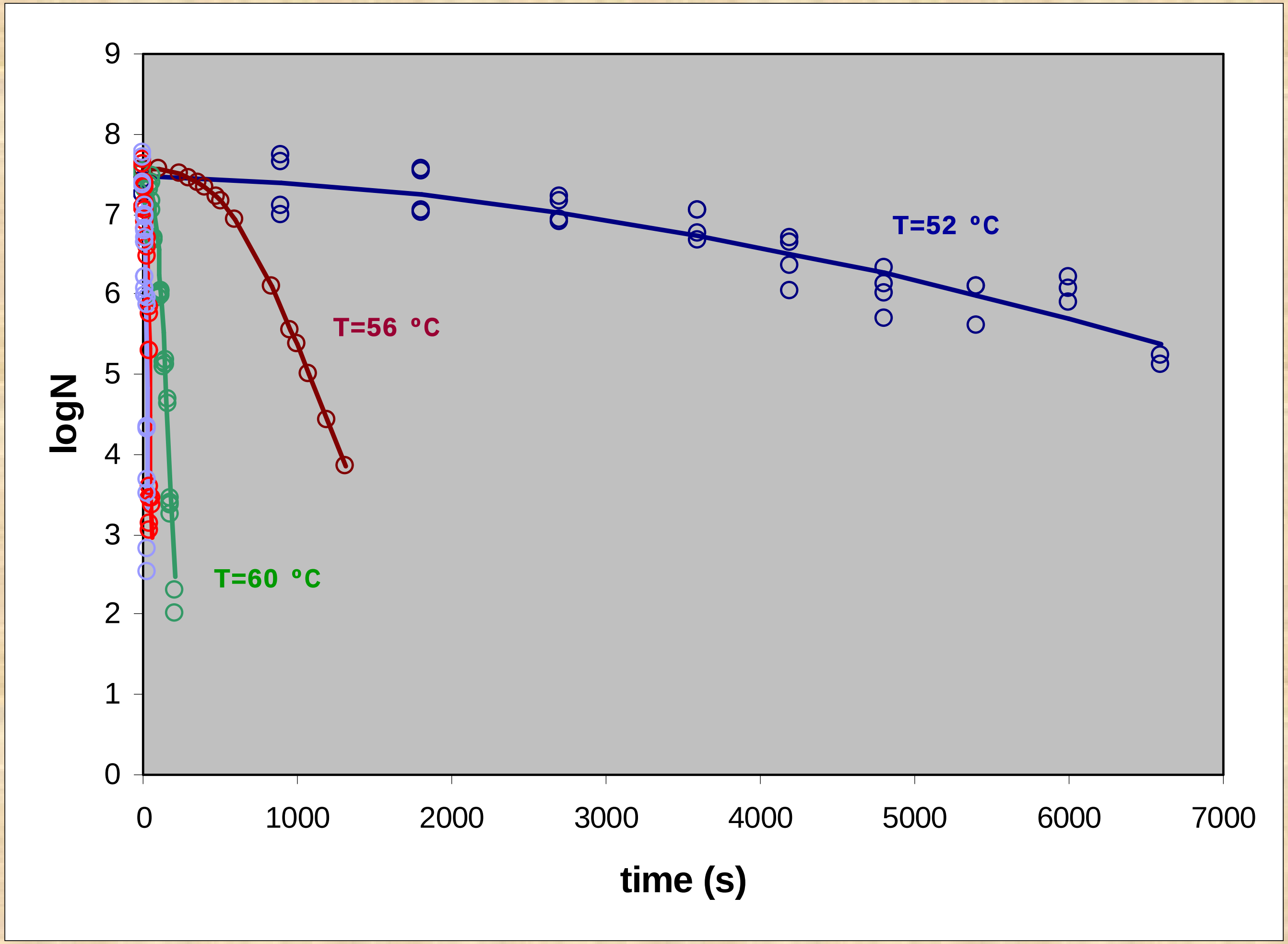
Examples

Data of *L.monocytogenes* Scott A at 52,56,60,64,68°C

(24 hours incubation at 5°C in half cream)

Casadei et al. (1998)

Gompertz



Gil (2002)

Statistica 6.0

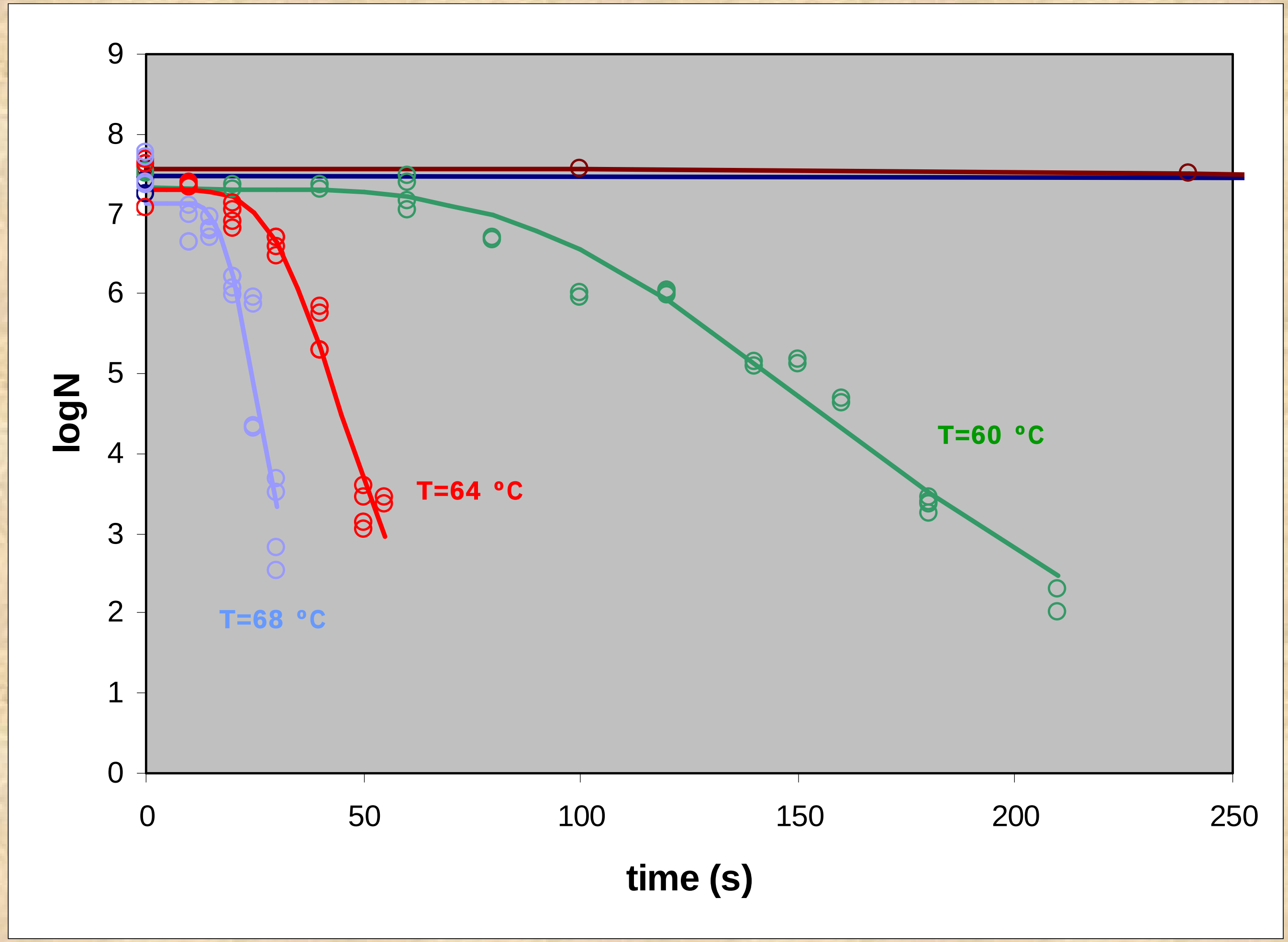
Examples

Data of *L.monocytogenes* Scott A at 52,56,60,64,68°C

(24 hours incubation at 5°C in half cream)

Casadei et al. (1998)

Gompertz



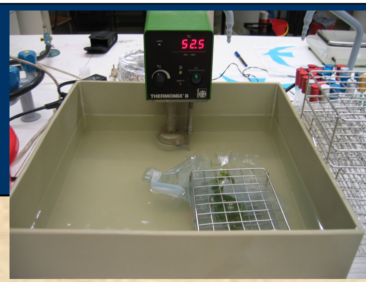
Gil (2002)

Statistica 6.0

Examples

Data of *L.innocua* at 52.5 and 60°C

Parsley (*Petroselinum crispum*) artificially contaminated



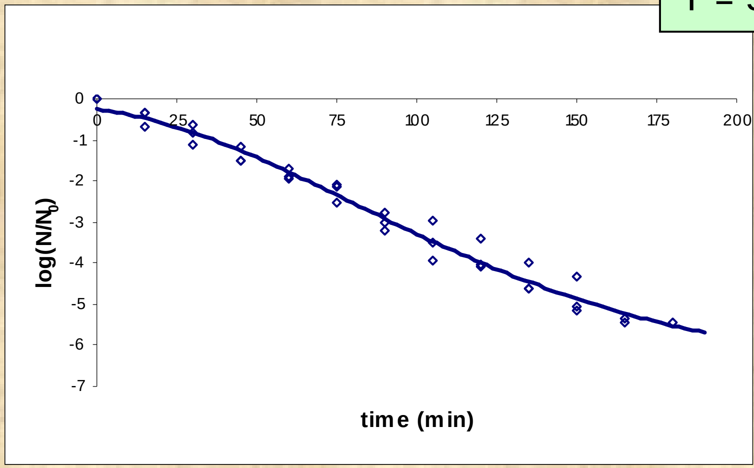
Gompertz

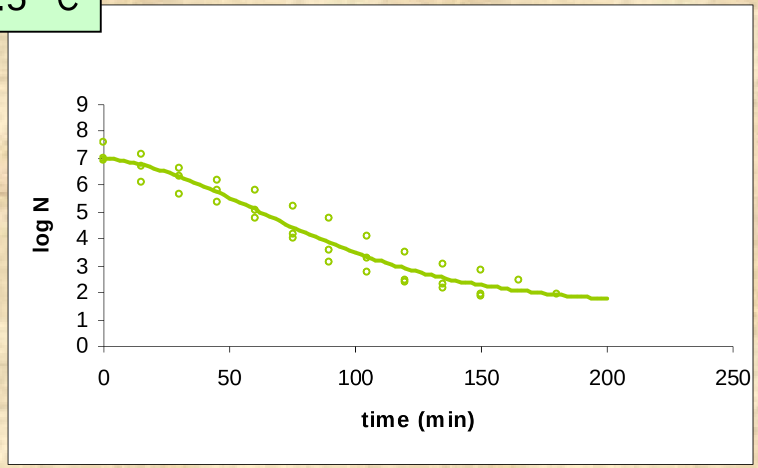
$$\log \frac{N}{N_0}$$

logN

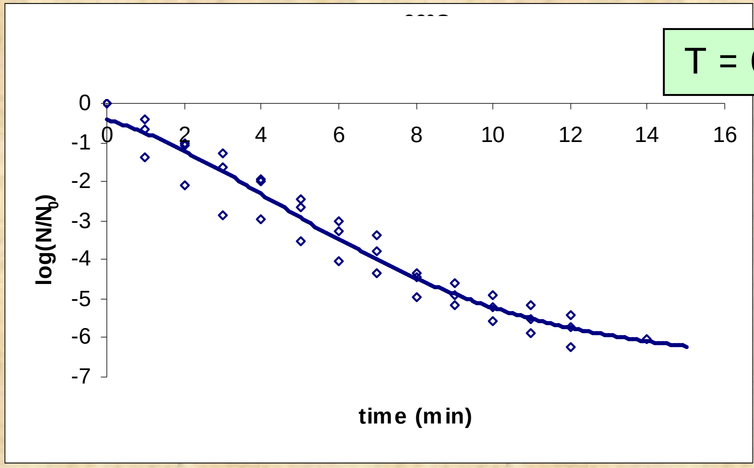
Miller (2006)

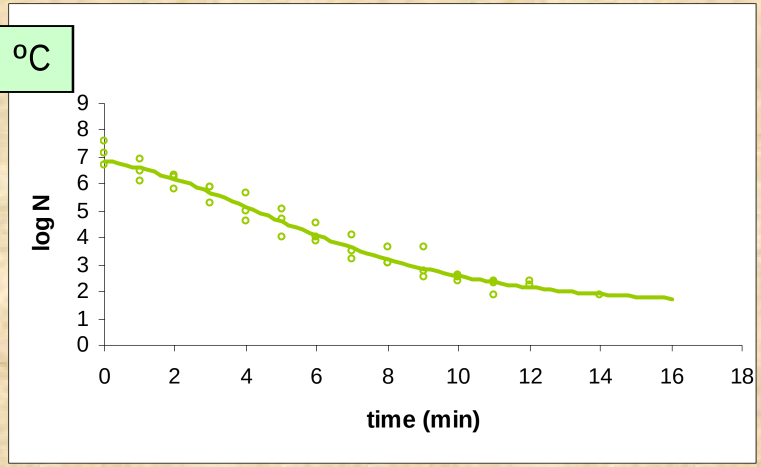
T = 52.5 ºC





T = 60 ºC

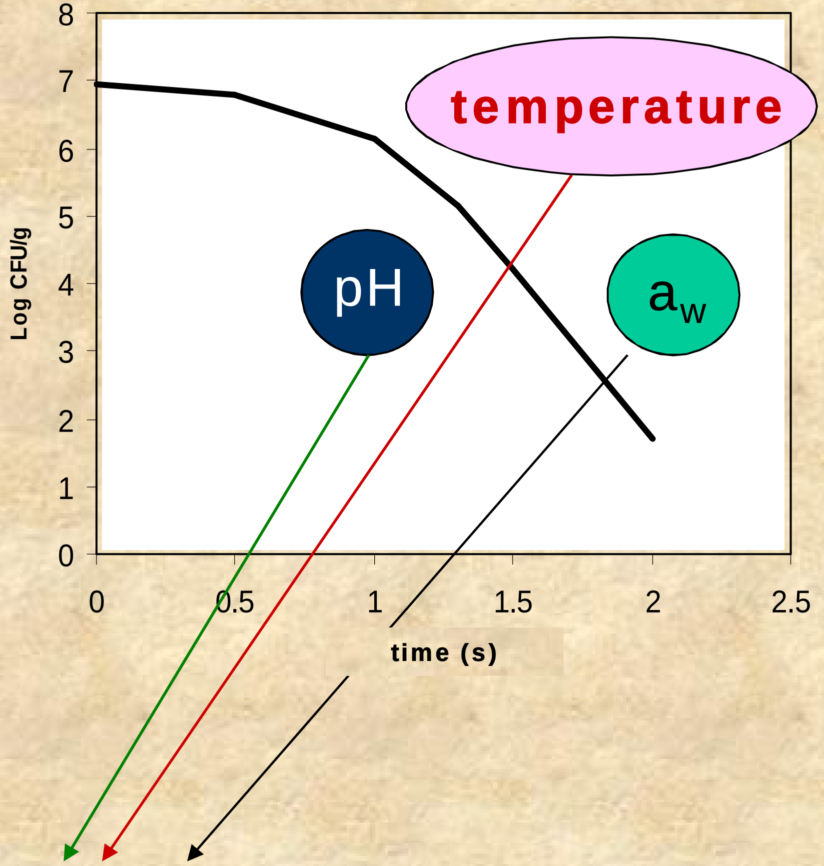




Statistica 6.0

Mathematical models

- **primary**



- **secundary**
  parameters

Mathematical models

secundary

Arrhenius

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \rightarrow \ln k = \ln k_0 - \frac{E_a}{RT}$$

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

Davey / Arrhenius modified

$$\ln k = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 a_w + C_4 a_w^{\;2}$$

"Square-root type models"

Ratkowsky *et al.* (1982)

$$\sqrt{k} = b(T - T_{min})$$

McMeekin *et al.* (1987)

$$\sqrt{k} = b(T - T_{min})\sqrt{(a_w - a_{w_{min}})}$$

Adams *et al.* (1991)

$$\sqrt{k} = b(T - T_{min})\sqrt{(pH - pH_{min})}$$

McMeekin et al. (1992)

$$\sqrt{k} = b(T - T_{min})\sqrt{(a_w - a_{w_{min}})}\sqrt{(pH - pH_{min})}$$

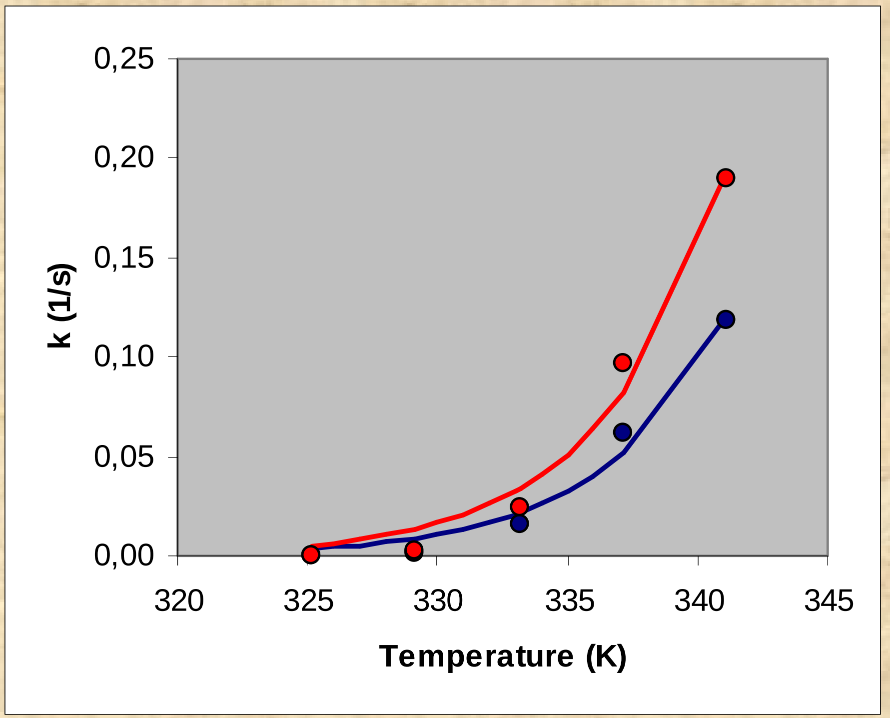
min – minimal value for growth

Examples

Data of *L.monocytogenes* Scott A

(24 hours incubation at 5°C in half cream)

Arrhenius



k = f(T)

Gompertz	k=**0.0216** exp(-**203.3**/R*(1/T-1/333.15))	DEa=28.85 kJ/mol $DK_{ref}=4.58 \times 10^{-3}\ s^{-1}$
Logistic	k=**0.0337** exp(-**206.6**/R*(1/T-1/333.15))	DEa=27.56 kJ/mol $DK_{ref}=7.31 \times 10^{-3}\ s^{-1}$

Gil (2002)

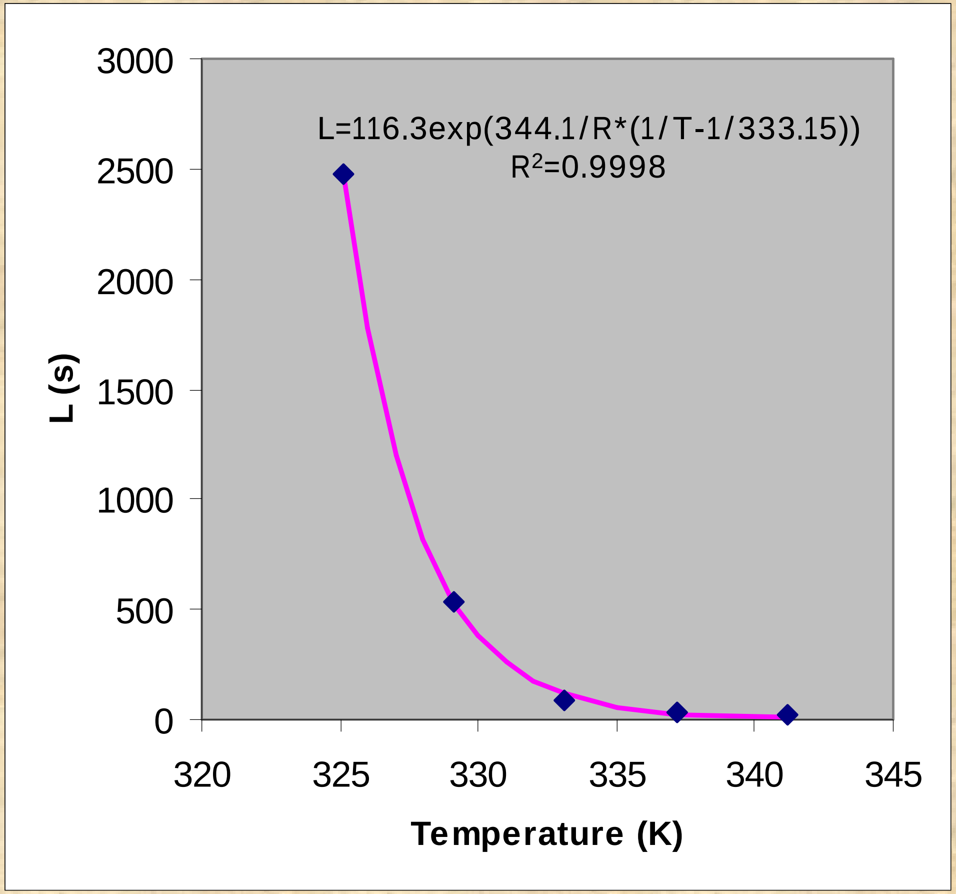
Statistica 6.0

Data of *L.monocytogenes* Scott A

(24 hours incubation at 5°C in half cream)

Gompertz

lag = f(T)



DEa=7.485 kJ/mol

DL_{ref}=7.595 s^{-1}

Gil (2002)

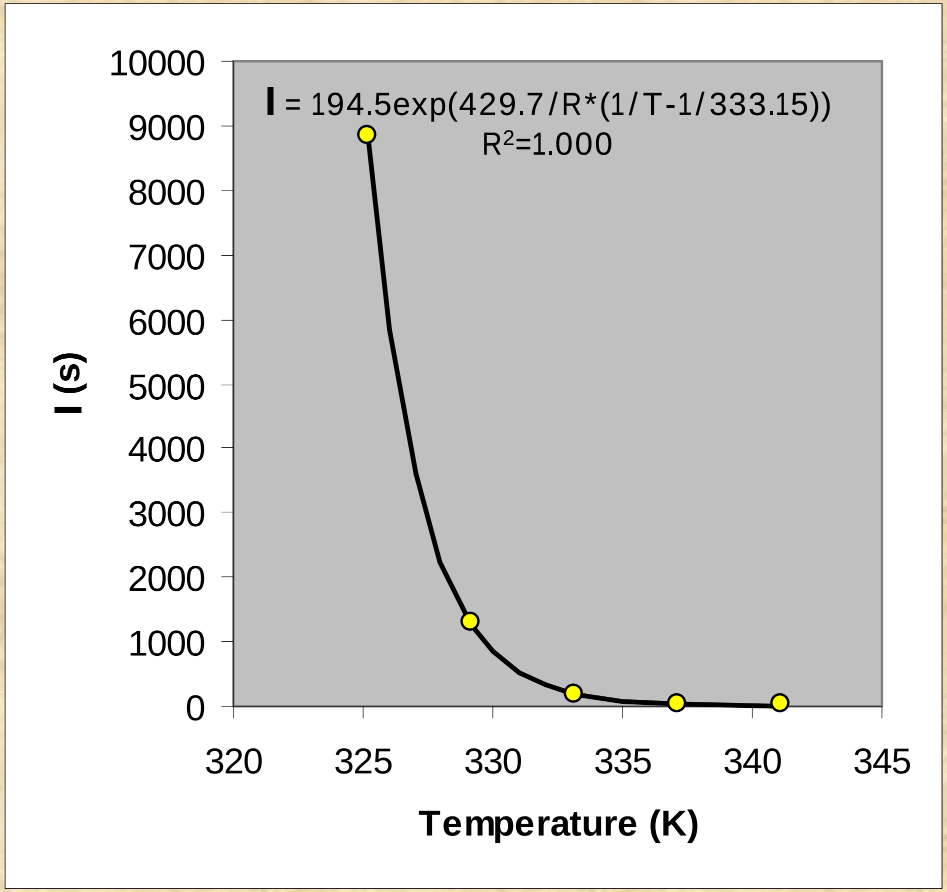
Statistica 6.0

Examples

Data of *L.monocytogenes* Scott A
(24 hours incubation at 5°C in half cream)

Logistic

lag = f(T)



DEa=3.591 kJ/mol

$D\mathbf{l}_{ref}$=6.154 s^{-1}

Gil (2002)

Statistica 6.0

Mathematical models

temperature

time-varying conditions

pH

a_w

More complexity !!

$$\frac{d(\log N)}{d(tempo)}$$

Mathematical models

Gompertz

time-varying temperature conditions

$$\frac{d(\log N)}{d(tempo)}$$

$$\log N = \log N_0 + \int_0^t \left[k \exp(1) \exp\left(-\frac{k \exp(1)}{\log\left(\frac{N_{res}}{N_0}\right)} (L - t') + 1 \right) \exp\left(-\exp\left(-\frac{k \exp(1)}{\log\left(\frac{N_{res}}{N_0}\right)} (L - t') + 1 \right)\right) \right] dt'$$

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

$$L = a \exp\left(b \left(\frac{1}{T} - \frac{1}{T_{ref}} \right)\right)$$

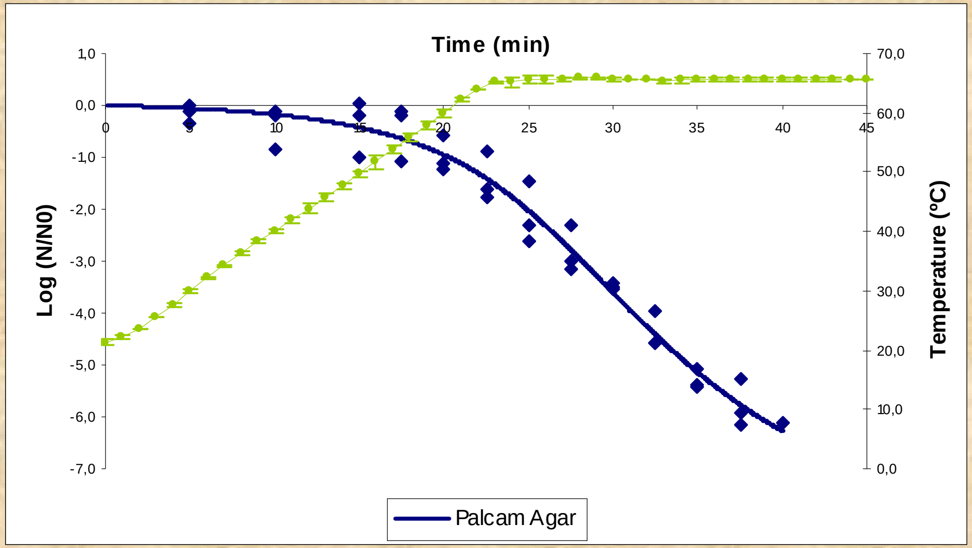
Mathematical models

Parsley (*Petroselinum crispum*) artificially contaminated with *L. innocua*

Miller (2006)

Gompertz

time-varying temperature conditions



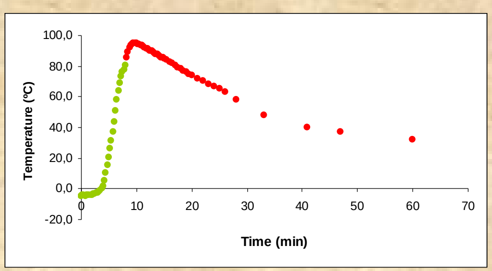
temperature increase rate ~ **2 ºCmin^{-1}**

Mathematical models

Meat pockets artificially contaminated with *L. innocua*

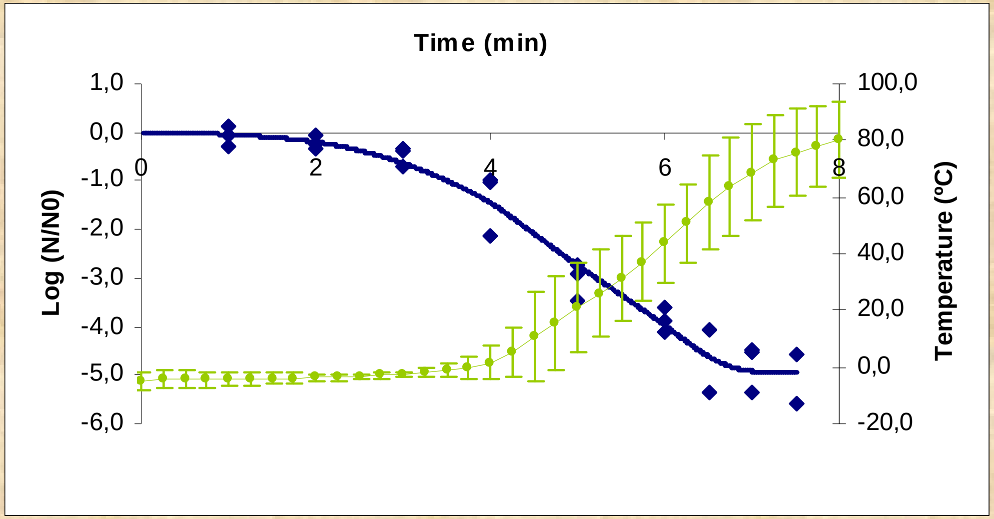
Gompertz

frying process



Miller (2006)

time-varying temperature conditions



maximum temperature increase rate ~ **20 ºCmin^{-1}**

Models for inactivation

- tertiary

softwares

Microbial growth √

Shelf life prediction

Microbial inactivation

lacunes

Drawbacks

- microbial interaction
- natural strains diversity
- complexity of food structure
- food / microorganism
- modelling ‘lag’
- modelling ‘tail’
- predictions in real time-varying environmental conditions

Participation of our research team in the project ...

2001-2004

BUGDEATH

www.frperc.bris.ac.uk/bugdeath.htm

Predictive microbiology

Surface pasteurization of foods

important boundary in food contamination

"BUGDEATH" (QLRT-2001-01415)

Micrograph of a Salmonella bacteria

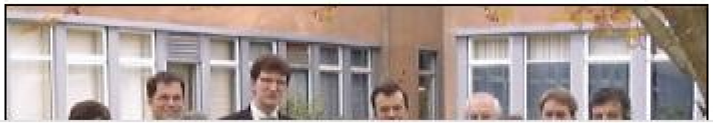
Predicting the reduction in microbes on the surface of foods during pasteurisation.

- Tell me more about the Bugdeath project.
- What are the objectives of the Bugdeath project?
- Who are the project partners?
- Meetings
- Industrial Advisory Group (IAG)
- Partner area of web site (specifically for project partners - password needed to enter)

Bugdeath

BUGDEATH is a research project funded by the European Commission to produce accurate predictive models of the reductions in microbial numbers that can be achieved on the surface of foods during surface pasteurisation processes.

Food poisoning is increasing throughout the European Union (EU). Over 60% of outbreaks are associated with meat, fresh fruit and salad vegetables. Most of the contamination by pathogenic and spoilage organisms is present on the surface of foods at the time of harvesting or is transferred to the surfaces during slaughter and processing. Accurate microbial death models would be of considerable help to the food industry in the development of surface pasteurisation systems for meat, fruit and vegetables. This will in turn lead to safer foods with improved quality and shelf life.



Personnel involved in'Bugdeath from left to right

Bugdeath

Partners

1. Food Refrigeration and Process Engineering Research Center (**FRPERC**)
   University of Bristol

2. Escola Superior de Biotecnologia (**ESB**)
   Universidade Católica Portuguesa

3. Department of Chemical Engineering
   Katholieke Universiteit Leuven (**KUL**)

4. Teagask, The National Food Center (**NFC**)

5. Campden & Chorleywood Food Research Association (**CCFRA**)

6. Faculty of Applied Science
   University of West England (**UWE**)

7. Laboratoire de Ginie des Procidis Alimentares (**ENITIA**)
   Ecole Nationale d'Inginieurs des Techniques des Industries Agricoles et Alimentaires

8. Institute National de la Recherche Agronomique (**INRA**)

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Objectives

- microbial inactivation studies at food surfaces
- development of precise and accurate inactivation models
- development of an equipment for surface food pasteurization
  'rig apparatus'
- software development for prediction

ESB task

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Foods under study

- potato
- chicken
- meat

microorganisms

- *Listeria monocytogenes*
- *E. coli*
- *Salmonella*
- *Campylobacter*

rig apparatus

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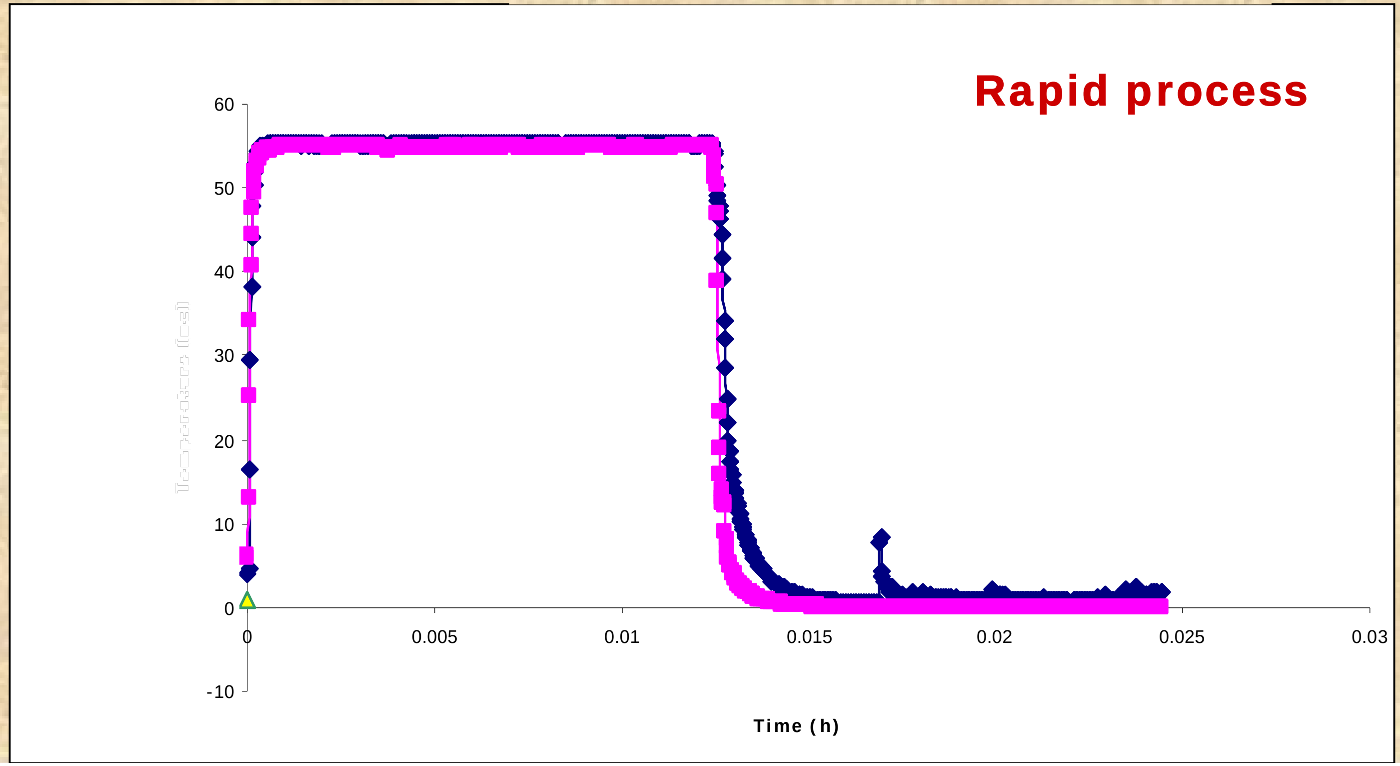
Thermal processes

- steam
- dry air

Temperature histories

rig apparatus



Different temperature histories can be tested

Escola Superior de Biotecnologia, Universidade Católica Portuguesa

Team

Pedro Pereira
Maria Manuel Gil
Teresa Brandão
Cristina Silva

Dissemination session
Chipping Campden, Agosto 2004

Development of a user-friendly combined heat, mass transfer and microbial death model

Objectives

- Create a software application

User-friendly tool

Simulation of inactivation kinetics of microorganisms on the surface of foods during pasteurisation treatments

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Quality of Life and Management of Living Resources Programme

Introduction

- Need to obtain reliable data on relationship between microbial death and the surface temperature of real foods

Lead to the design, construction and commission of equipment

Software application to simulate results obtained in the rig apparatus

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Modelling

Global model

Prediction/simulation purposes

Heat transfer phenomena

Integrates

Microbial inactivation under constant and time-varying temperature conditions

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Modelling

two modelling approaches

- Accurate modelling of heat transfer
  - Description of the phenomena induced to the food surface by a thermal process

- Modelling microbial inactivation behaviour under such temperature conditions

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Heat transfer model

estimation of surface temperature

- One dimensional heat transfer model
- Combination of different phenomena
  - Conduction
  - Convection
  - Evaporation/condensation of water or steam
  - Radiation (not considered)

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Heat transfer model

- Simplified model

  Conduction/convection - Fourier

  Geometry – plane sheet

  No mass transfer phenomena considered

- Limits

  Air velocity ranged from 15 m/s to 25 m/s

  Extrapolation locked outside of conditions that can be verified in the test rig

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Heat transfer model

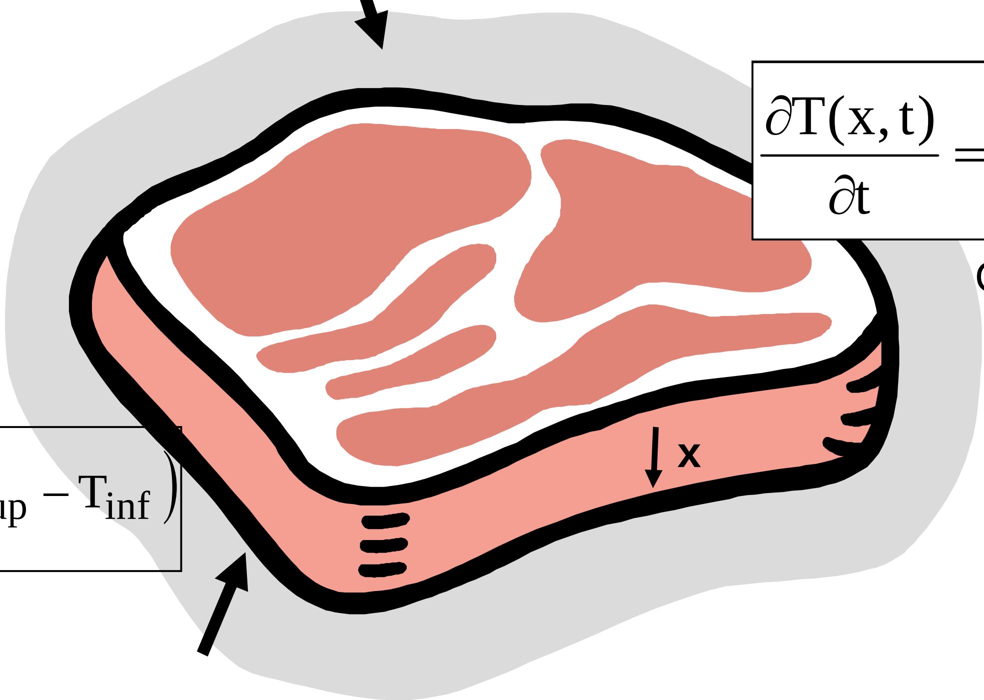
③

$$\lambda\left(\frac{\partial T}{\partial x}\right)_{top} = h_{eff}\left(T_{air\ or\ steam} - T_{sur}\right)$$

①

$$\frac{\partial T(x,t)}{\partial t} = \alpha \frac{\partial^2 T(x,t)}{\partial x^2}$$

Crank-Nicholson method



②

$$\lambda\left(\frac{\partial T}{\partial x}\right)_{bot} = h_{inf}\left(T_{sup} - T_{inf}\right)$$

t – time
T – temperature
h – heat transfer coefficient
α – thermal diffusivity
λ – Thermal conductivity

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Boundary conditions - bottom

Exchanges between the bottom of the product and the support can be neglected – product thickness < 0.5 cm

Experimentally observed in the rig

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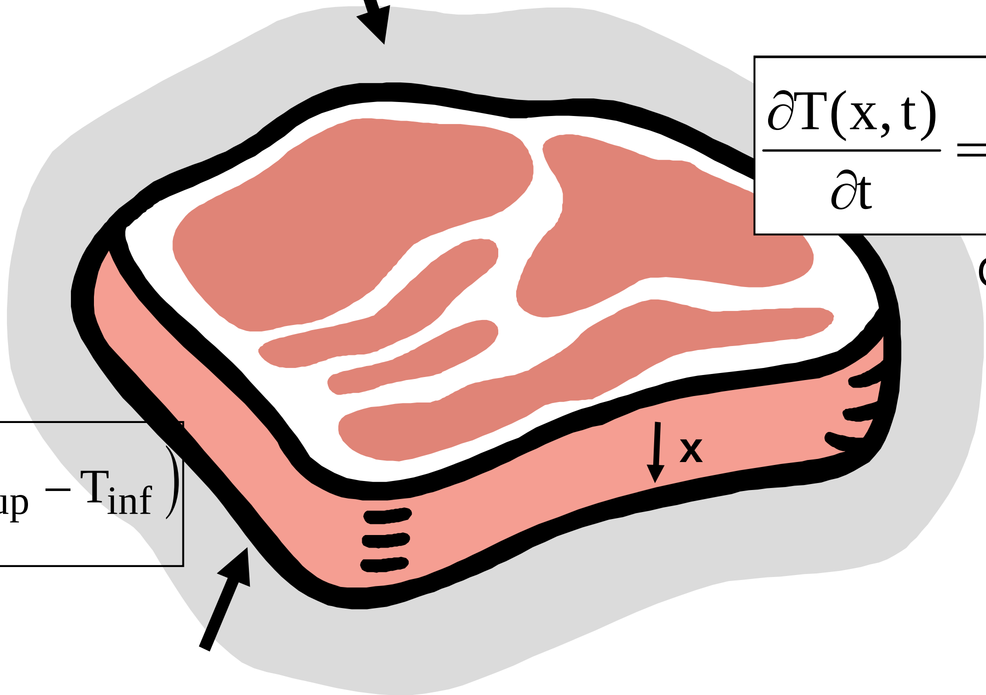
Heat transfer model

③

$$\lambda\left(\frac{\partial T}{\partial x}\right)_{top} = h_{eff}\left(T_{air\ or\ steam} - T_{sur}\right)$$



①

$$\frac{\partial T(x,t)}{\partial t} = \alpha\frac{\partial^2 T(x,t)}{\partial x^2}$$

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Effective coefficient – dry conditions

$$h_{eff} = h\frac{T_{air} - T_{sur}}{T_{max} - T_{sur}} + K_m \Delta H \frac{P_T - a_{w_{sur}} P_{T_{sur}}}{T_{max} - T_{sur}}$$

convection

evaporation

k_m – mass transfer coefficient
ΔH – latent heat of water evaporation

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Effective coefficient – wet conditions (steam)

~~h_{eff}~~

Due to complexity of mathematical expressions to be incorporated in the software

$$T_{sur} = T_{steam} - 3^{o}C$$

→ Experimentally observed in the rig

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Microbial inactivation model

- To predict microbial content at the surface of foods

- It has the advantage of dealing with time-varying temperature conditions

- Typical of pasteurisation treatments

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Microbial inactivation model

$$\frac{dN}{dt} = -k_{\max} k_Q(Q) N$$

$$\frac{dQ}{dt} = -k_{\max} Q$$

N – microbial cell density
Q – variable related to the physiological state of the cells
k_{max} – inactivation rate constant

$$\log \frac{N}{N_0} = \log(\exp(-k_{\max} t) \frac{1+Q(0)}{1+Q(0)\exp(-k_{\max} t)})$$

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Microbial inactivation model

$$k_{\max}(T, a_w) = \frac{\ln 10}{D_{ref}} \exp\left(\frac{\ln 10}{z} \left(T - T_{ref} \right) \right) \exp\left(\frac{\ln 10}{z_{a_w}} \left(a_w - 1 \right) \right) + c1$$

T = Tsur → Calculated on the basis of all considerations of heat transport

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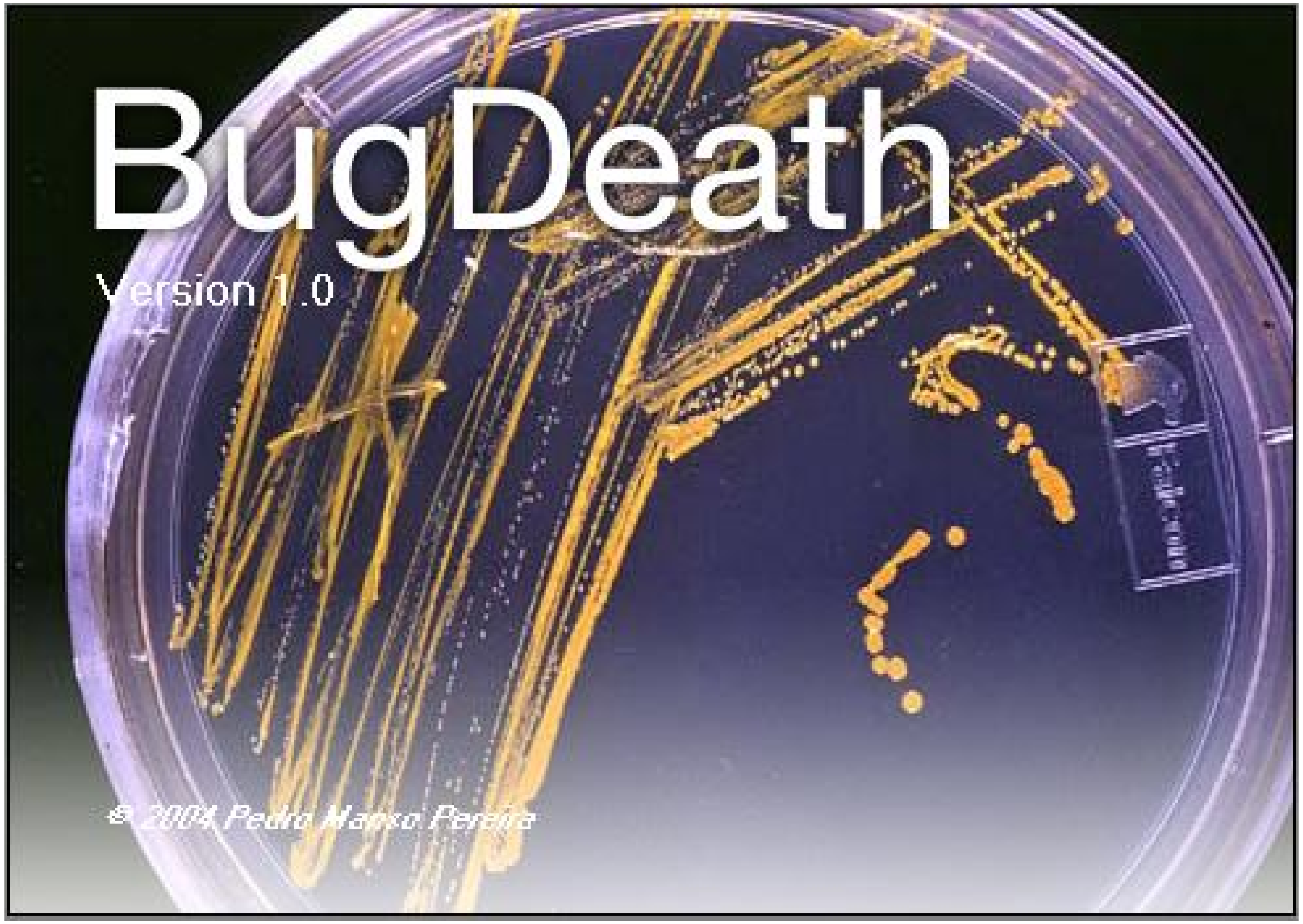
Software Program



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Software Program

- The programme was developed using Real Basic® 5.2 application
- Food/microorganism selection is allowed
  (database of thermal properties and kinetic parameters)
- On the basis of the selection of a heating regime of the medium, the programme allows prediction of the food surface temperature and simulates the microbial load content along the whole process time

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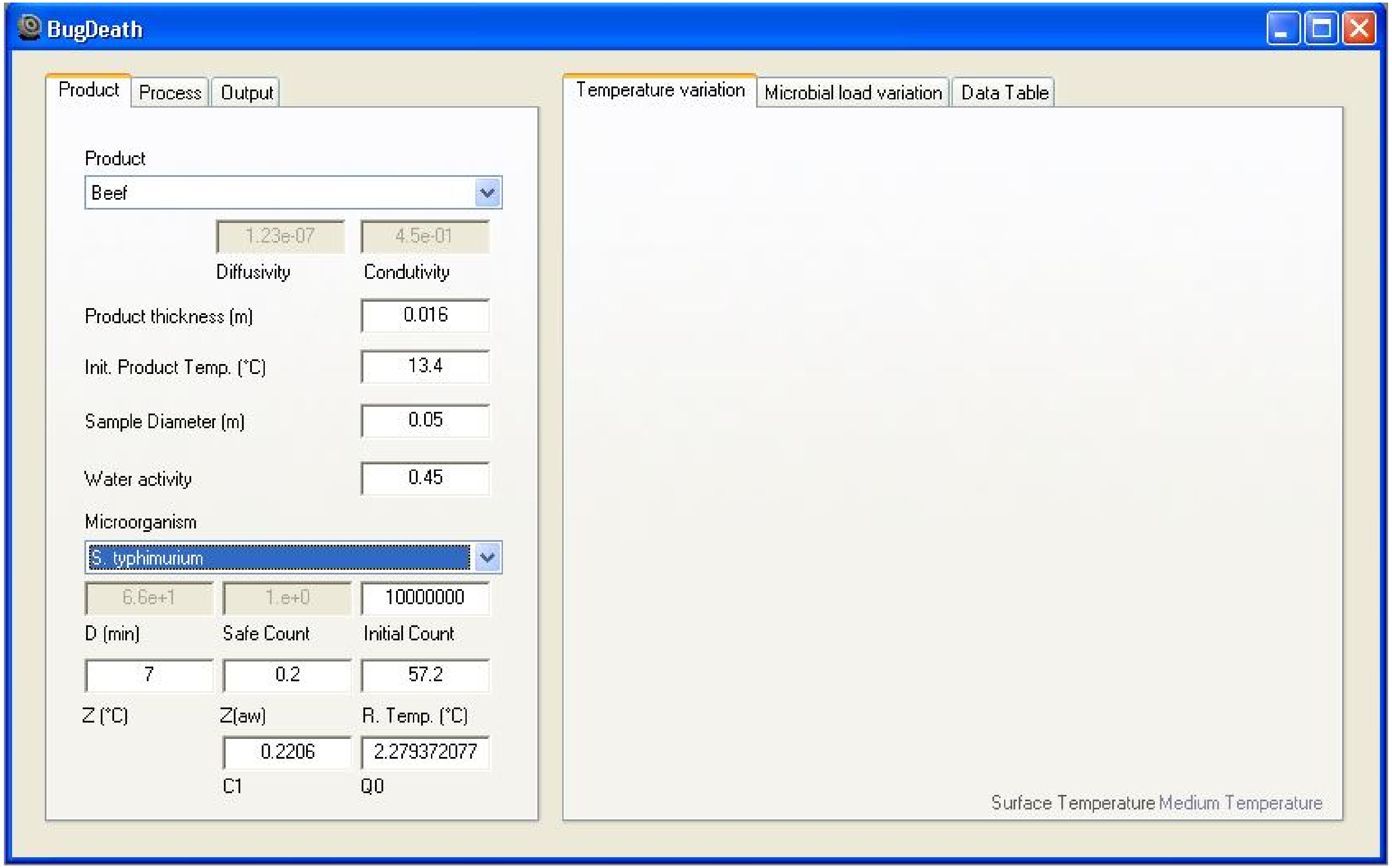
First screen – product / microorganism



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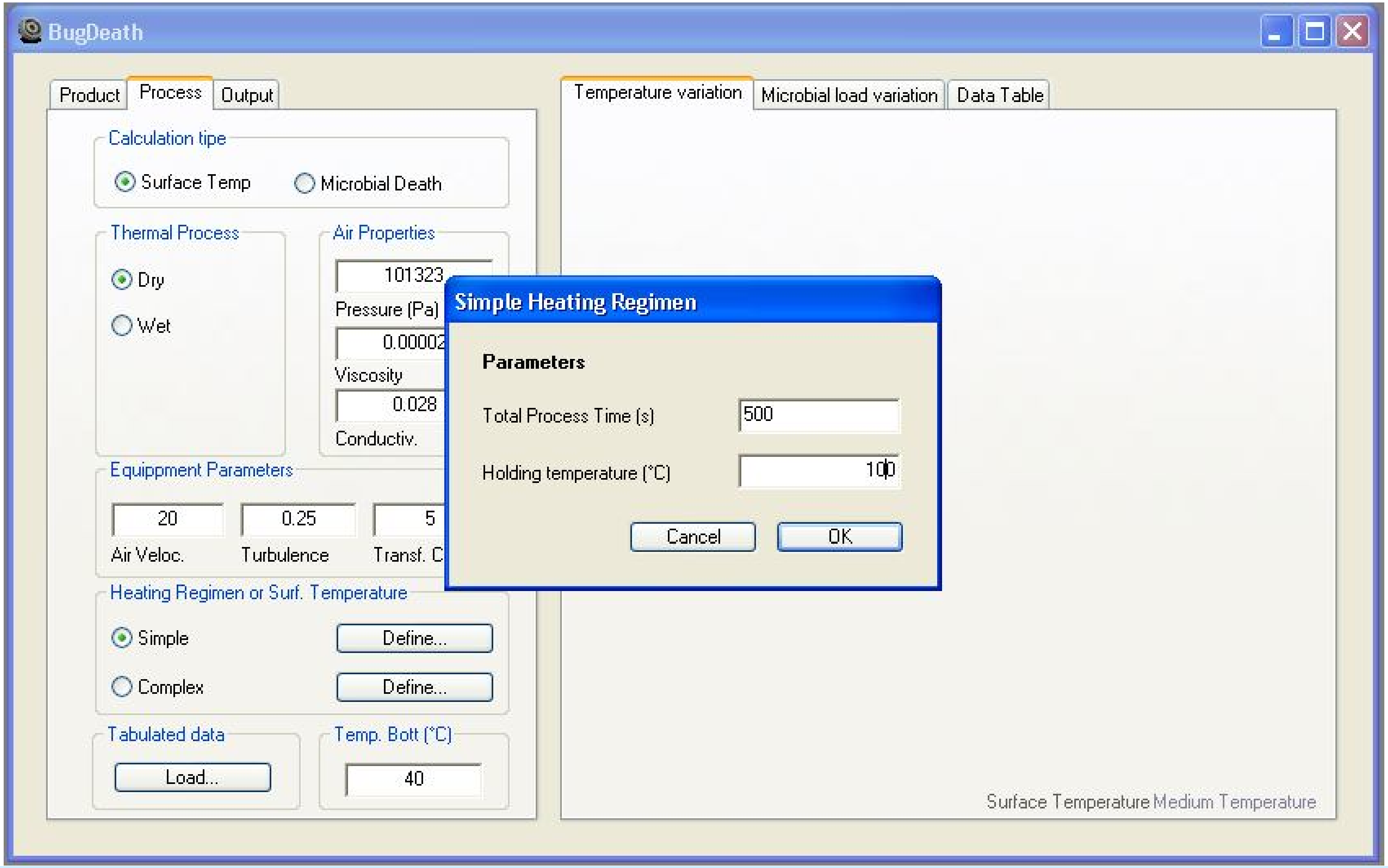
Second screen – process



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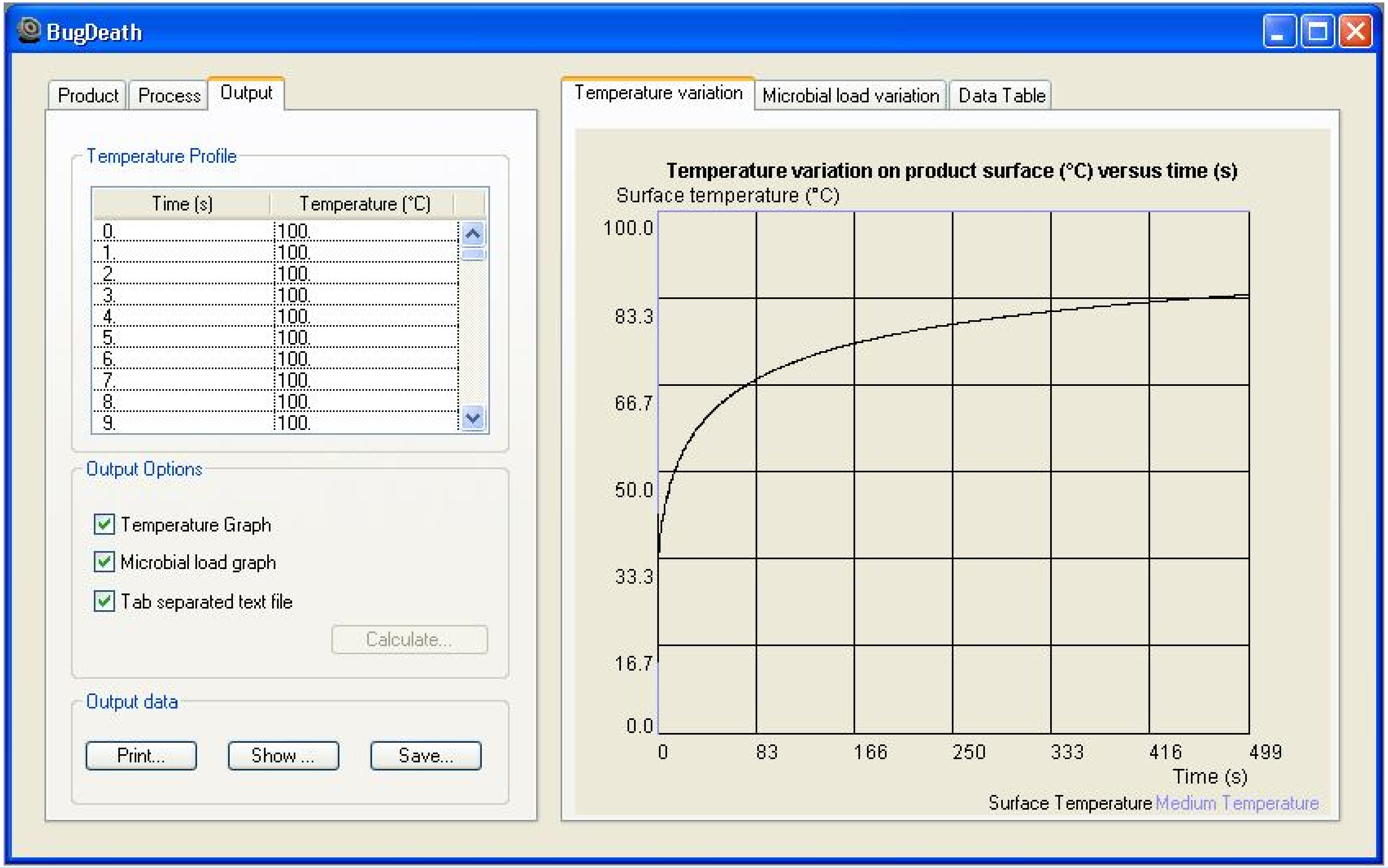
Output – graphic / temperature



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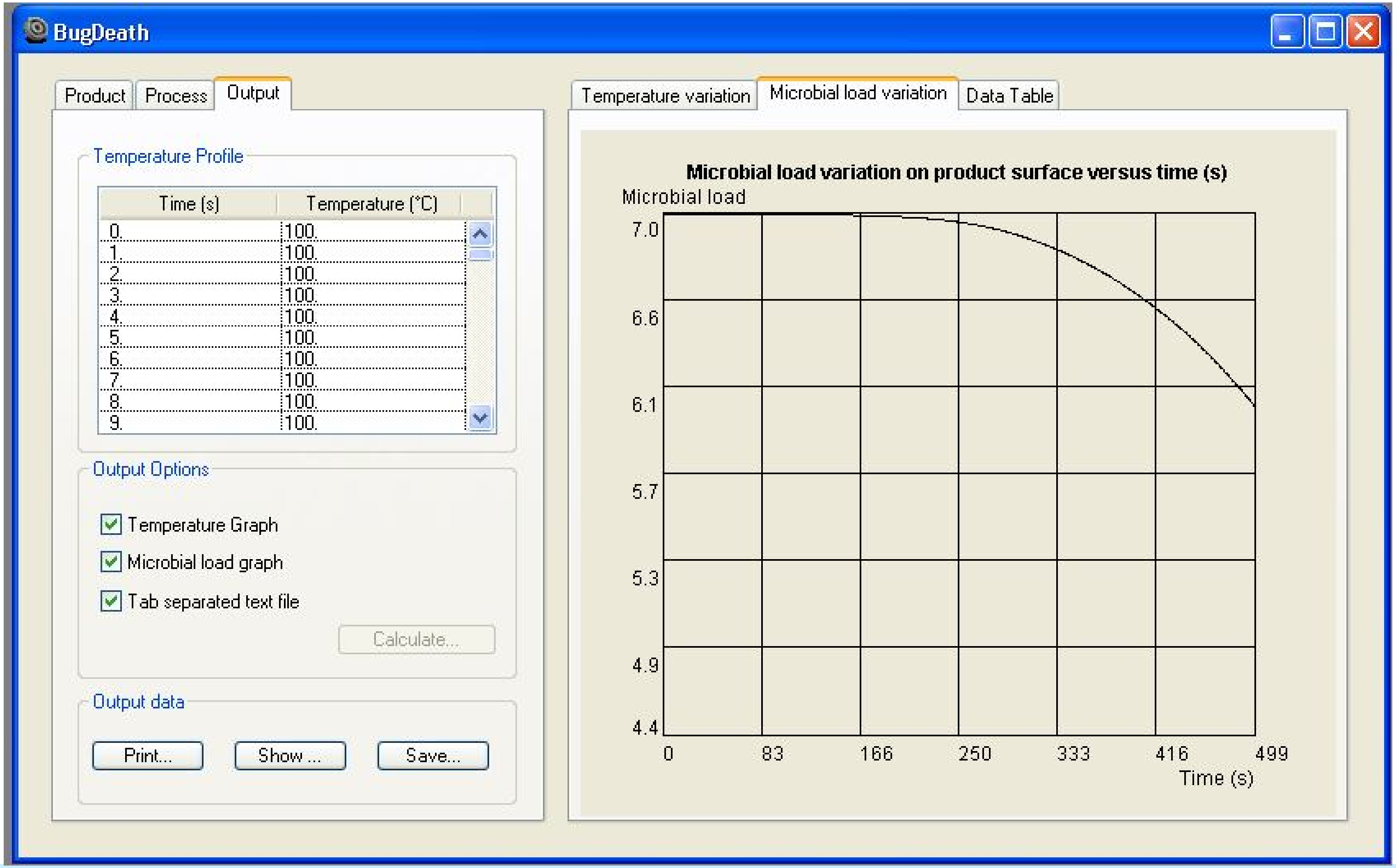
Output – graphic / microbial load



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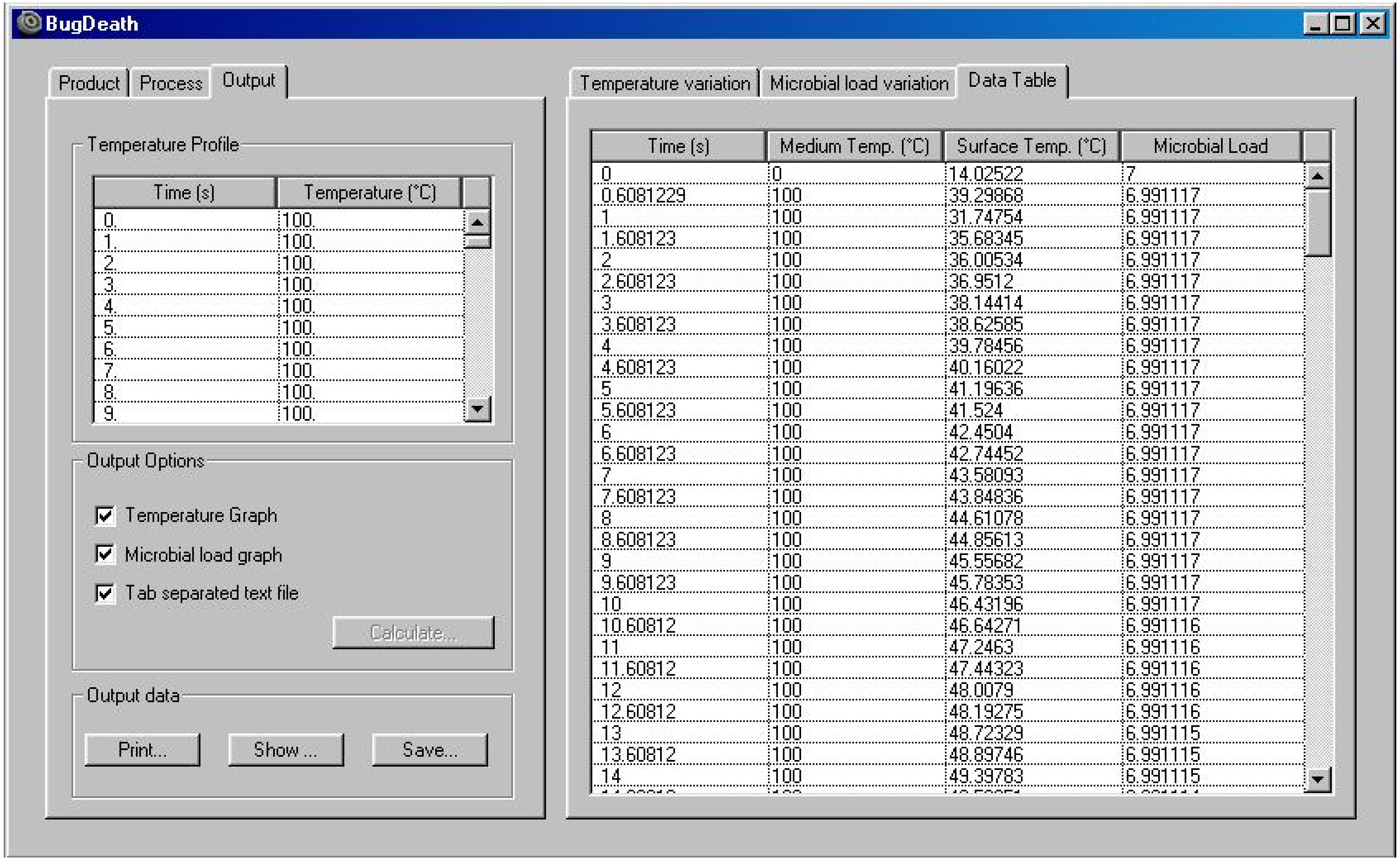
Output – data table



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Conclusions / outputs

- Software application simulates the results obtained in the rig apparatus
- Valuable for developing appropriate and safety thermal processes
- Marketed and commercially available
- Educational purposes (simulation of real food processes)

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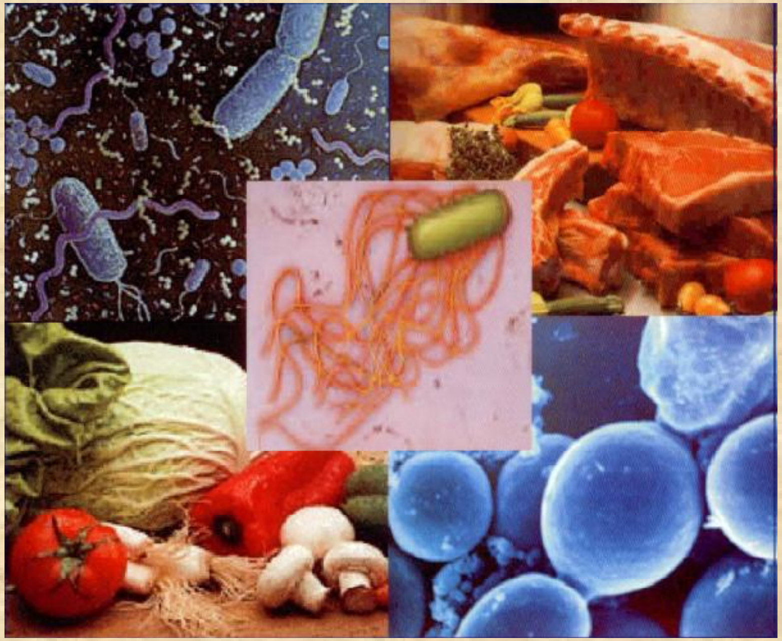

Predictive Microbiology

A tool to support food safety decisions

Thank you

Teresa Brandão and Cristina Silva

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Escola Superior de Biotecnologia

7th December 2007