Thermal inactivation of *Alicyclobacillus* spores in fruit product processing

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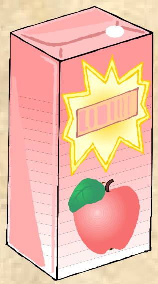
Brighton 4th – 7th July 2005

Alicyclobacillus acidoterrestris

- non-pathogenic
- spore-forming bacterium
- thermoacidophilic

found in
commercial pasteurized
fruit juices



Historical background

Alicyclobacillus acidoterrestris

in the 1980s ...

fruit juice industry faced a serious problem !!

consumers complained about spoilage juices

off flavor
loss of color
no gas production

before shelf life had expired

only a **spore former** could survive a thermal treatment in the pasteurization range

it had to be **acidophilic** to grow in acid juices

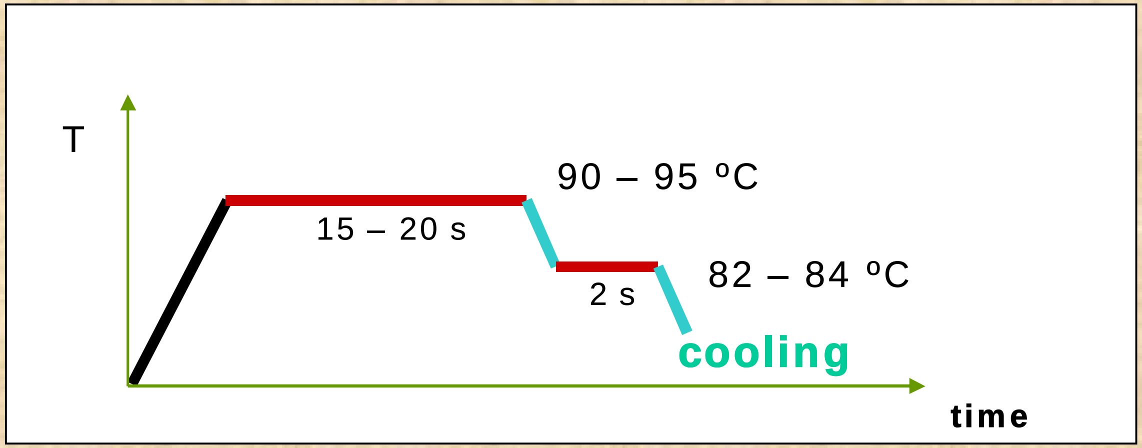
Design of the pasteurization processes

Fruit products — **juices, nectars, concentrates of purées**

acidic pH < 4.6

- ~~*Clostridium botulinum*~~
- **yeasts**
- **molds**
- **nonspore-forming bacteria**

Pasteurization



adequate for stabilisation at ambient temperature

Design of the pasteurization processes

Fruit products **juices, nectars, concentrates of purées**

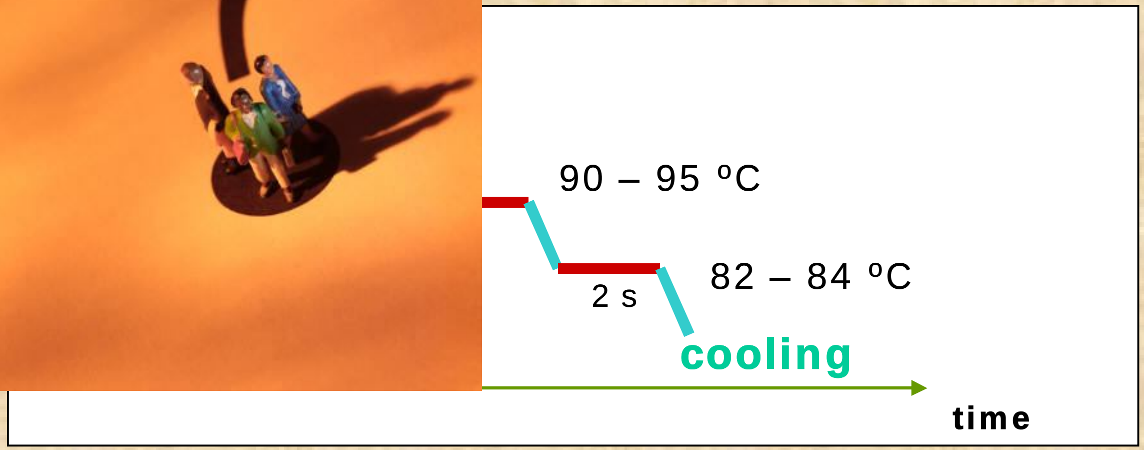
What was happening

acidic pH < 4.6

Clostridium botulinum

ming bacteria

Pasteurization



adequate for stabilisation at ambient temperature

What was happening

Historical background

Alicyclobacillus acidoterrestris

1984 The microbial growth was isolated and identified as a **new type of spoilage bacterium**

First studies in aseptically packaged apple juice

(Cerny et al.)

1987 The species was first named as ***Bacillus acidoterrestris***

(Deinhard et al.)

1992 Reclassified as a new genus Alicyclobacillus, becoming ...

Alicyclobacillus acidoterrestris

(Wisotzkey et al.)

Pasteurization

… should inactivate **microorganisms’ vegetative cells**

bacteria
yeasts
molds

safety

and **enzymes**

quality

prevent the degradation of the original **organoleptic** and **nutritive** fruit characteristics

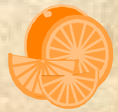
however ...

thermal processes affect negatively quality factors

Design and optimization of pasteurization processes

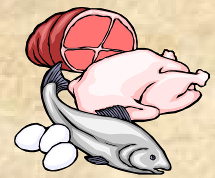
Pasteurization

acidic foods



↓

inactivation of non-pathogenic microorganisms and enzymes



non-acidic foods

↓

inactivation of pathogenic microorganisms

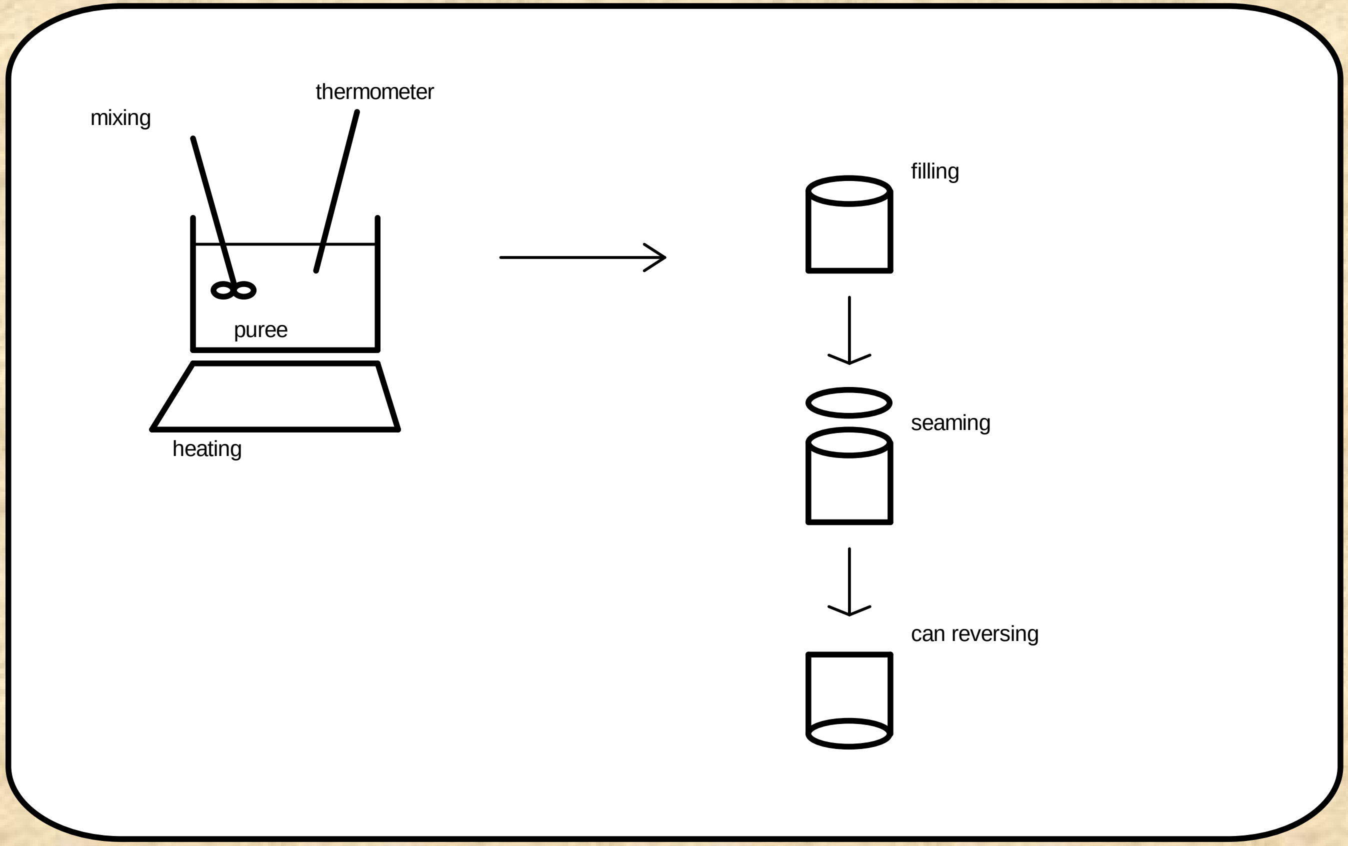
Pasteurization methods

... applied to several fruit products

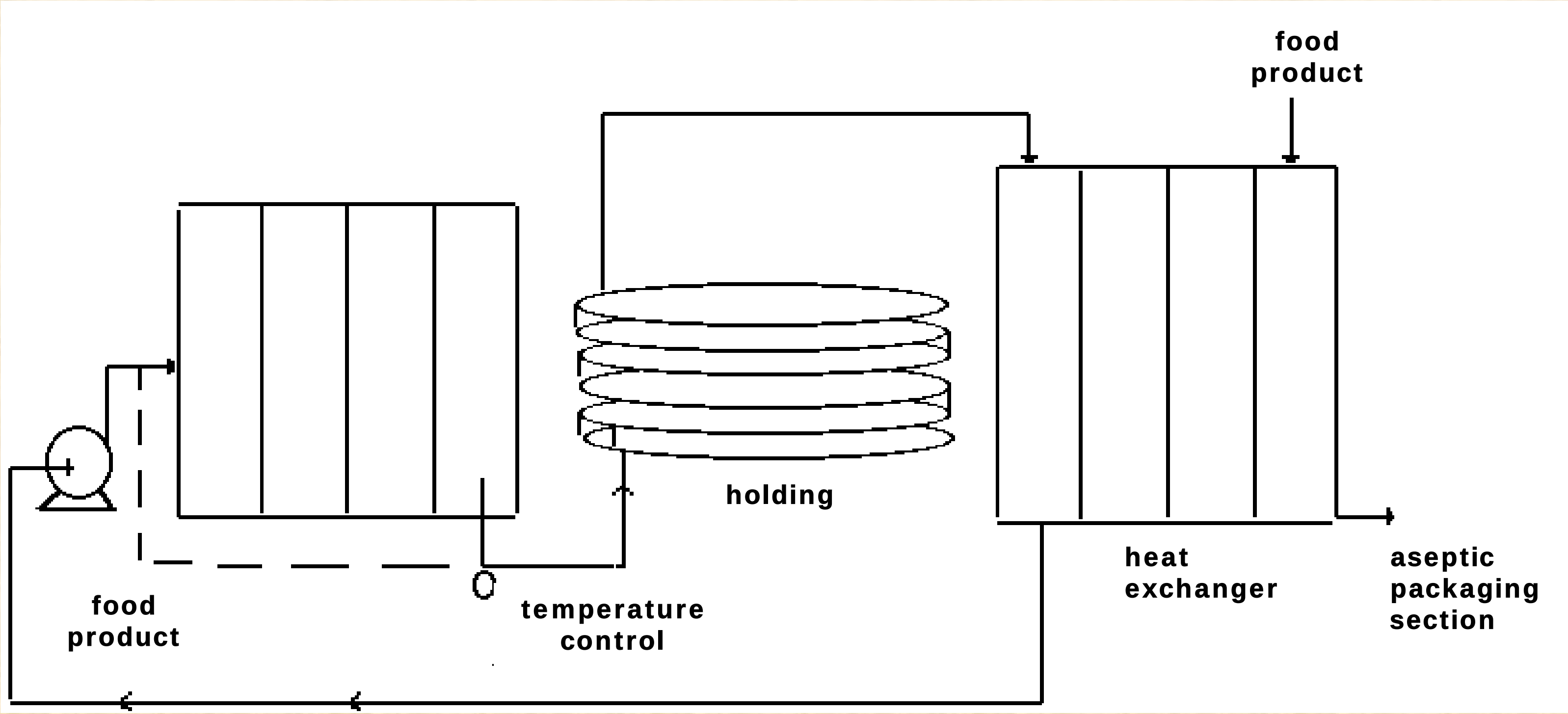
- hot filling
- aseptic process
- traditional canning

temperatures < 100 °C

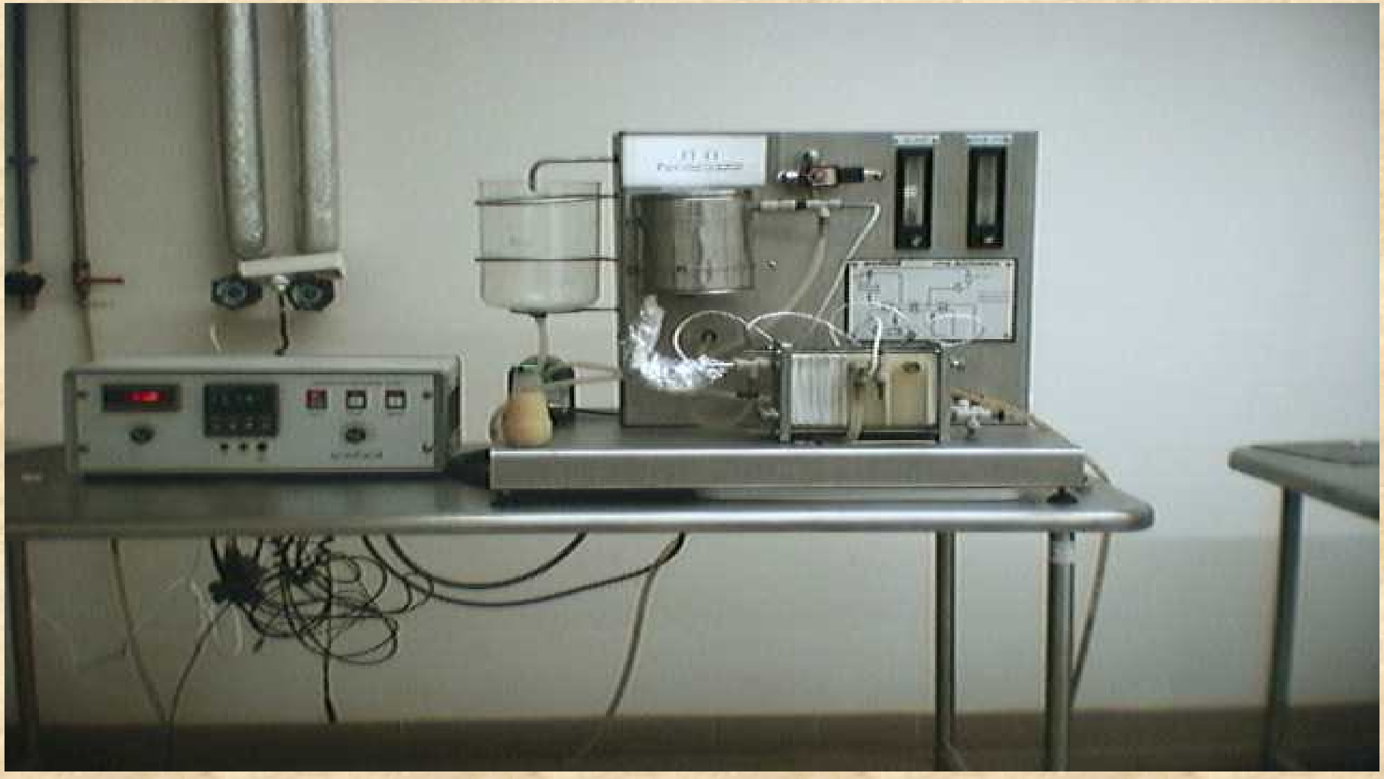
- **hot filling**


thermometer
mixing
puree
heating
filling
seaming
can reversing

- **aseptic process**



- **aseptic process**



- **traditional canning**

Target

... for shelf-stable high acidic fruit products

not very clear !

- Target **microorganisms/enzymes** and its inactivation requirements are not defined or vary with product

- No regulation available – depends usually on industrial experience

time / temperature ?

Alicyclobacillus acidoterrestris spores as pasteurization target

in 2000

establishment of a new pasteurization criterion for shelf-stable high-acidic fruit products

↑

***Alicyclobacillus acidoterrestris* spores**

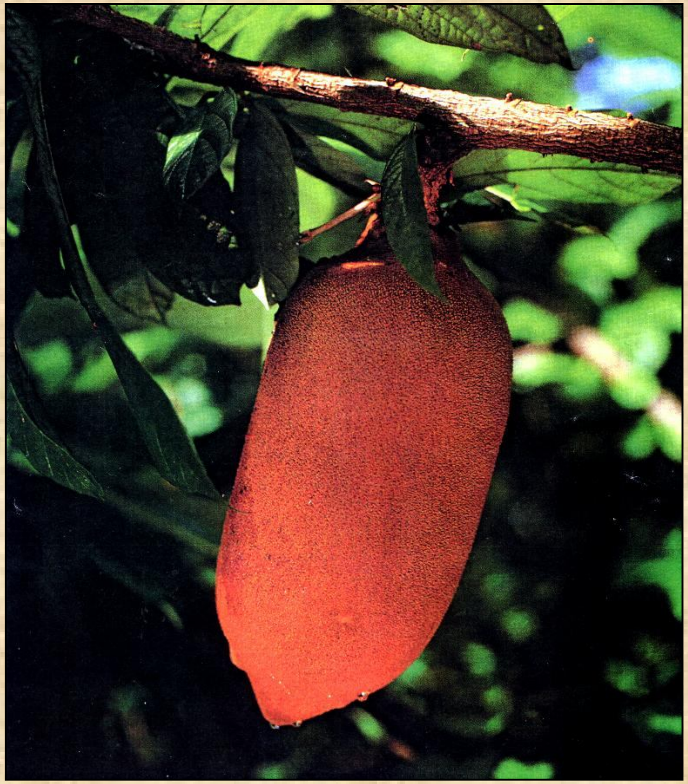
Filipa Silva (2000) **Ph.D. thesis** developed in Escola Superior de Biotecnologia Universidade Católica Portuguesa, Portugal.

Research project:

Multidisciplinary Study of the Transformation of Amazonian Fruits for their Commercial Valorization Aiming at the Development of Local Rural Communities

EU (DGXII) - program STD3

Portugal, Belgium, France and Brazil



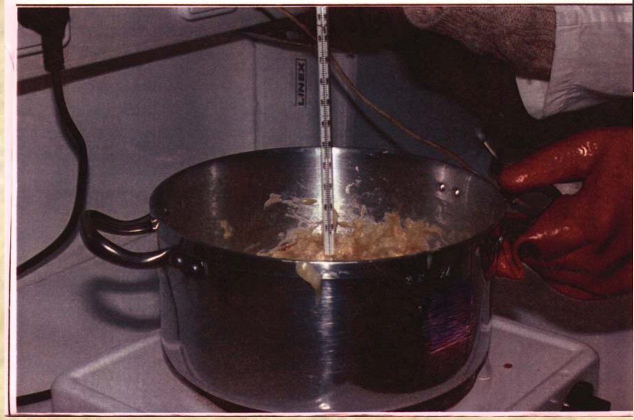
Cupuaçu

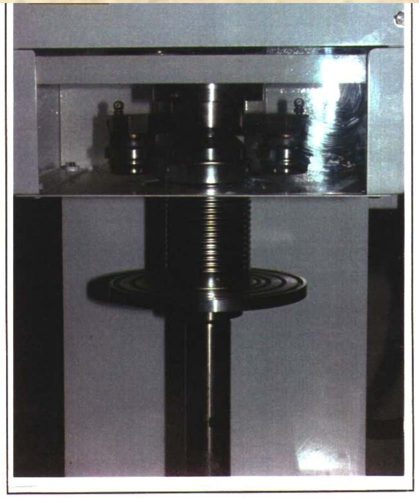
Theobroma grandiflorum

Design and optimization of pasteurization processes

Design and optimization of pasteurization processes

- **hot filling**





pasteurization conditions for cupuaçu pulp

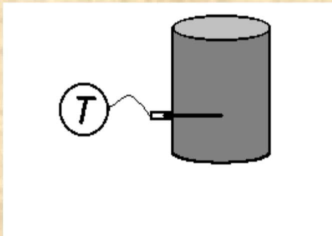
treatment	heating time (min)	holding time (min)	holding temperature (ºC)
hot-filling	8.0	15	70
	8.2	10	75
	10.3	5	80
	11.3	2	85
	13.8	0	87
isothermal pasteurization conditions	3.7	1.3	70
	3.7	1.3	90

thermostatic water baths of TDT cans filled with cupuaçu pulp

pasteurized cupuaçu pulp

evaluation of the process

at the coldest point



estimation of
***Alicyclobacillus acidoterrestris* spores load**

How was it achieved ????

function of time

$$\frac{N}{N_0} = 10^{\left(\frac{-1}{D_{T_{ref}}} \int_0^{PT} 10^{\frac{T - T_{ref}}{z}} dt\right)}$$

estimated parameters

D decimal reduction time
z- value

N	number of viable spore cells
N_0	number of initial viable spore cells
T	temperature
T_{ref}	reference temperature
PT	total process time

pasteurized cupuaçu pulp

ambience storage for 6 months

microbial inactivation

- total counts
- moulds
- yeasts

product stabilisation

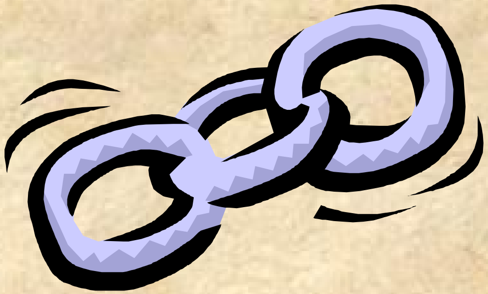
- colour
- soluble solids
- pH
- reducing sugars

evaluation of the process

The role of mathematical modelling

predictive microbiology

microbiology



statistics

mathematics

Predictive microbiology

The use of **mathematical models** in the description of **microbial responses** to environmental stressing factors

is gaining considerable importance in the food processing domain, particularly in the **design** of **efficient** and **safe inactivation treatments**

The role of mathematical modelling

objective

precise and accurate description of observations

model adequacy

quality of model parameters

The role of mathematical modelling

advantages

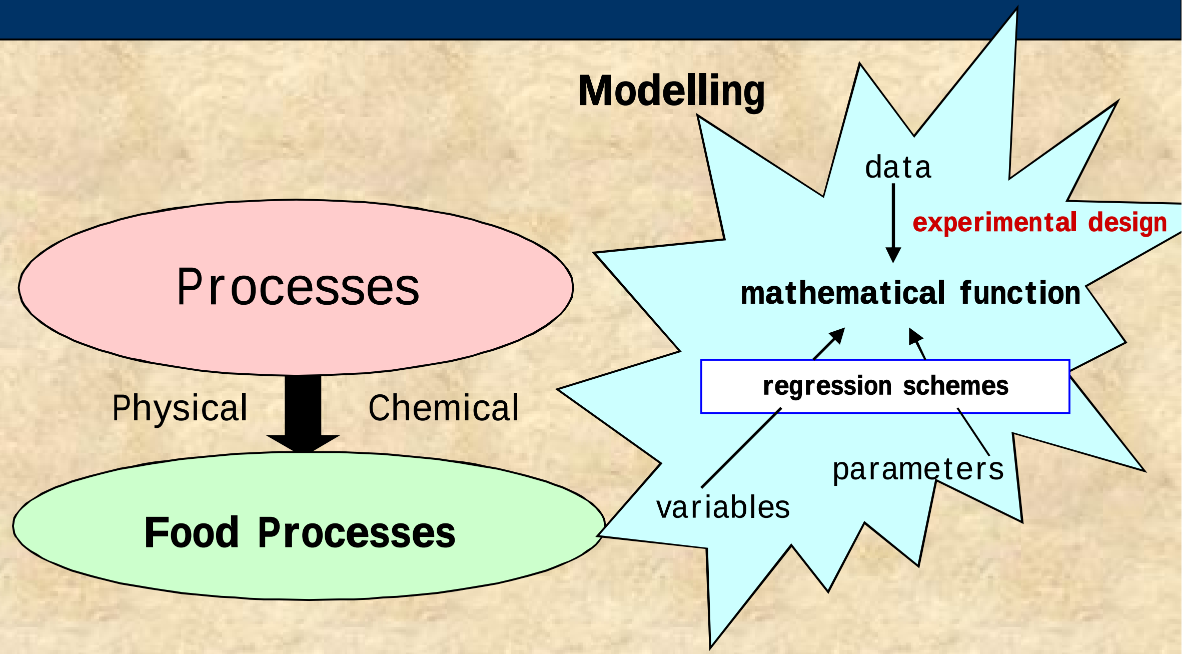
- knowledge of the process
- process effect on product
- control of process variables

The role of mathematical modelling

Transport Phenomena
- heat
- mass
- *momentum*

Reaction kinetics

Properties



The role of mathematical modelling

Transport Phenomena
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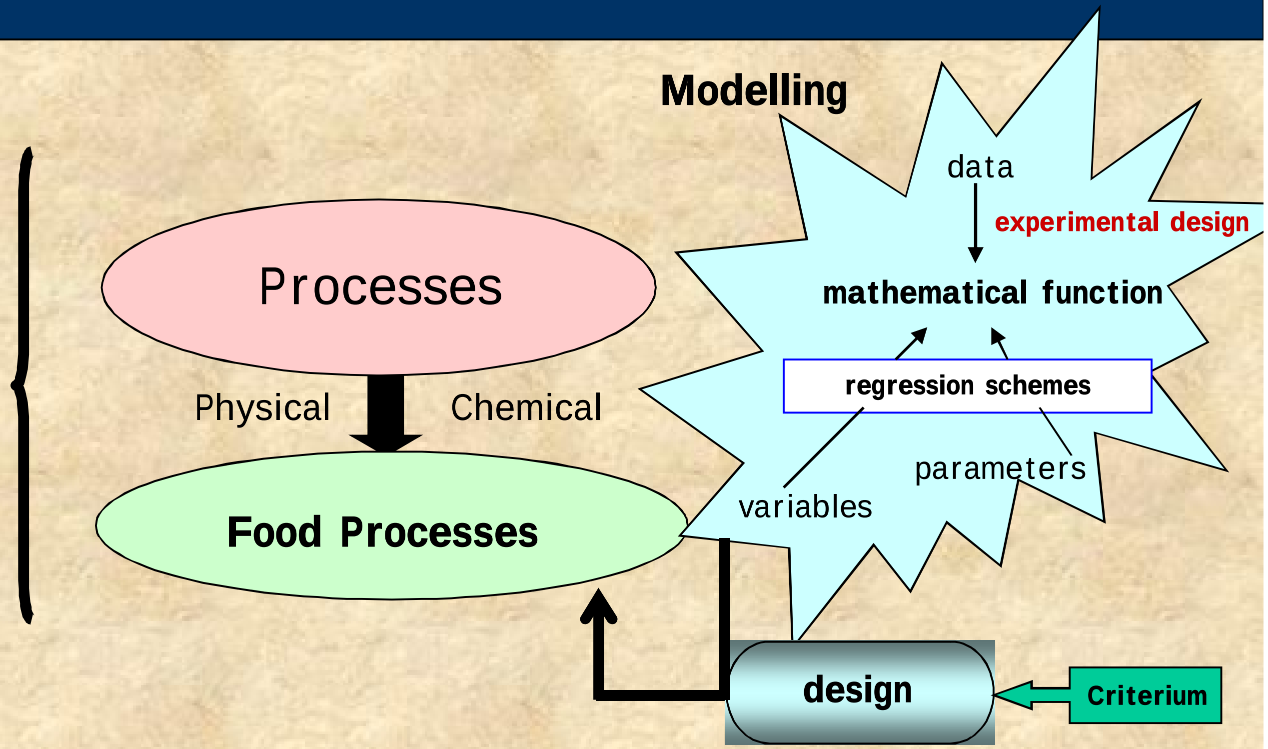
Reaction kinetics

Properties



The role of mathematical modelling

Transport Phenomena
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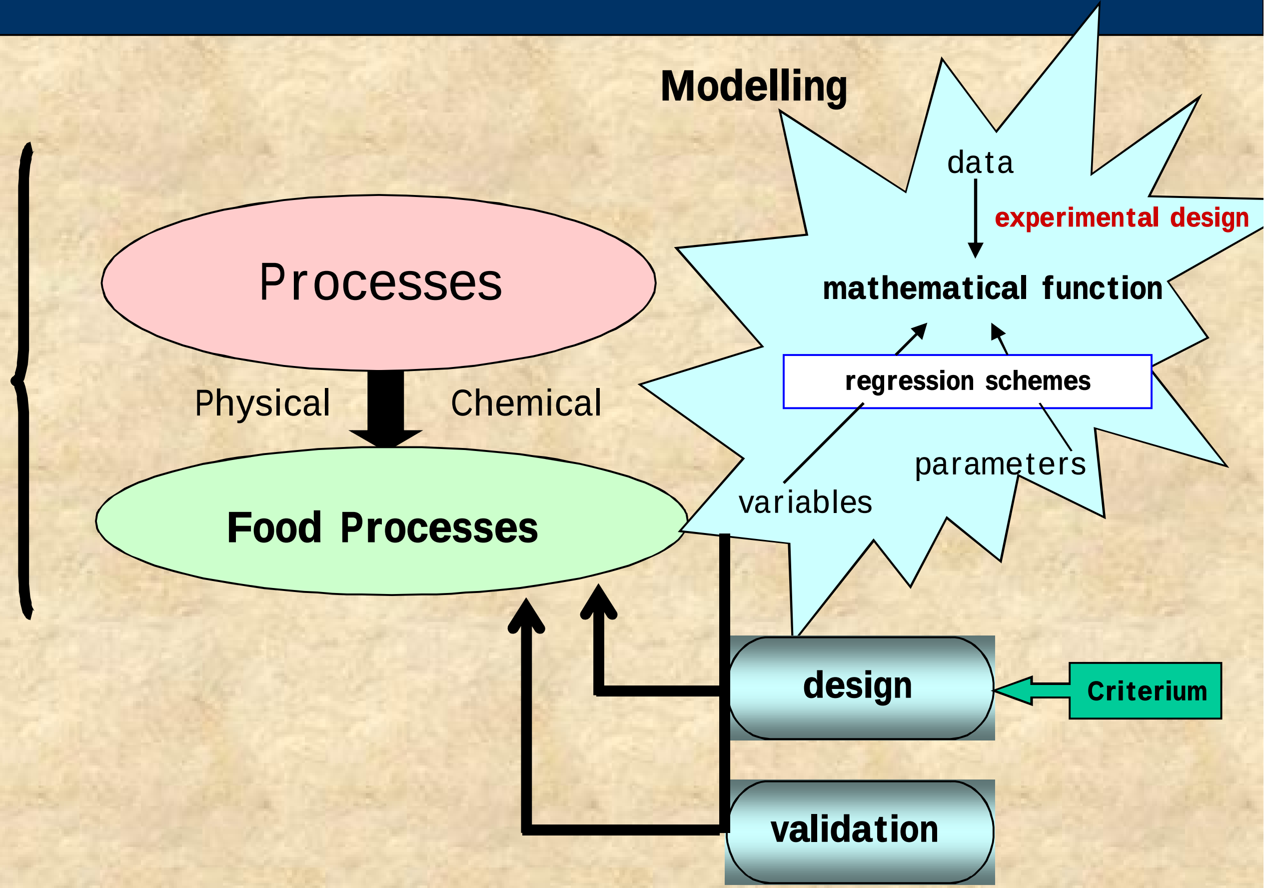

The role of mathematical modelling

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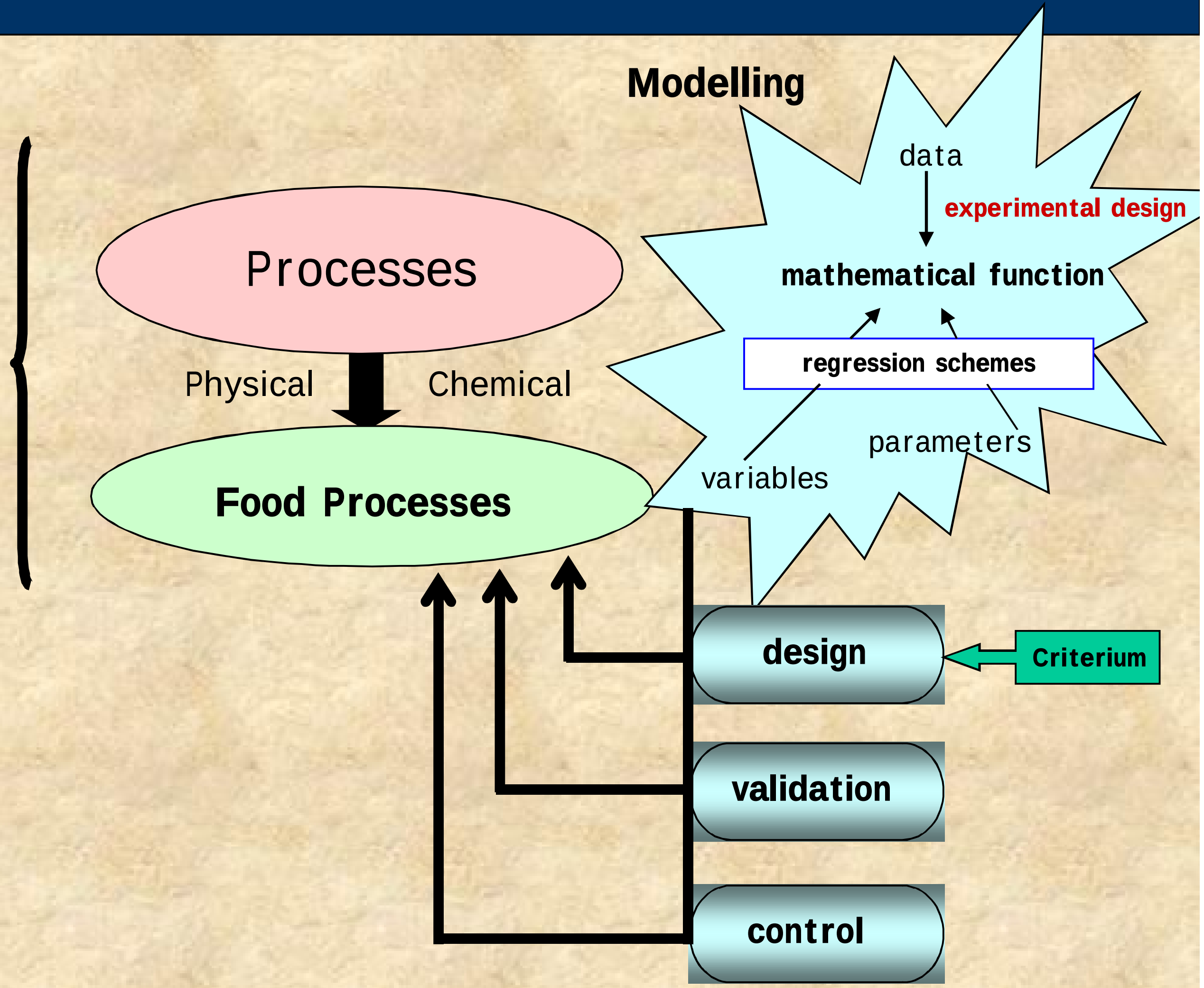

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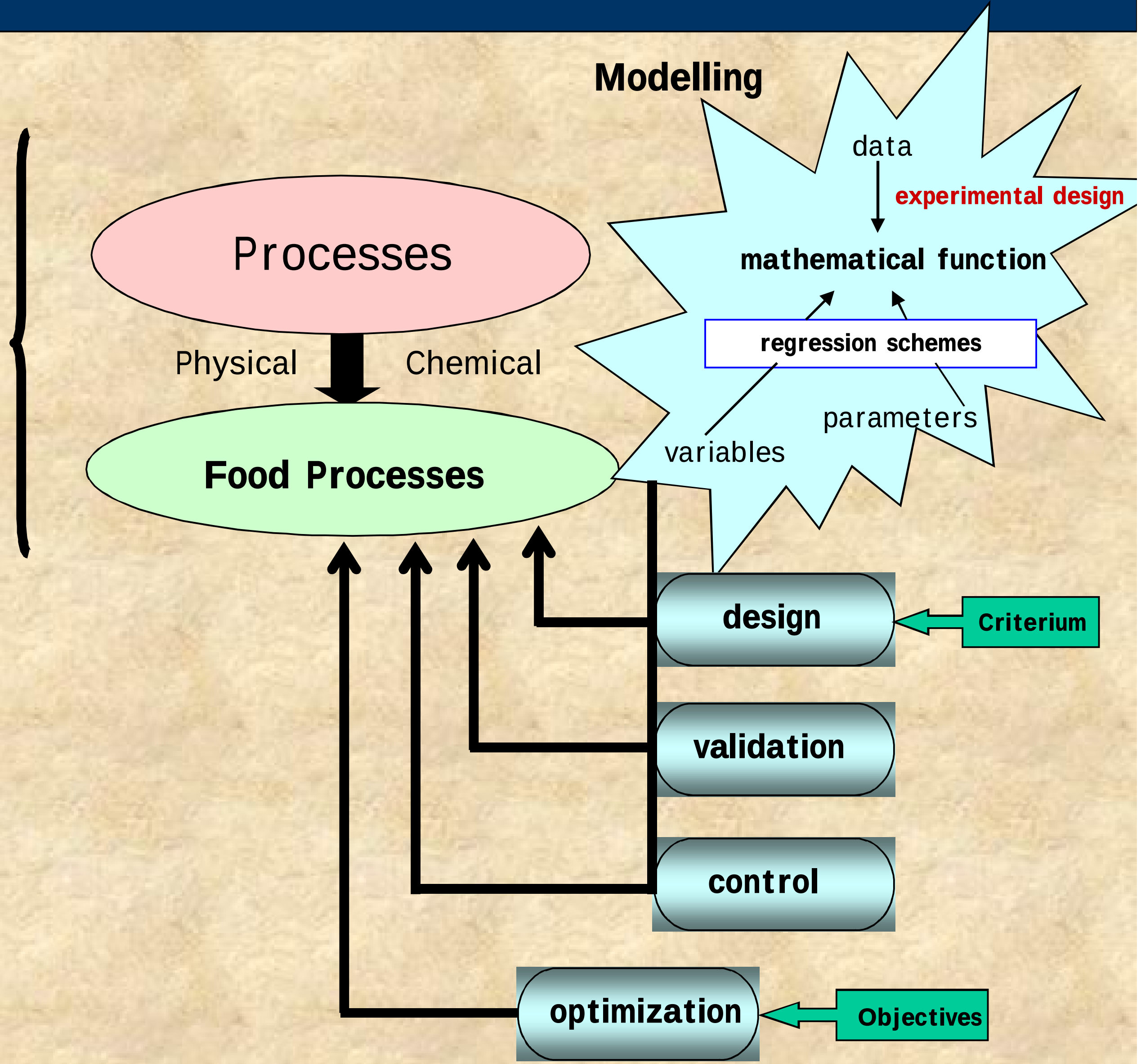

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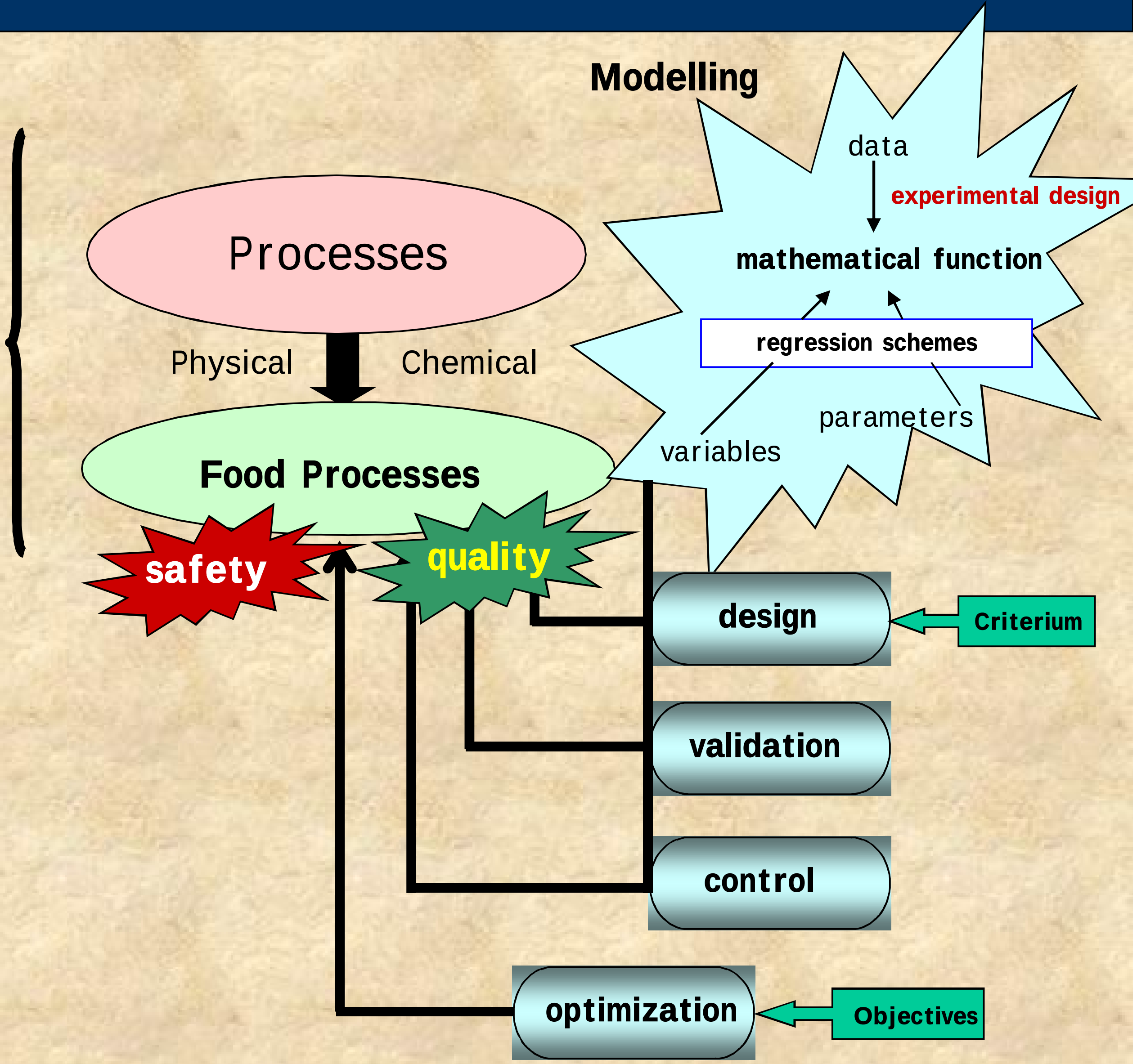


The role of mathematical modelling

aplication

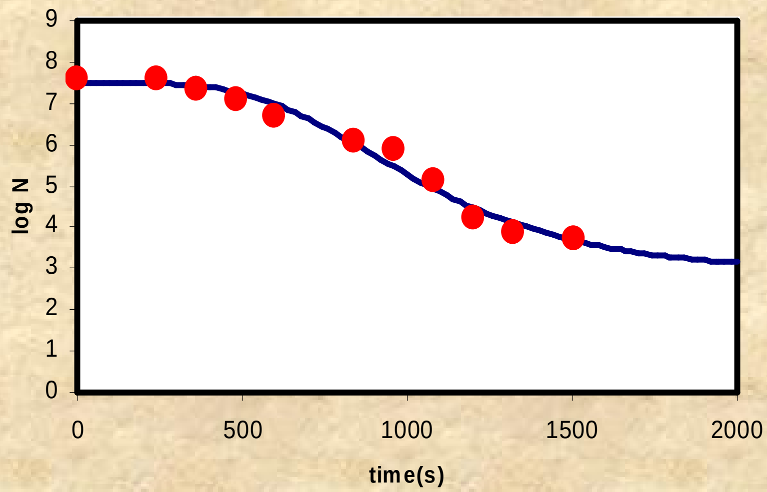
- prediction / simulation
- development of efficient inactivation processes

↓

contribution to safety

inactivation

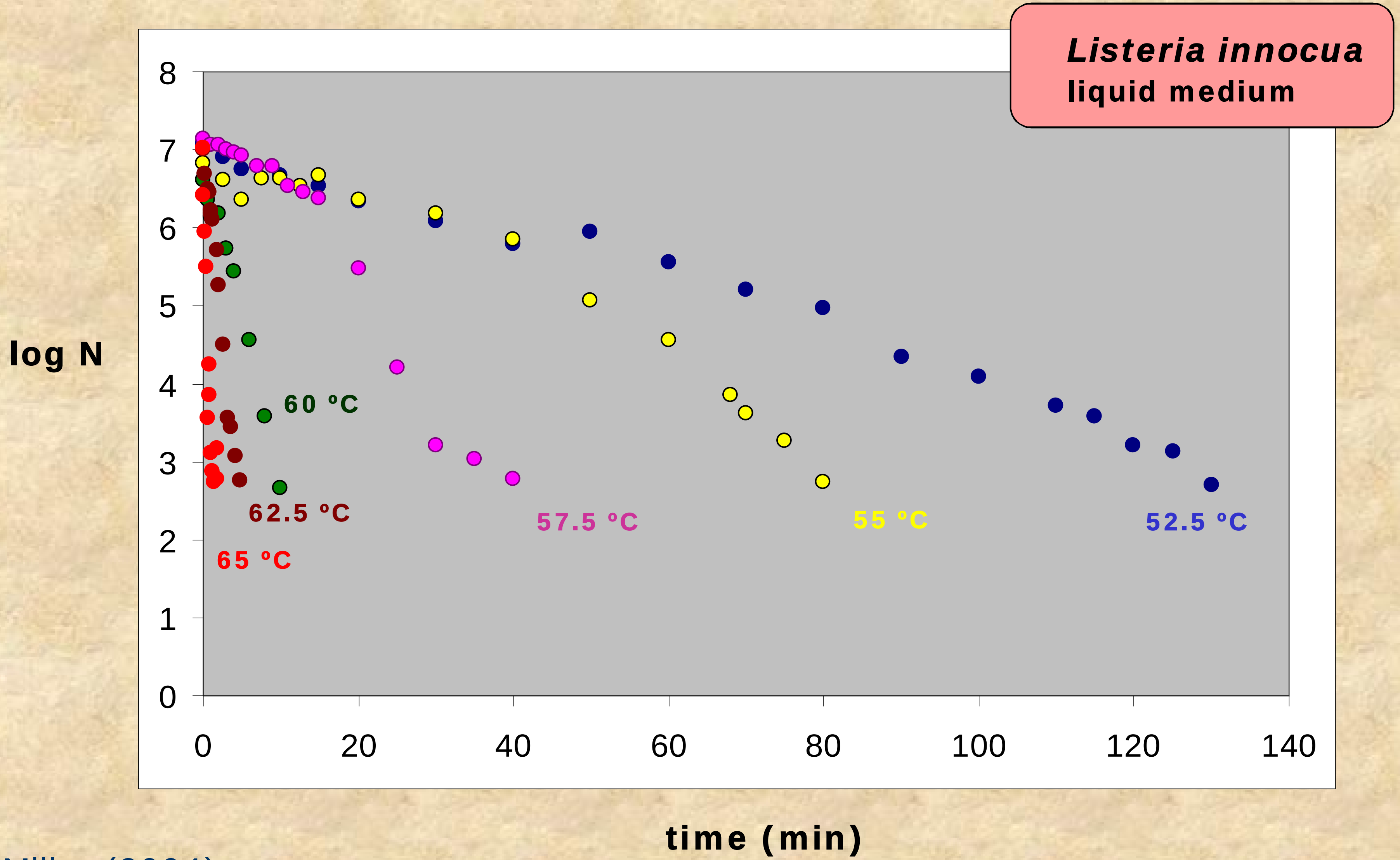
sigmoidal behaviour



presence of aggregated microorganisms or sub populations

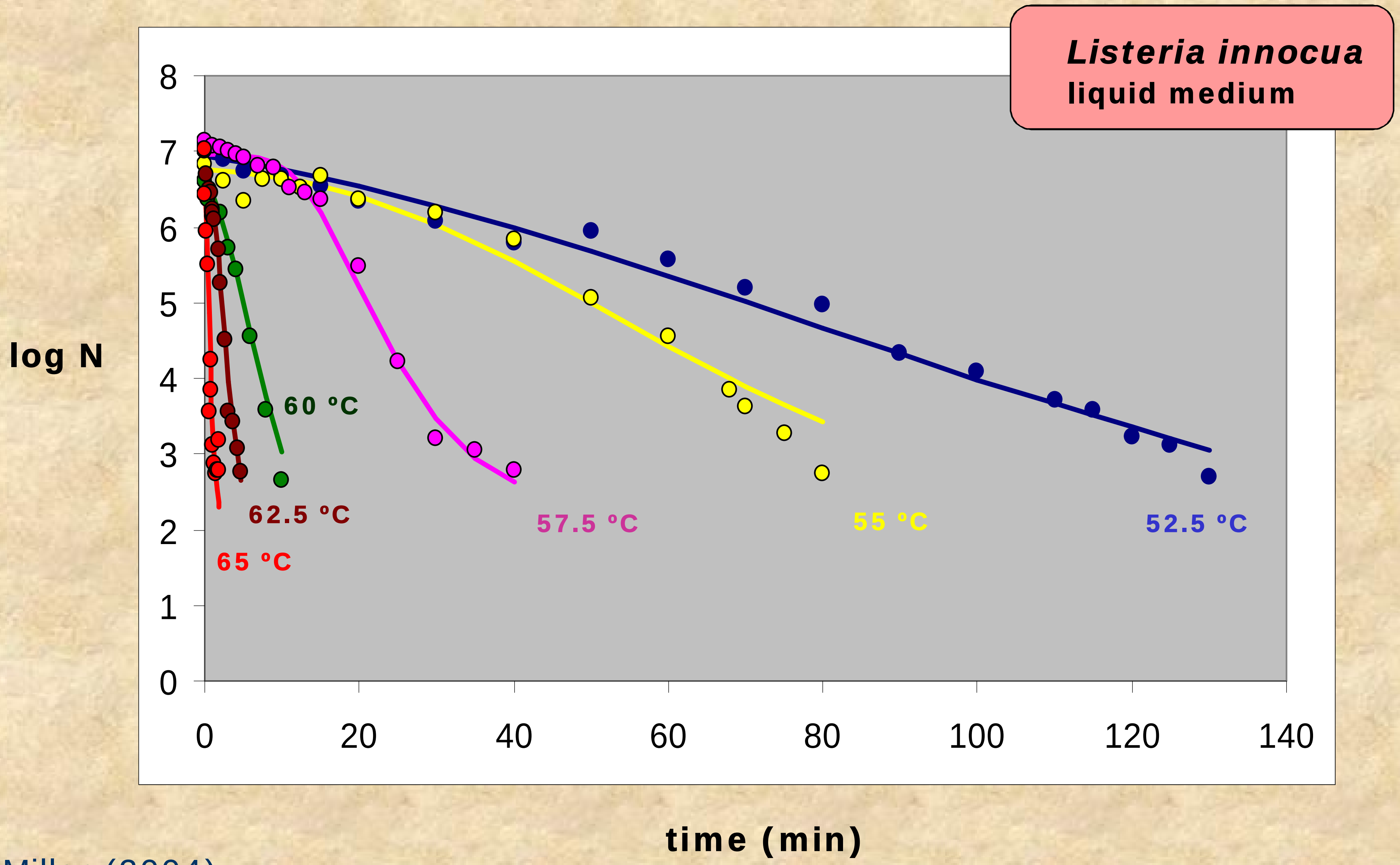
more **heat** (or other **stress factor**) **resistent**

Examples



Miller (2004)

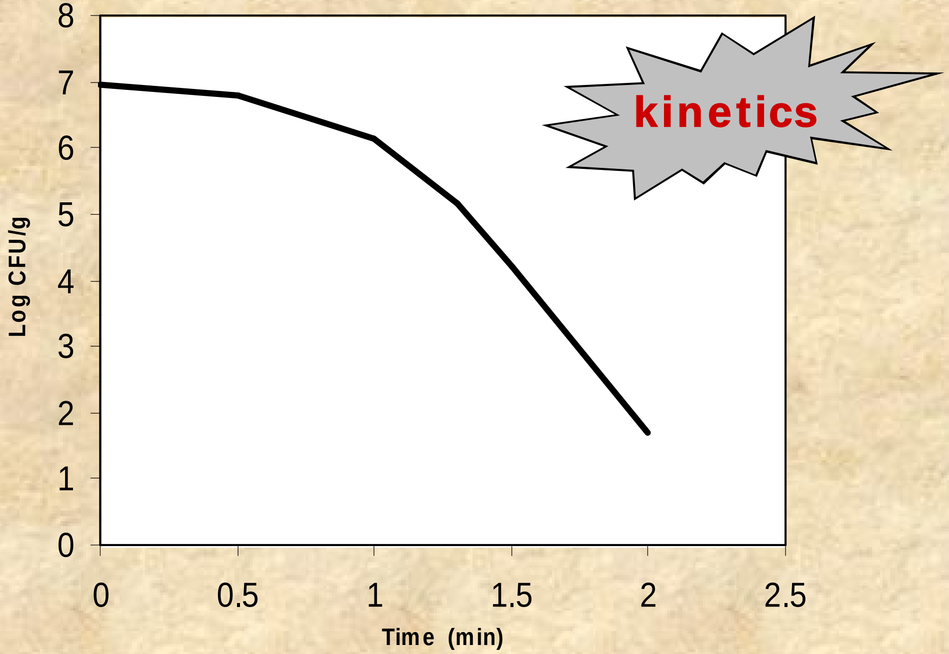
Examples



Miller (2004)

Mathematical models

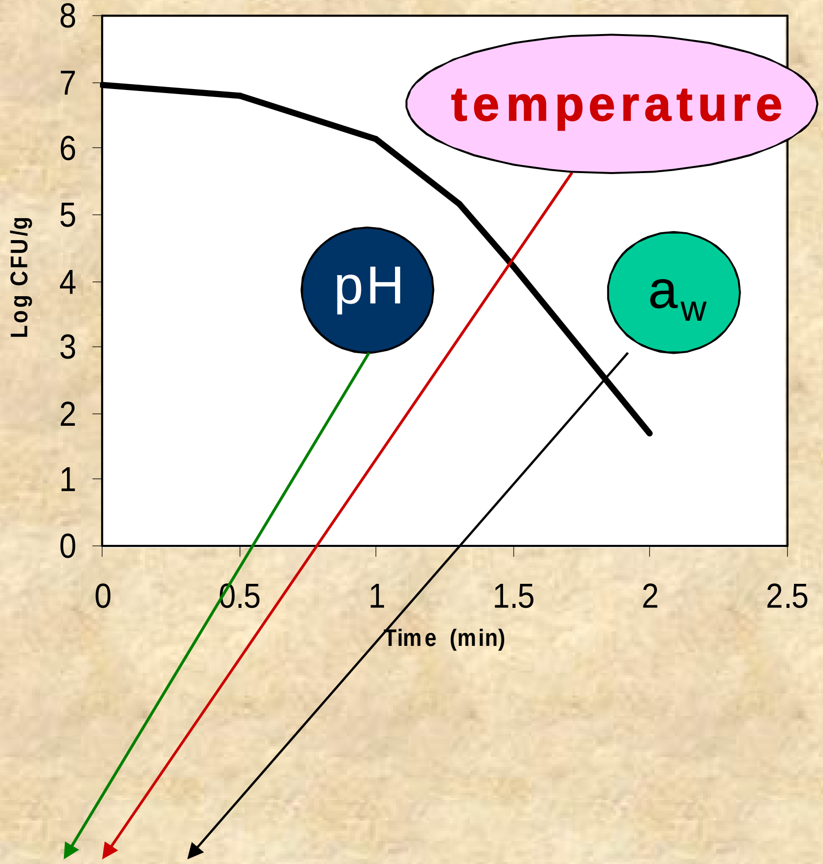
❖ **primary**



parameters

Mathematical models

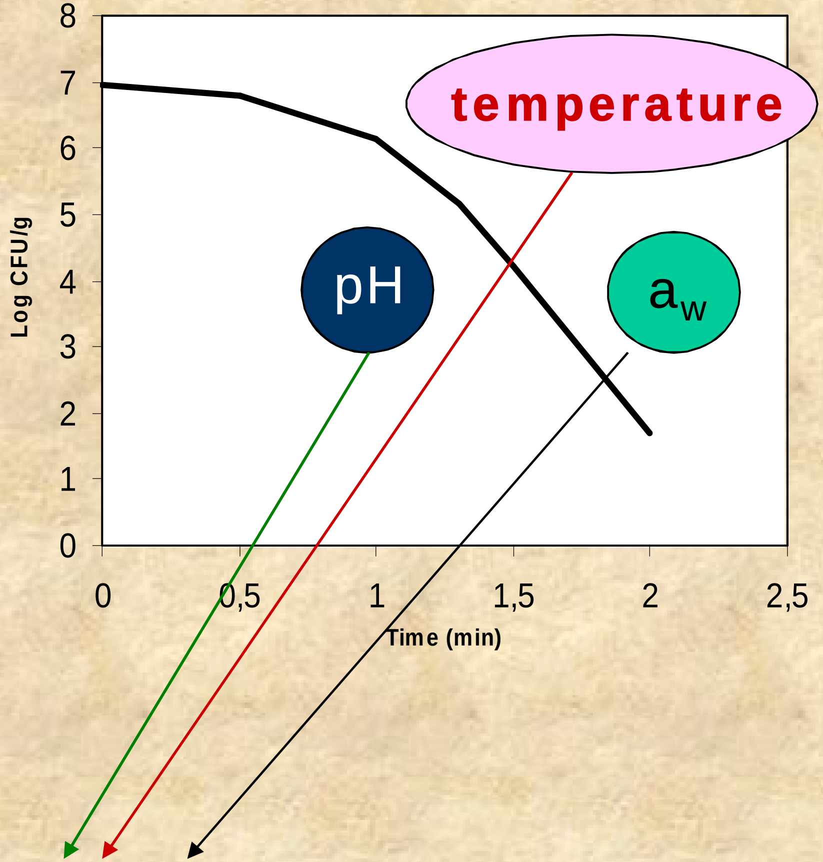
❖ **primary**



❖ **secondary**

parameters

❖ **primary**



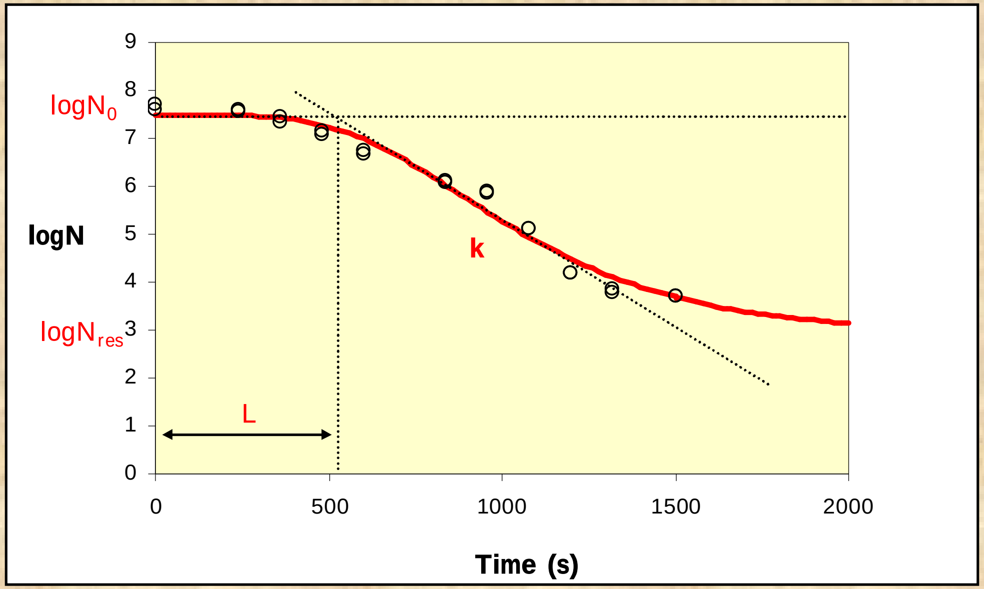
❖ **secondary**

parameters

❖ **terciary -** integration of the previous models - **software**

Inactivation models

❖ **primary**



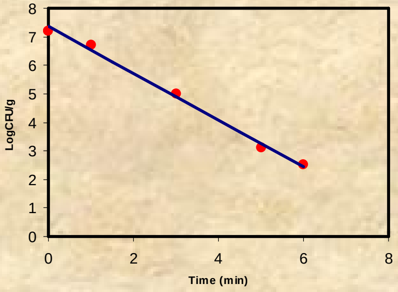
N_0 number of initial viable spore cells
N_{res} number of residual spore cells
k maximum inactivation rate
L lag or shoulder

empirical

fundamental

Inactivation models

- **primary**



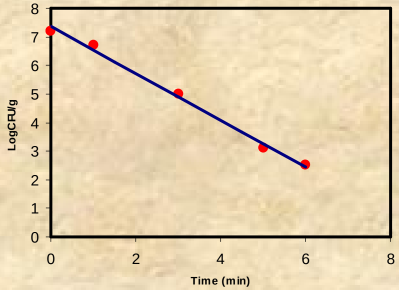
$$N = N_0 \exp(-kt) \quad \Leftrightarrow \quad \log N = \log N_0 - \frac{t}{D}$$

First order

D – decimal reduction time

Inactivation models

❖ primary



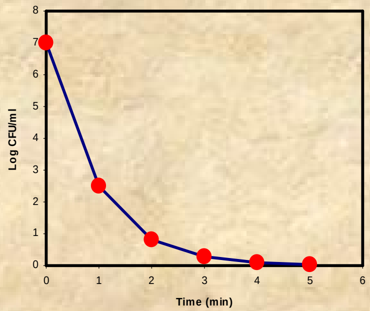
$$N = N_0 \exp(-kt) \iff \log N = \log N_0 - \frac{t}{D}$$

First 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

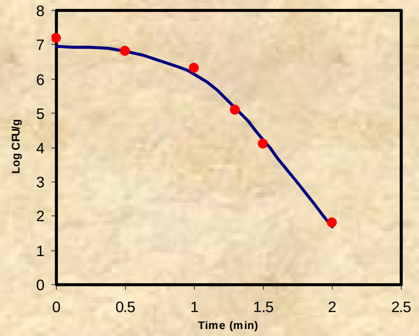
F_1 – fraction of inactivated microorganisms
k_1 e k_2– kinetic constants

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

Kamau et al. (1990)

Inactivation models

❖ **primary**



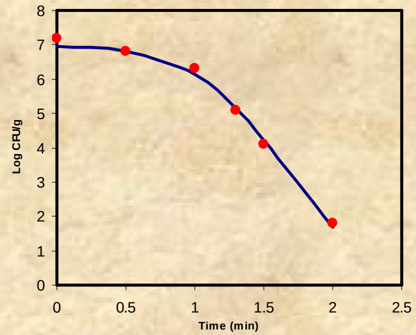
Whiting & Buchanan (1992)

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

L – lag or shoulder

Inactivation models

- **primary**



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

L – lag or shoulder

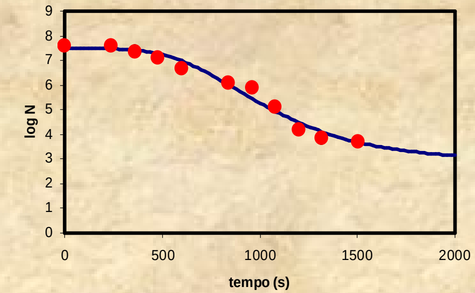
Cole et al. (1993)

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

distribution of heat sensibility of microbial populations

Inactivation models

- **primary**



Baranyi et al. (1993)

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

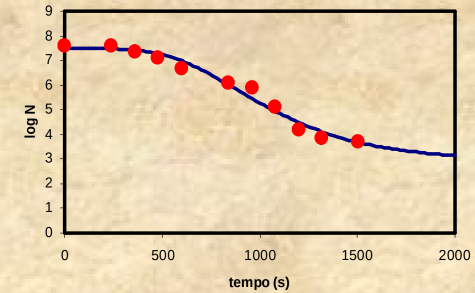
$$N(t = 0) = N_0$$

'tail' function ($\beta(t)$)

'lag' function ($\alpha(t)$)

Inactivation models

- **primary**



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

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

'tail' function

'lag' function

Baranyi et al. (1993)

Geeraerd et al. (2000)

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

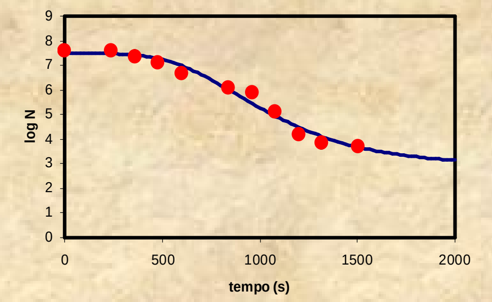
$$\frac{dQ}{dt} = -k_{max}\; Q$$

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

Q – variable related to the physiological state of the cells

Inactivation models

❖ **primary**



Gompertz

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

↓

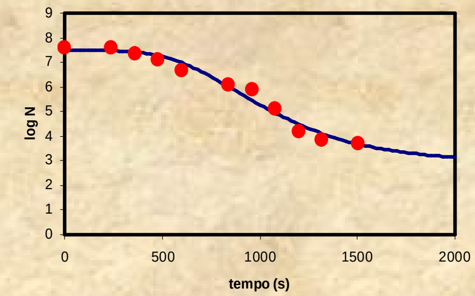
Listeria monocytogenes

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

reparameterized for inactivation based in Zwitering (1990)

Inactivation models

❖ **primary**



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reparameterized for inactivation based in Zwitering (1990)

Logistic

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

c – constant

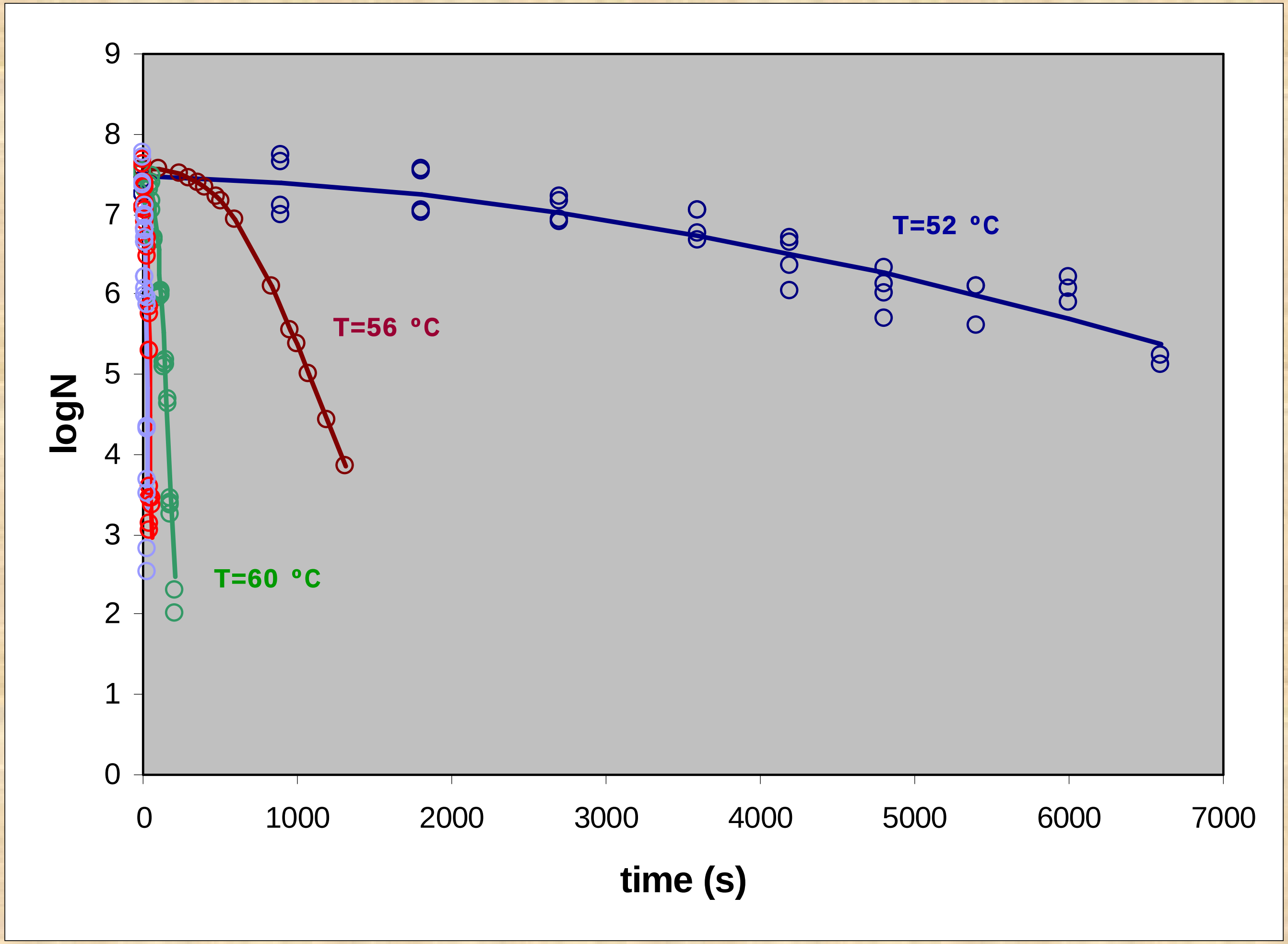
Examples

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 *Alicyclobacillus acidoterrestris* spores at 85,91,95,97°C

Cupuaçu extract pH=3.6 11.3 °Brix

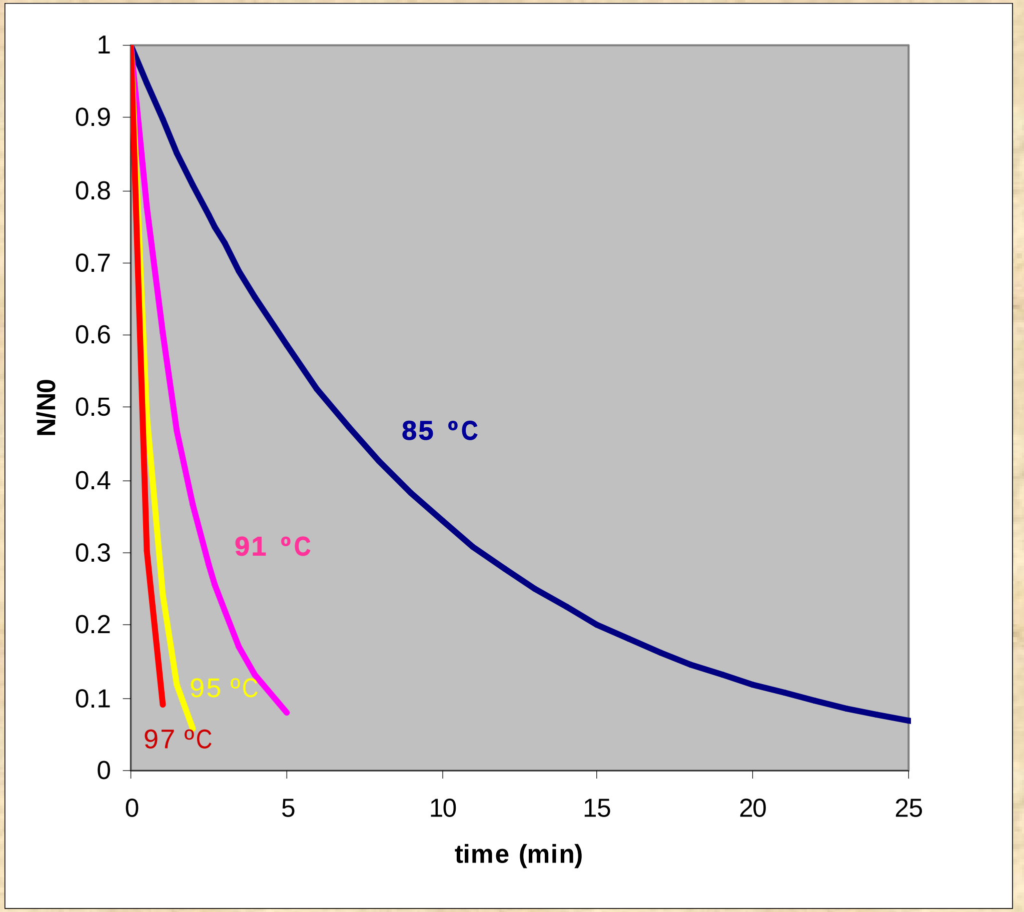
First order

↓

Bigelow

$$\frac{N}{N_0} = 10^{-\frac{t}{D}}$$

$$k = \frac{\ln(10)}{D}$$

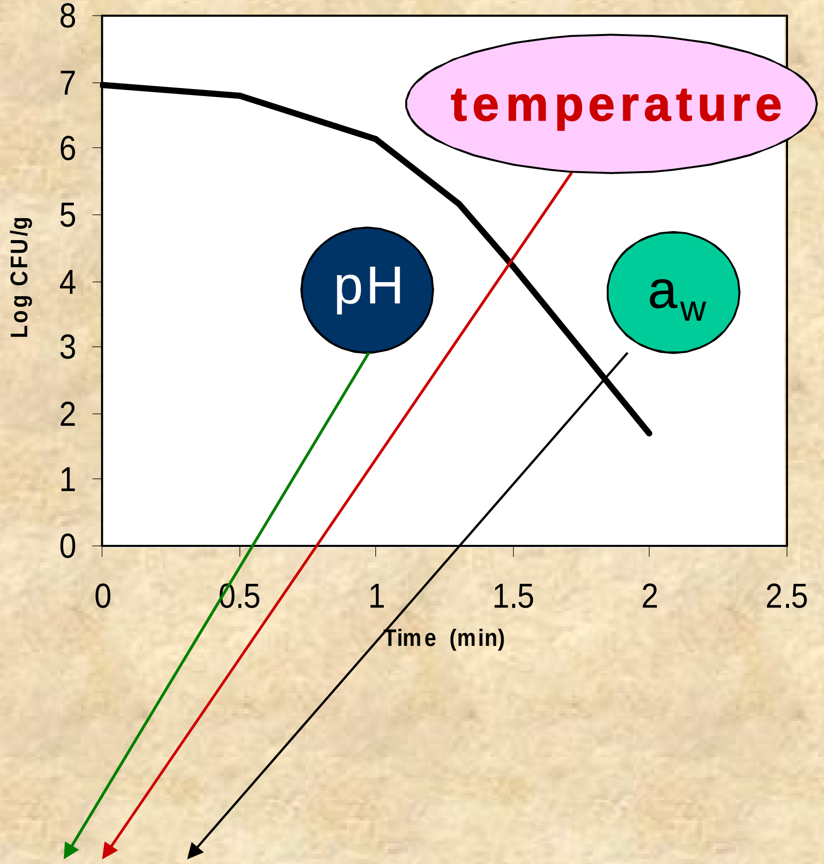


Silva (2000)

Statistica 6.0

Mathematical models

- primary



- secondary

  parameters

Mathematical models

❖ secondary

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)

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

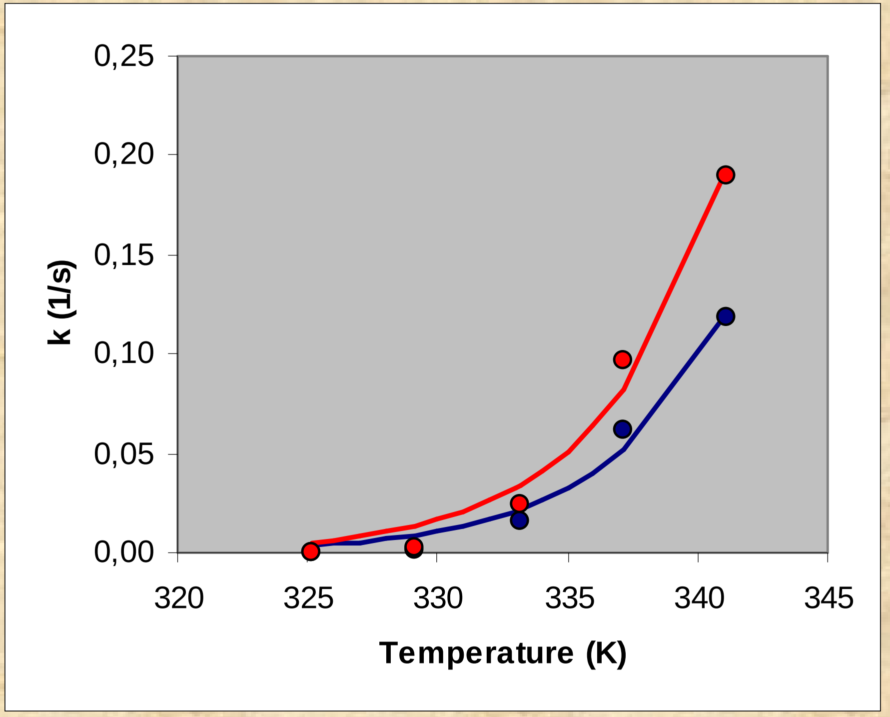
min – minimal value for growth

Data of *L.monocytogenes* Scott A

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

Temperature effect on k

Arrhenius



Gompertz k=**0.0216** exp(-**203.3**/R*(1/T-1/333.15)) | DEa=28.85 kJ/mol, $DK_{ref}=4.58x10^{-3}\ s^{-1}$

Logística k=**0.0337** exp(-**206.6**/R*(1/T-1/333.15)) | DEa=27.56 kJ/mol, $DK_{ref}=7.31x10^{-3}\ s^{-1}$

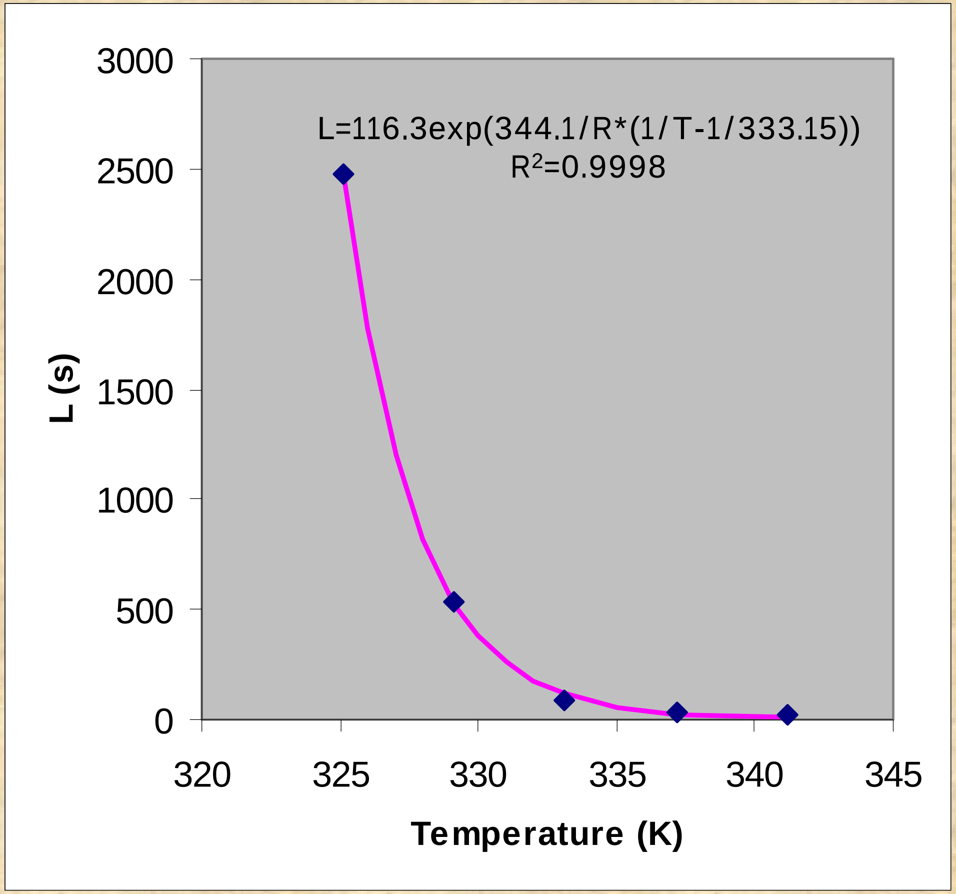
Gil (2002)

Statistica 6.0

Data of *L.monocytogenes* Scott A

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

Temperature effect on lag



$DEa=7.485$ kJ/mol

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

Gil (2002)

Statistica 6.0

Examples

Data of *Alicyclobacillus acidoterrestris* spores at 85,91,95,97°C

Cupuaçu extract pH=3.6 11.3 °Brix

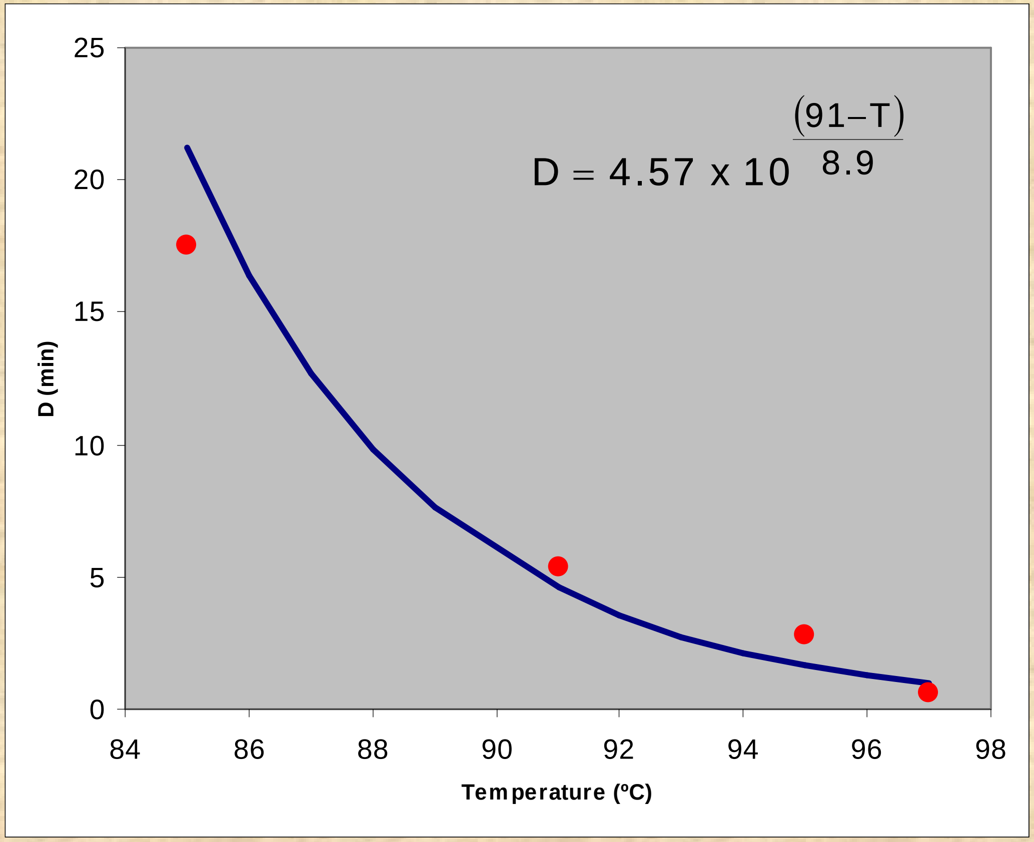
Temperature effect on D-value of *A.acidoterrestris* spores

$$D = D_{ref} 10^{\frac{(T_{ref} - T)}{z}}$$

T_{ref}=91 ºC

DD_{ref}=1.12 min

Dz=1.45 ºC



Silva (2000)

Examples

Temperature, Soluble Solids and pH effect on D-value of *A.acidoterrestris* spores

malt extract broth

ranges

85 < T (ºC) < 97
5 < SS (ºBrix) < 60
2.5 < pH < 6.0

Response surface methodology

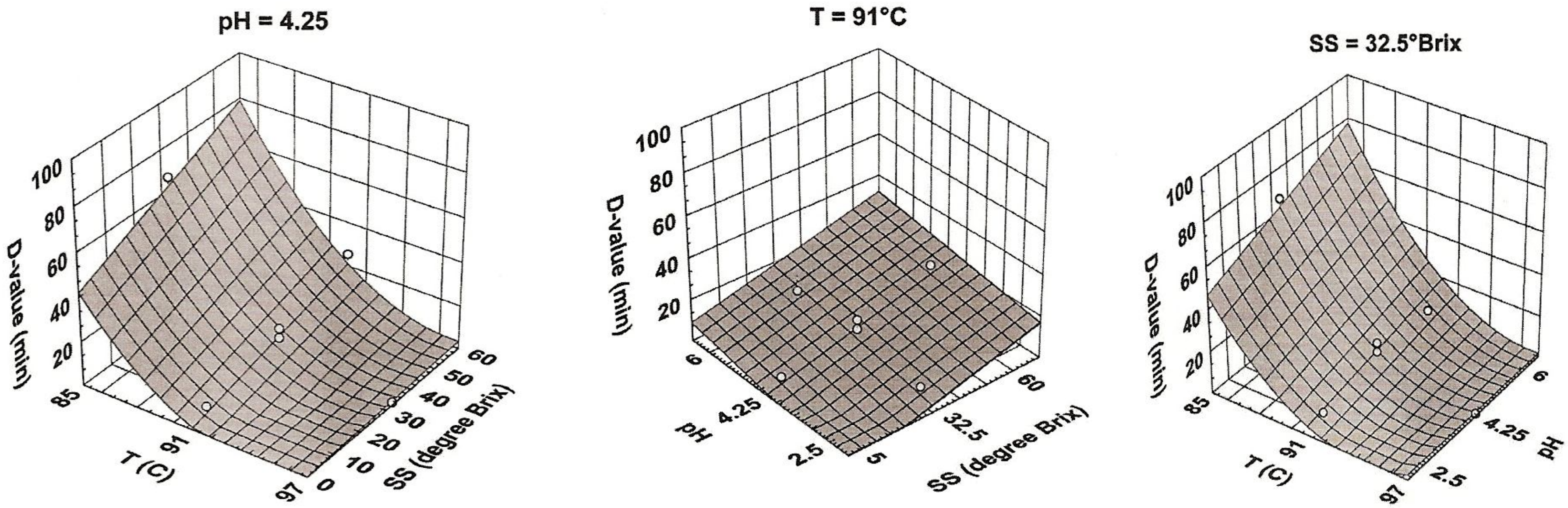
$$D = 4715.1 - (102.96\ T) + (0.56042\ T^2) + (4.6096\ SS) - (0.04699\ T\ SS) + (57.147\ pH) - (0.59083\ T\ pH)$$

Stata 3.0

Silva et al. (1999) IJFM

Examples

Temperature, Soluble Solids and pH effect on D-value of *A.acidoterrestris* spores



Silva et al. (1999) IJFM

Mathematical models

temperature

Variable conditions of

pH

a_w

Complexity !! … more realistic conditions!!

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

Mathematical models

Gompertz

dynamic situation of temperature

$$\frac{d(\log N)}{d(\text{time})}$$

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

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

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

Mathematical models

Linear

dynamic situation of temperature

$$\frac{d(\log N)}{d(\text{time})}$$

$$\frac{N}{N_0} = 10^{\left(\frac{-1}{D_{T_{ref}}} \int_0^{PT} 10^{\frac{T - T_{ref}}{Z}} dt \right)}$$

$$D = D_{ref}\, 10^{-\frac{1}{z}(T - T_{ref})}$$

different temperature histories

approach by Vieira et al. (2002)

Cupuaçu nectar

Design and optimization of pasteurization processes

reminding ….

ambience storage for 6 months

samples

microbial inactivation

total counts
moulds
yeasts

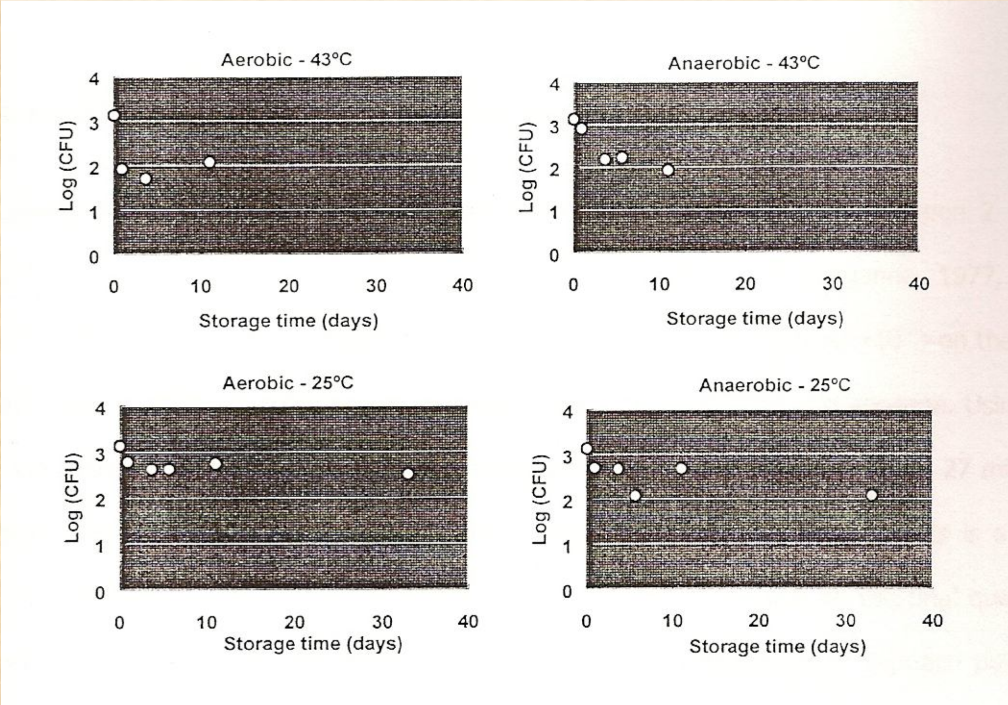
product stabilisation

colour
soluble solids
pH
reducing sugars

evaluation of the process

Design and optimization of pasteurization processes

Criterion



$$\frac{N}{N_0} = 0.1$$

Alicyclobacillus acidoterrestris **spores**

Mathematical models

❖ **tertiary**

softwares

Microbial growth

Shelf life prediction

√

Microbial inactivation

lacunas

:heiro Editar Ver Favoritos Ferramentas Ajuda

roceder Avançar Parar Actualizar Home page Procurar Favoritos Histórico Correio Imprimir Editar Debate

ereço http://www.frperc.bris.ac.uk/bugdeath.htm Ir para Hiperligaçõ

"BUGDEATH" (QLRT-2001-01415)

Micrograph of a Salmonella bacteria

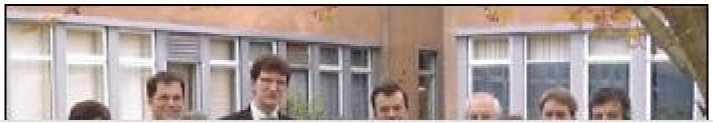
Predicting the reduction in microbes on the surface of foods during pasteurisation.

- 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.

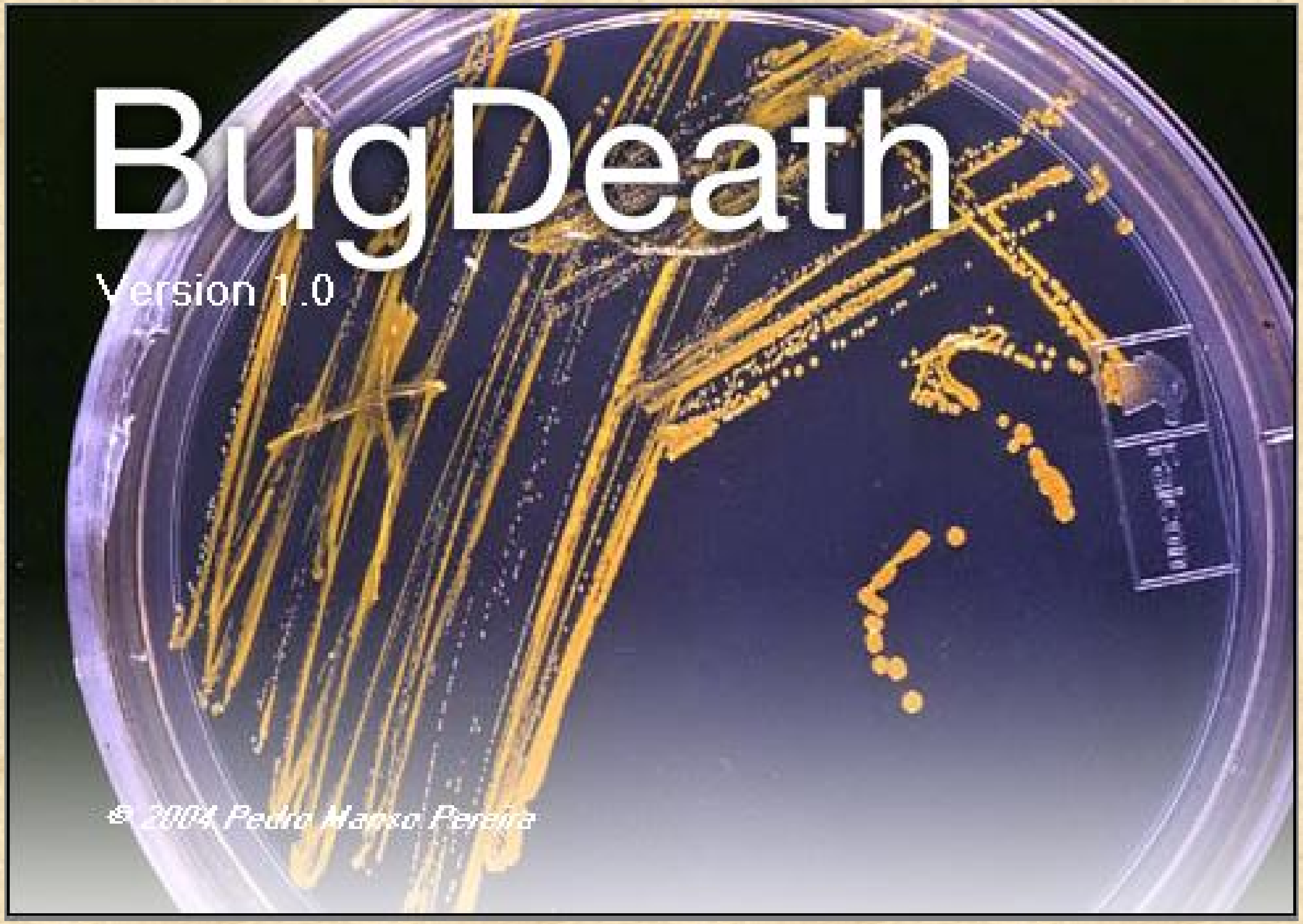
Food poisoning is increasing throughout the European Union (EU). Over 60% of outbreaks are associated with meat, fresh fruit and salad vegetables. Most of the contamination by pathogenic and spoilage organisms is present on the surface of foods at the time of harvesting or is transferred to the surfaces 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

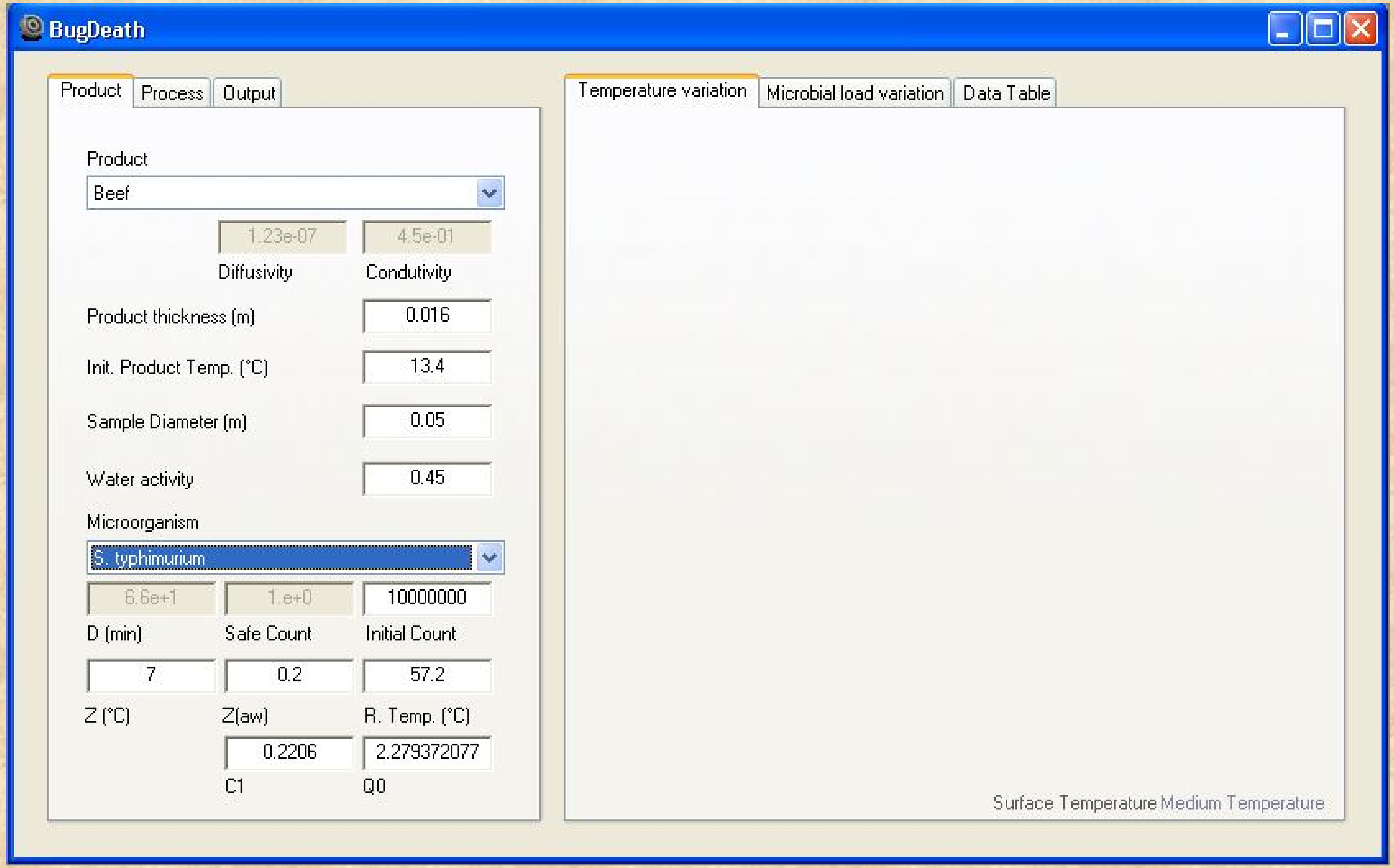
Internet

Software Program



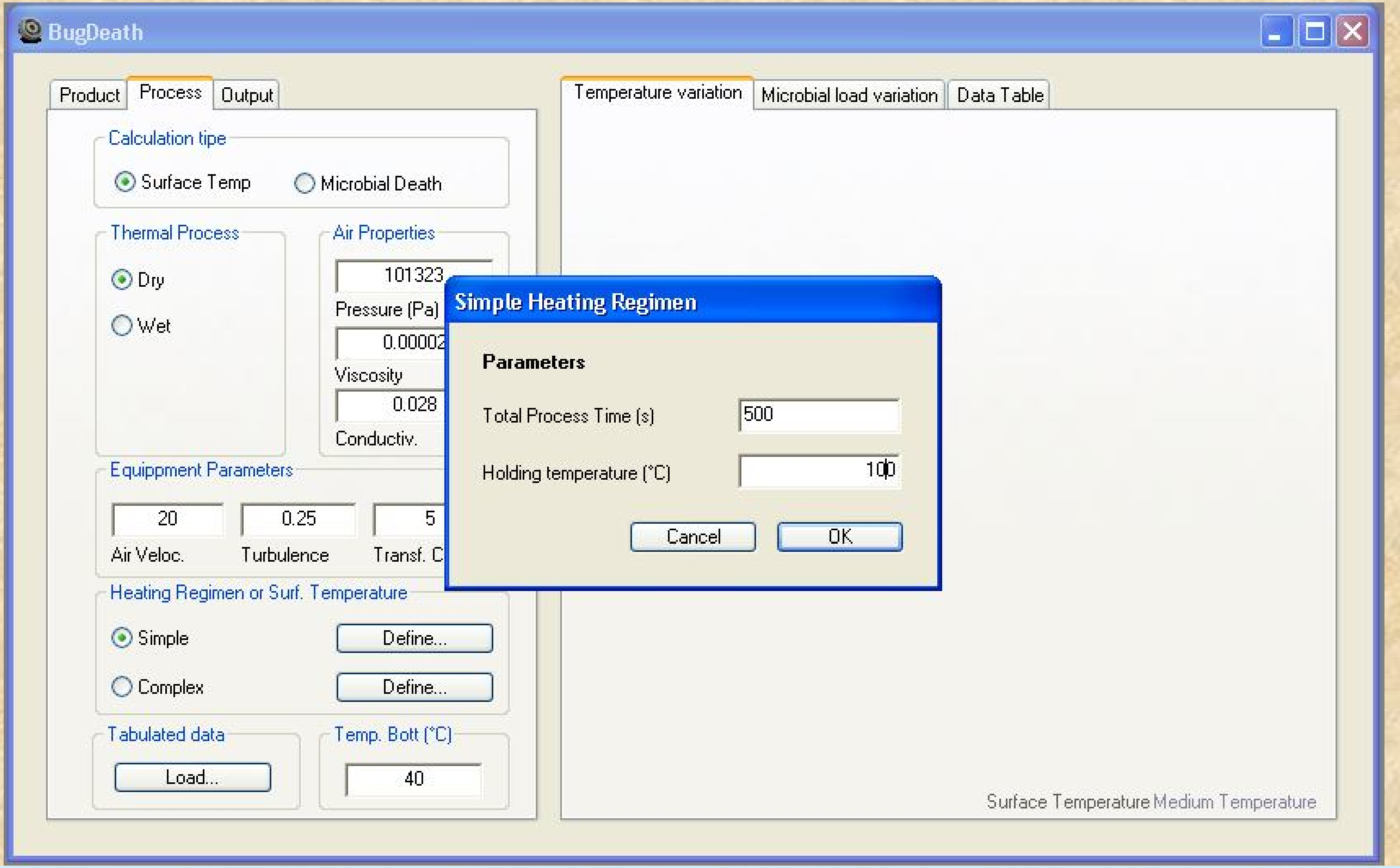
'Bugdeath' - funded by the European Commission under the EC Framework 5;
Quality of Life and Management of Living Resources Programme

First screen – product / microorganism



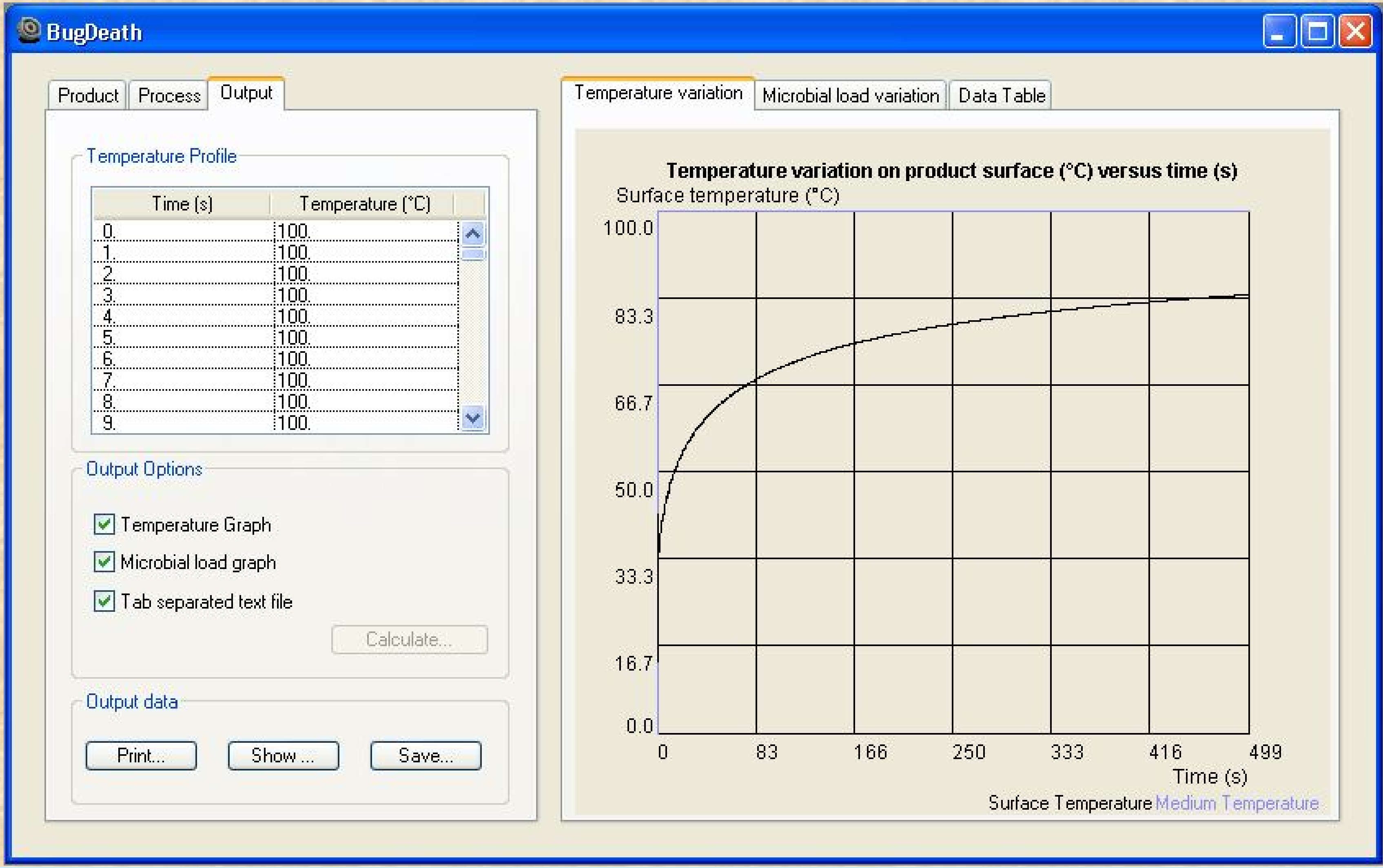
'Bugdeath' - funded by the European Commission under the EC Framework 5;
Quality of Life and Management of Living Resources Programme

Second screen – process



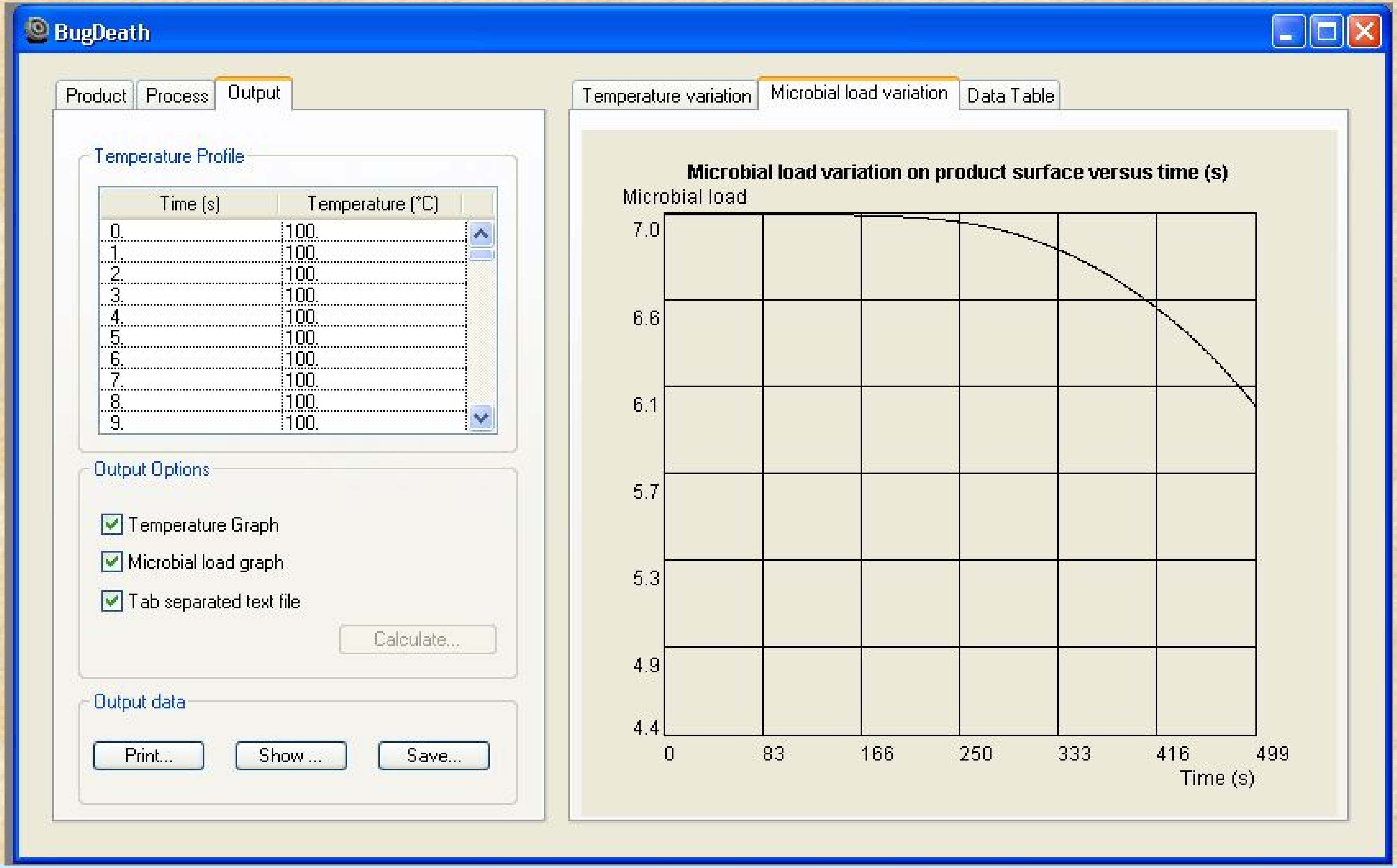
'Bugdeath' - funded by the European Commission under the EC Framework 5;
Quality of Life and Management of Living Resources Programme

Output – graphic / temperature



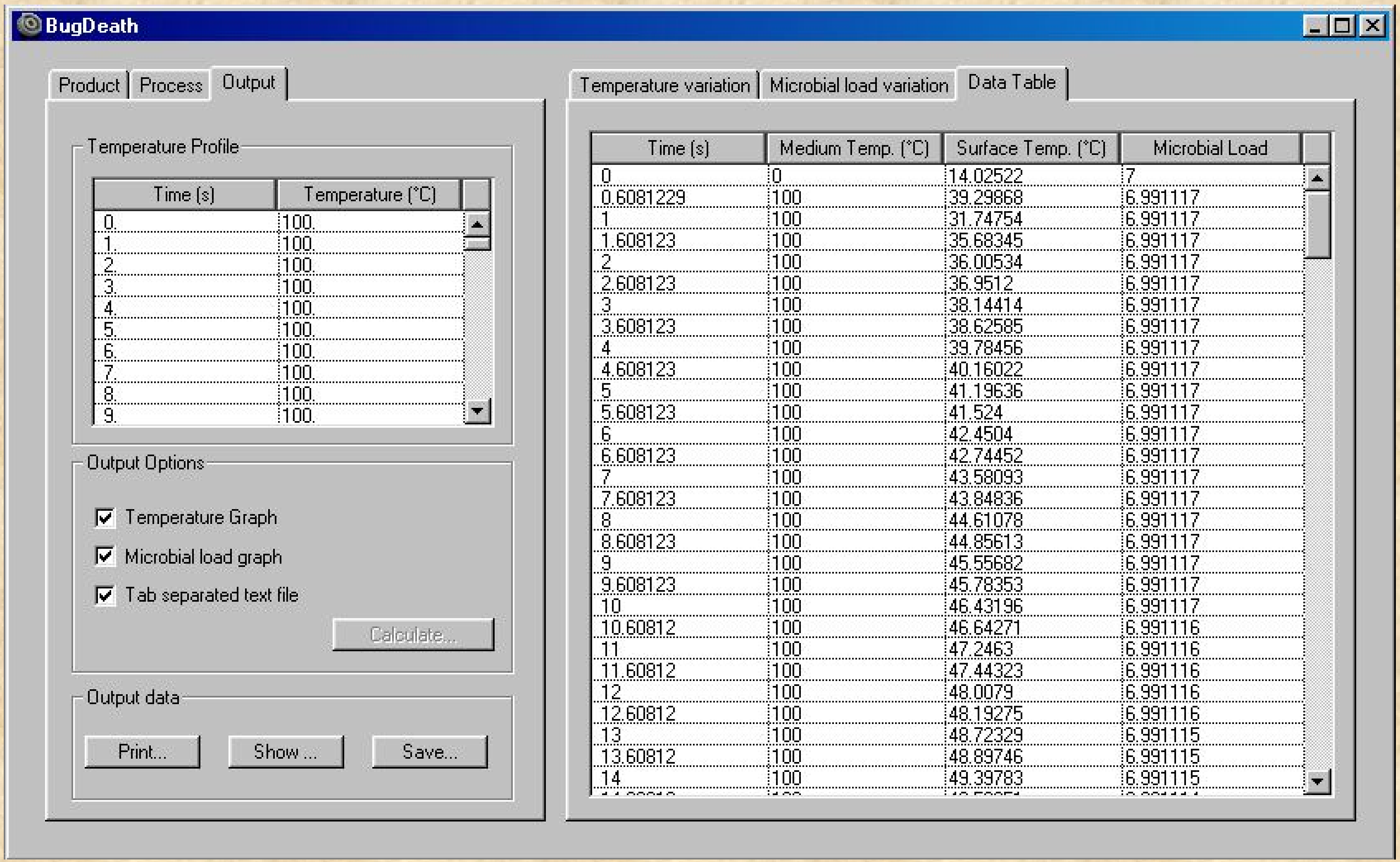
'Bugdeath' - funded by the European Commission under the EC Framework 5;
Quality of Life and Management of Living Resources Programme

Output – graphic / microbial load



'Bugdeath' - funded by the European Commission under the EC Framework 5;
Quality of Life and Management of Living Resources Programme

Output – data table



Time (s)	Temperature (°C)
0.	100.
1.	100.
2.	100.
3.	100.
4.	100.
5.	100.
6.	100.
7.	100.
8.	100.
9.	100.

Time (s)	Medium Temp. (°C)	Surface Temp. (°C)	Microbial Load
0	0	14.02522	7
0.6081229	100	39.29868	6.991117
1	100	31.74754	6.991117
1.608123	100	35.68345	6.991117
2	100	36.00534	6.991117
2.608123	100	36.9512	6.991117
3	100	38.14414	6.991117
3.608123	100	38.62585	6.991117
4	100	39.78456	6.991117
4.608123	100	40.16022	6.991117
5	100	41.19636	6.991117
5.608123	100	41.524	6.991117
6	100	42.4504	6.991117
6.608123	100	42.74452	6.991117
7	100	43.58093	6.991117
7.608123	100	43.84836	6.991117
8	100	44.61078	6.991117
8.608123	100	44.85613	6.991117
9	100	45.55682	6.991117
9.608123	100	45.78353	6.991117
10	100	46.43196	6.991117
10.60812	100	46.64271	6.991116
11	100	47.2463	6.991116
11.60812	100	47.44323	6.991116
12	100	48.0079	6.991116
12.60812	100	48.19275	6.991116
13	100	48.72329	6.991115
13.60812	100	48.89746	6.991115
14	100	49.39783	6.991115

'Bugdeath' - funded by the European Commission under the EC Framework 5;
Quality of Life and Management of Living Resources Programme

Drawbacks

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

Future challenges

non-thermal treatments

safety

efficient microbial inactivation

quality

maximum quality retention

avoiding the negative effects of heat at tissues level

Future challenges

non-thermal treatments

- **ozone**

{ aquous solution
  atmosphere

Future challenges

non-thermal treatments

- **ultrasounds**

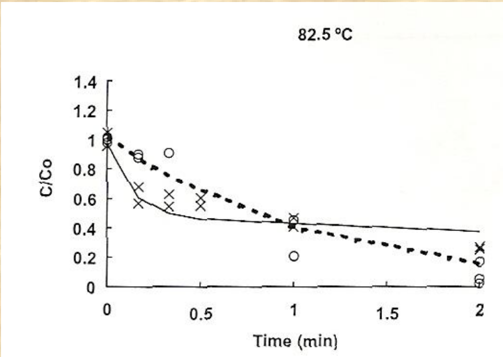
+

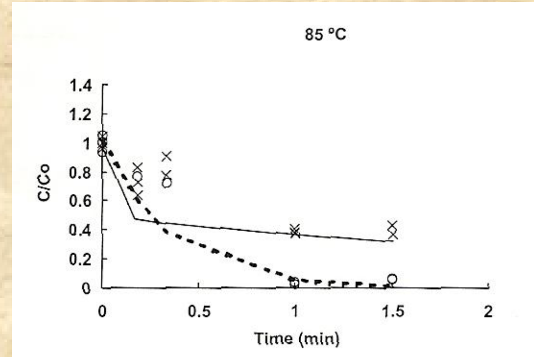
heat treatments

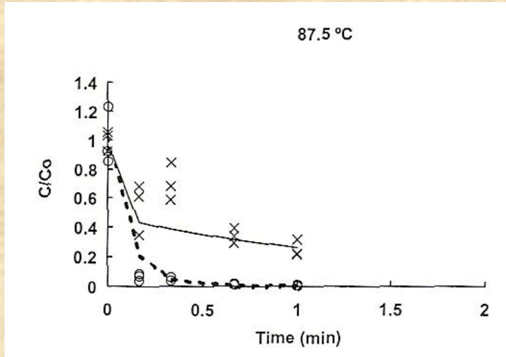
thermosonication

thermosonication

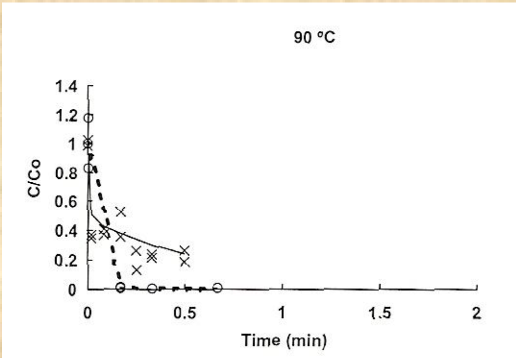
peroxidase inactivation in watercress

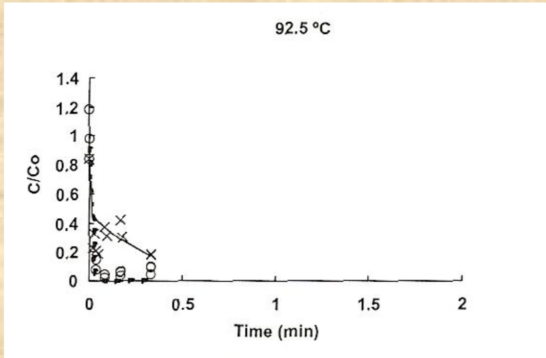






x blanching
○ thermosonication



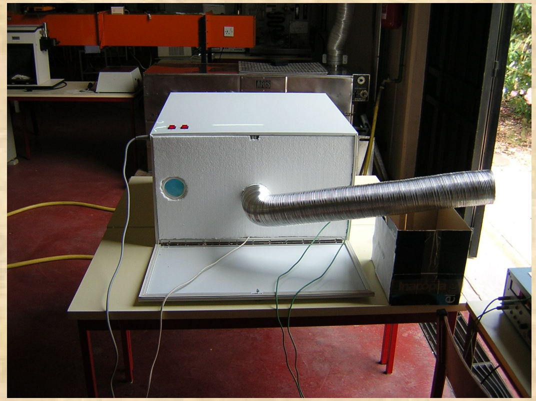


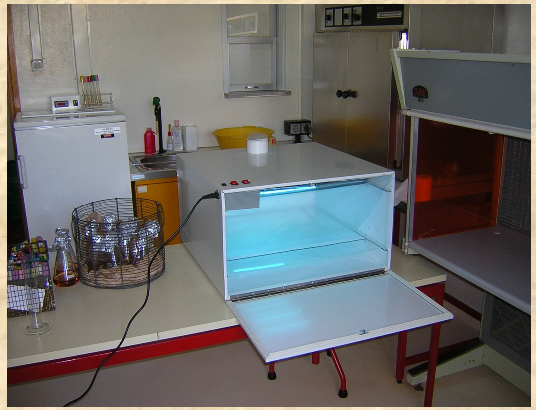
Cruz, Vieira & Silva (2005) JFOE

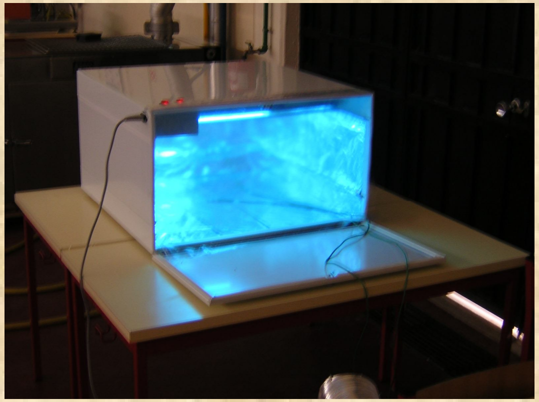
Future challenges

non-thermal treatments

- UV radiation



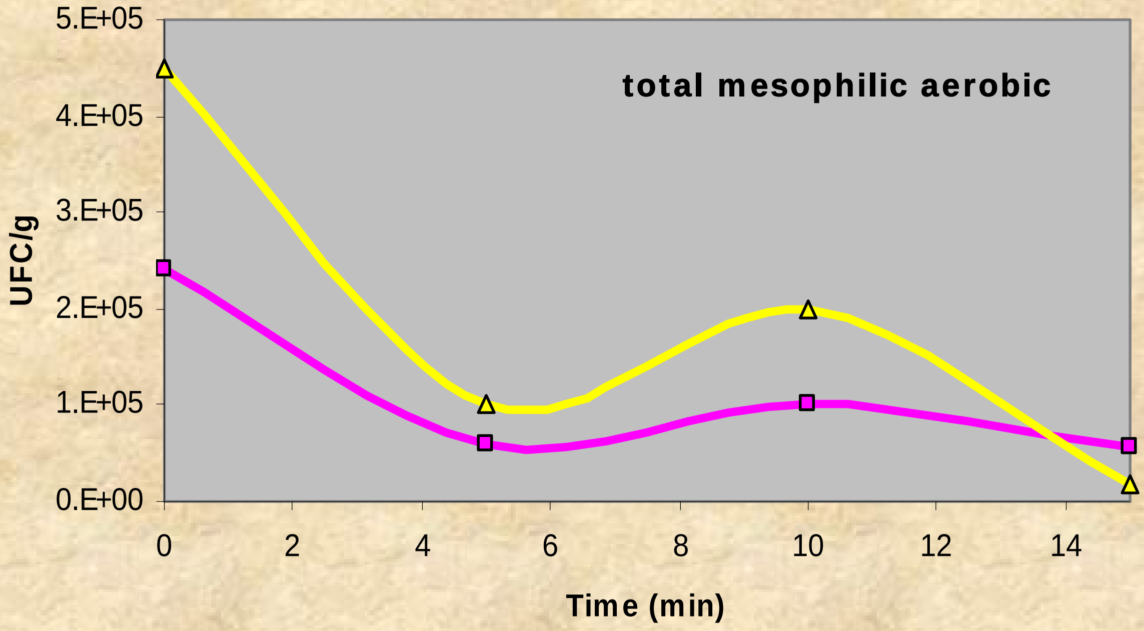




Future challenges

non-thermal treatments

- **UV radiation**



8.4 W UV-C

courgettes

EMERCON

New Processing Technologies for Frozen Fruits and Vegetables

Thank you

Teresa Brandão

Cristina Silva