CONCIA 2012
Congreso Nacional de Calidad e Inocuidad Alimentaria

OBTENTION AND DATA MANAGEMENT IN PREDICTIVE MICROBIOLOGY MODELS

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Centro Regional do Porto da Universidade Católica Portuguesa

OUTLINE

- Objectives of food industry
- The challenge
- Predictive microbiology
- How to obtain the data
- Available software
- Validation studies
- Acknowledgement by regulation
- The complexity of dynamic conditions
- Conclusions

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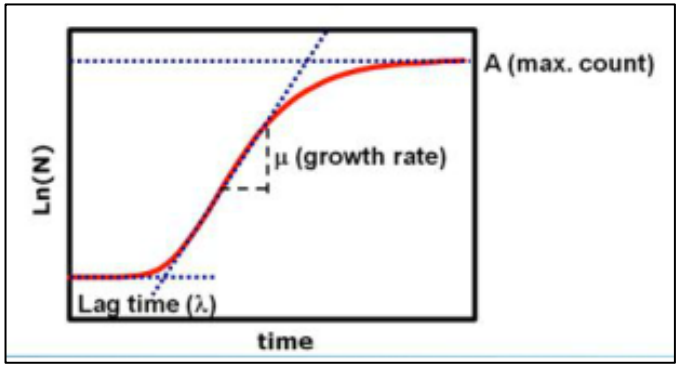
Objectives of food industry

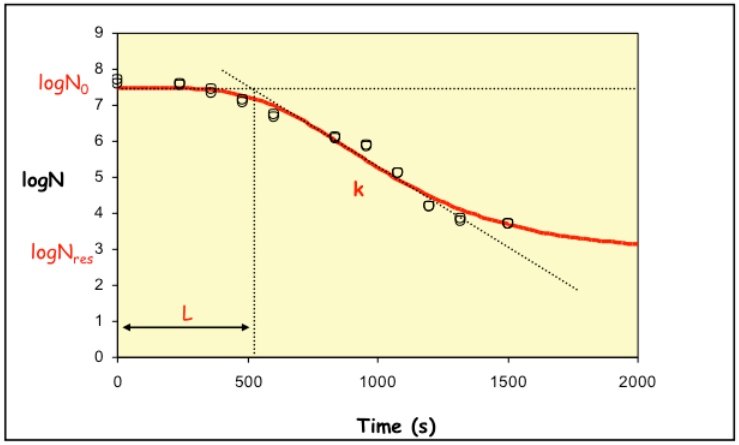
- Prediction of shelf life

- Preservation of foods

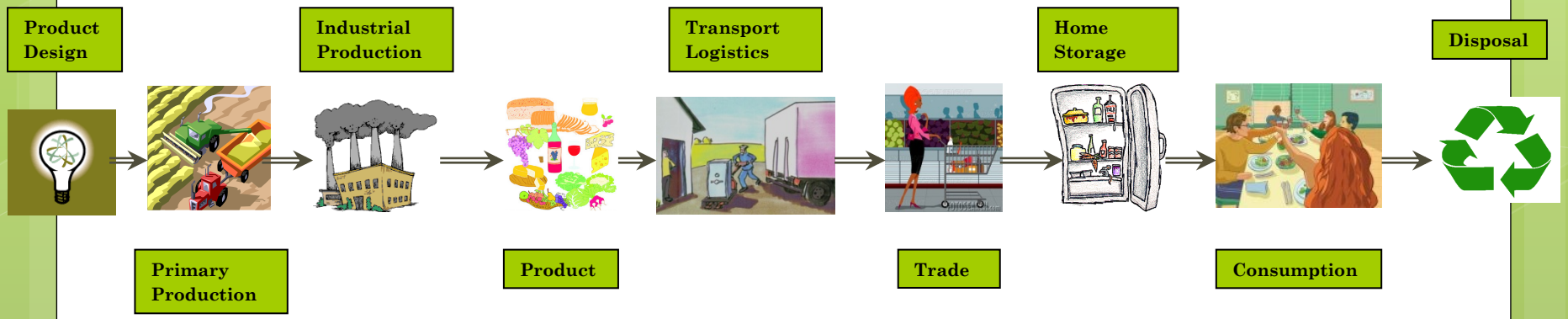
- Control/monitor the growth of microorganisms



- Predict microbial death



- Distribution chain



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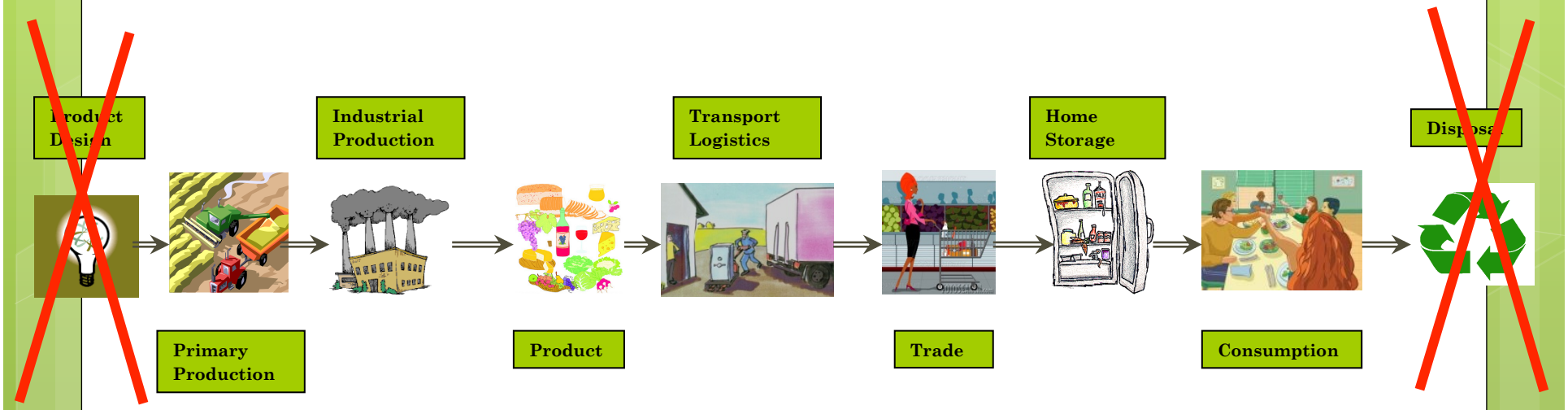

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The challenge

- Microorganisms response depends on:

  - **Intrinsic** factors

    pH
    a_w
    others

  - **Extrinsic** factors

    T
    pH
    humidity
    salt
    gas concentration
    others

  - **System dynamics**

- microbial interaction
- natural strains diversity
- history of initial population
- complexity of food structure
- interaction food/microorganism
- predictions in real and varying environmental conditions

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Predictive microbiology

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

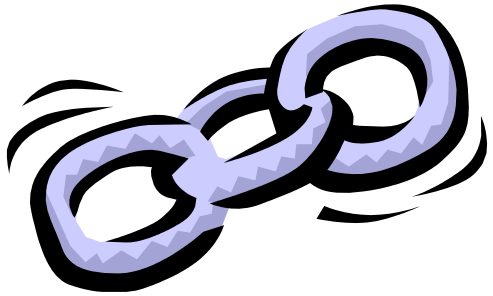
The idea is not even recent!

- Thermal lethality
  - D and z values – Bigelow model

$$F_c = \int_0^{tp} 10^{(T_c - T_{refm})/z_m} dt$$

predictive microbiology

microbiology



statistics

mathematics

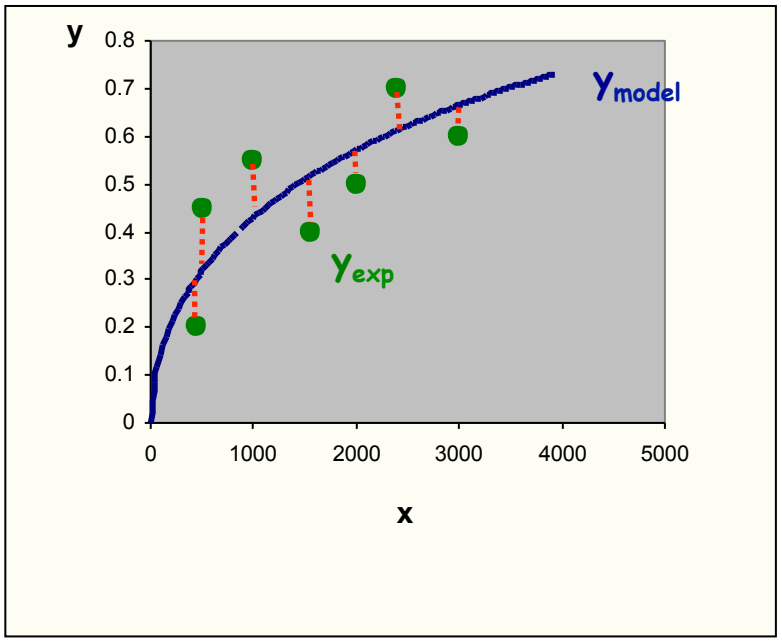
Model → mathematical expression

$$y_i = f(x_{ij}, \theta_k) + \varepsilon_i$$

i=1,2,…,n (number of experimental runs/observations)

j=1,2,…,v

k=1,2,…,p



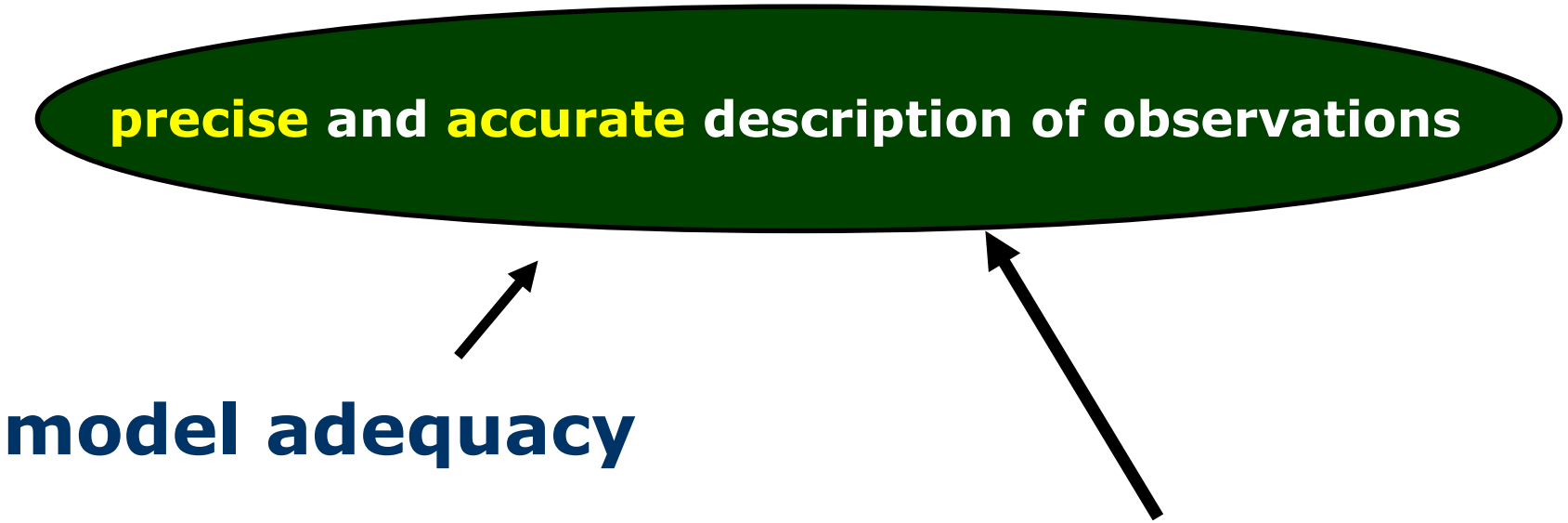
Minimize differences

θ^*

Precise ?

Accurate ?

objective


precise and accurate description of observations
model adequacy
quality of model parameters

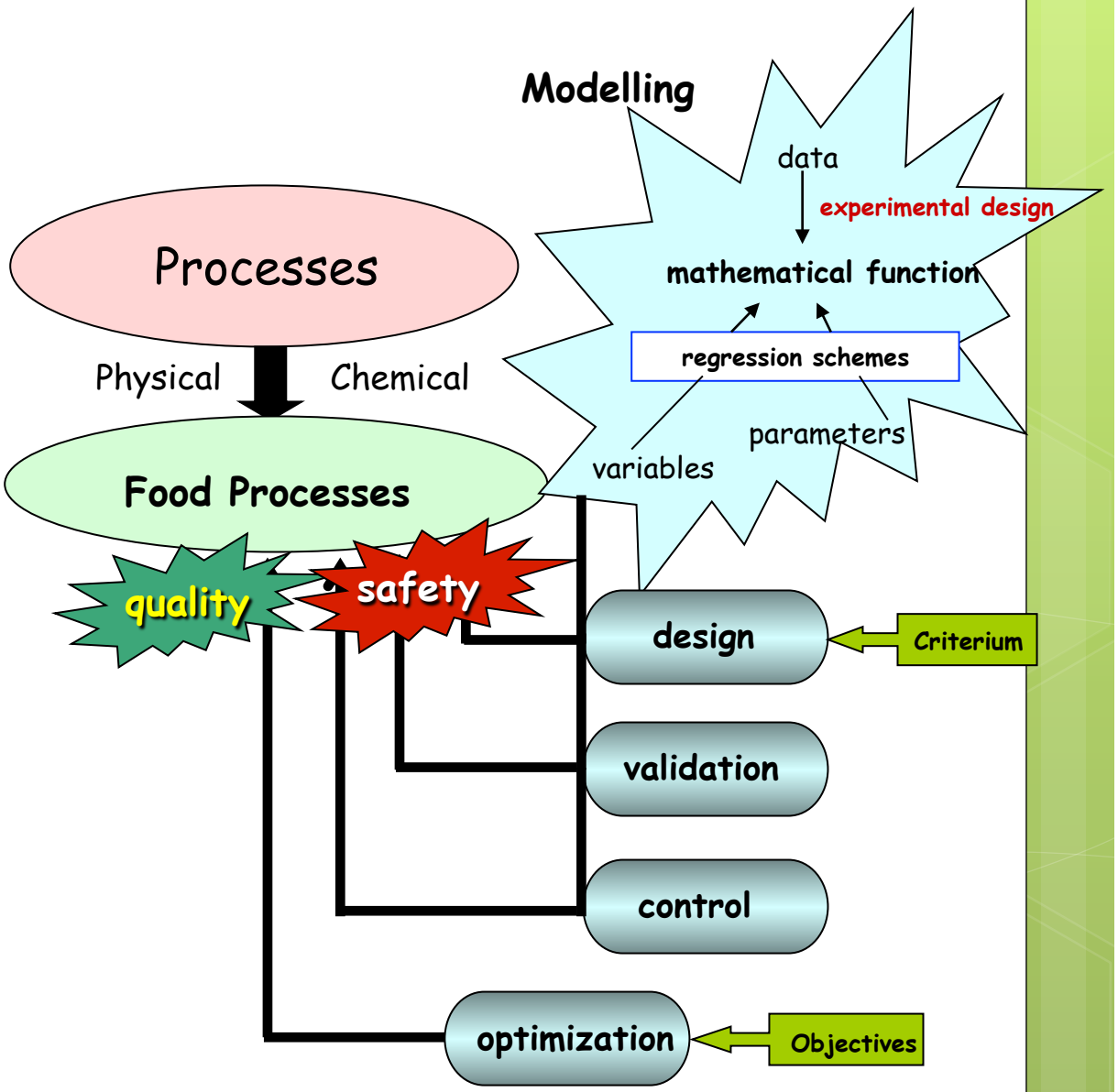
advantages

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

Transport Phenomena
- heat
- mass
- *momentum*

Reaction kinetics

Properties



aplication

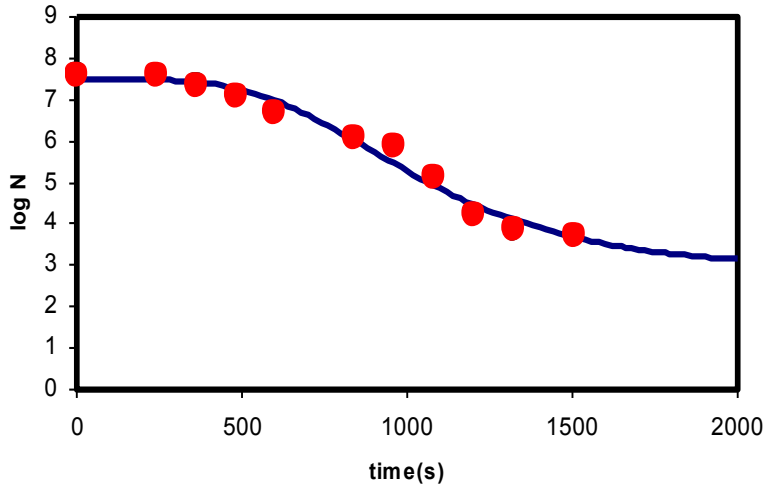
- prediction / simulation
- development of efficient processes

↓

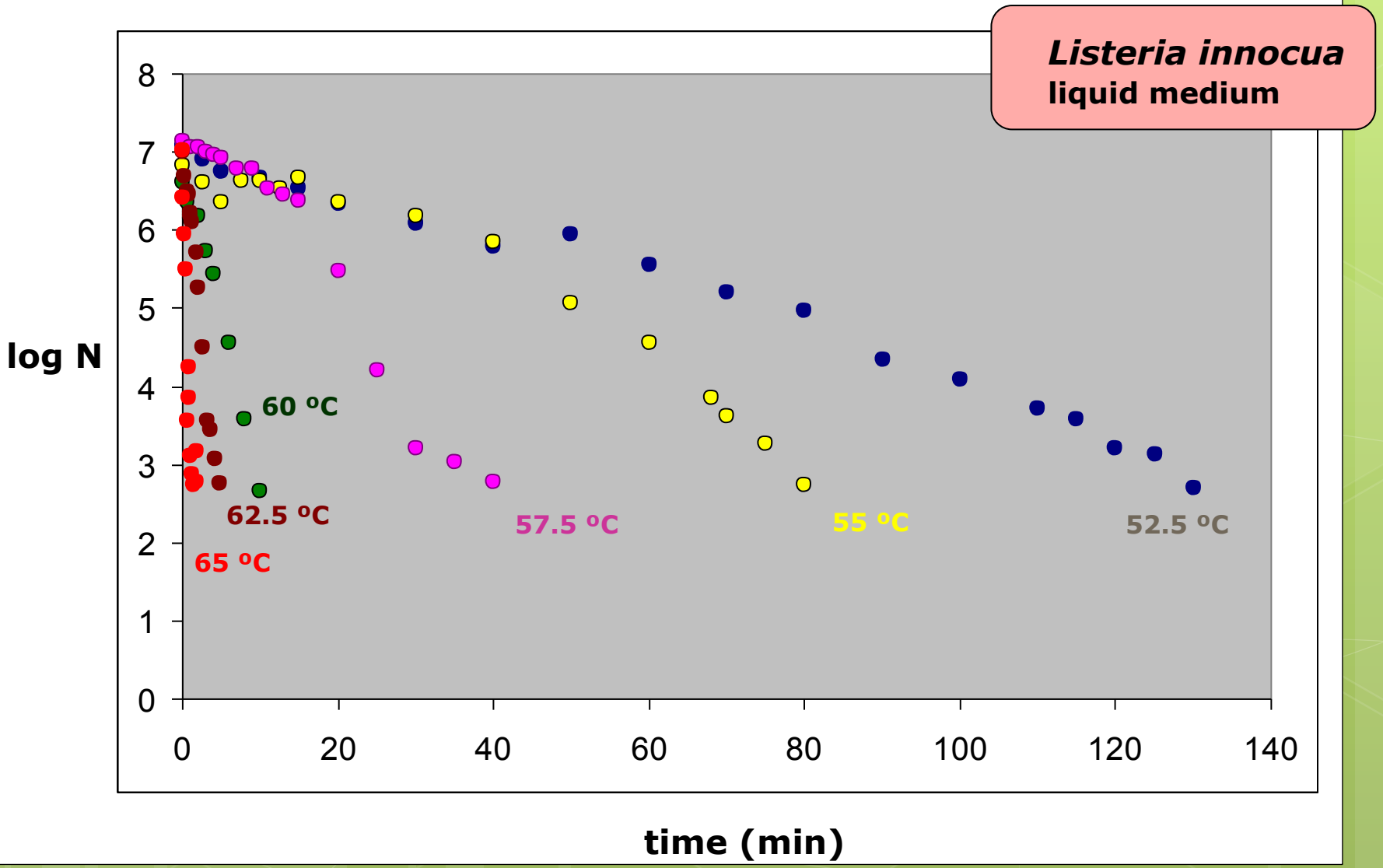
contribution to **safety**

inactivation

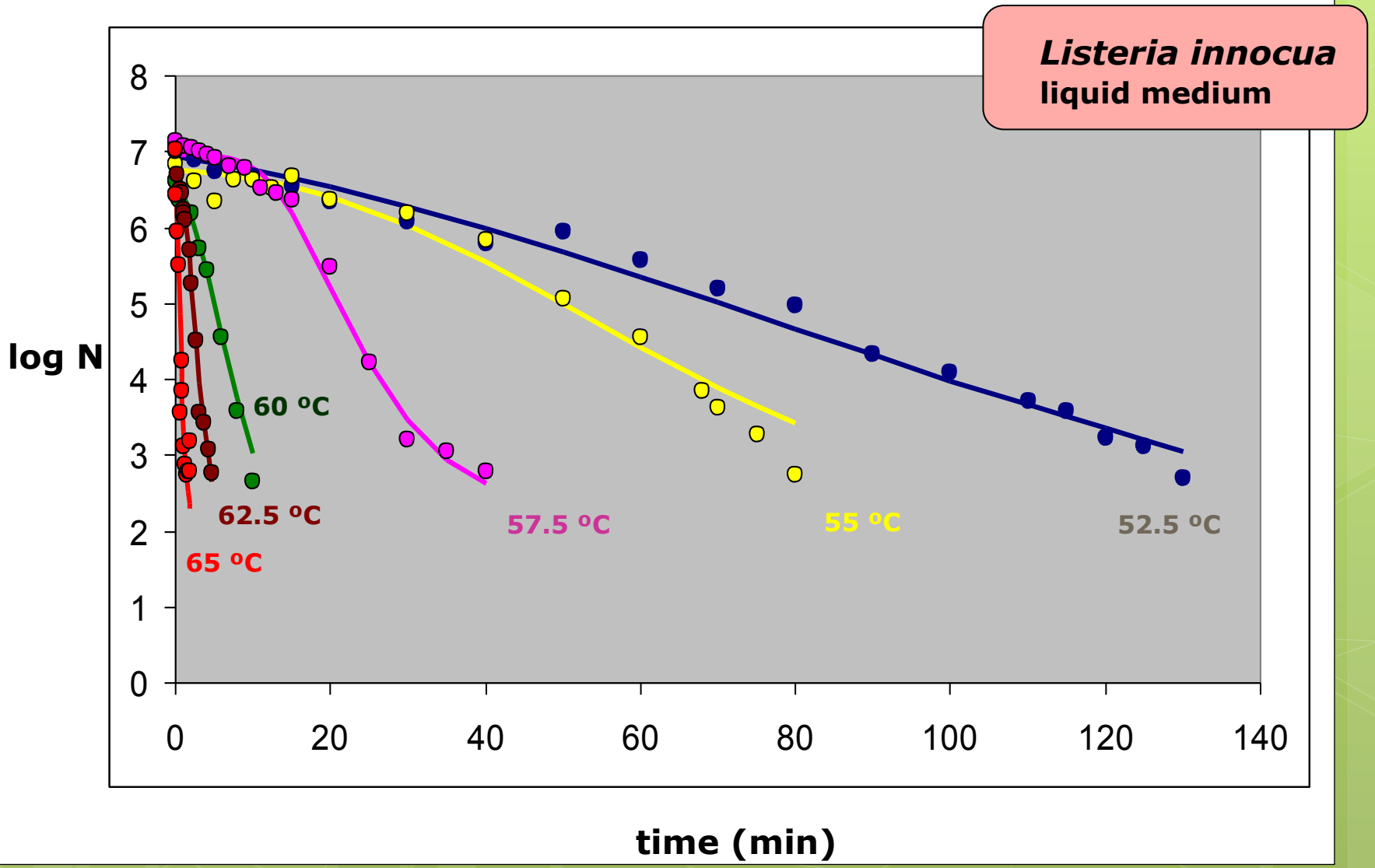
sigmoidal behaviour



presence of aggregated microorganisms or sub populations more **heat** (or other **stress factor**) **resistent**


Listeria innocua
liquid medium
log N
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
140
60 ºC
62.5 ºC
65 ºC
57.5 ºC
55 ºC
52.5 ºC
time (min)

Miller (2004)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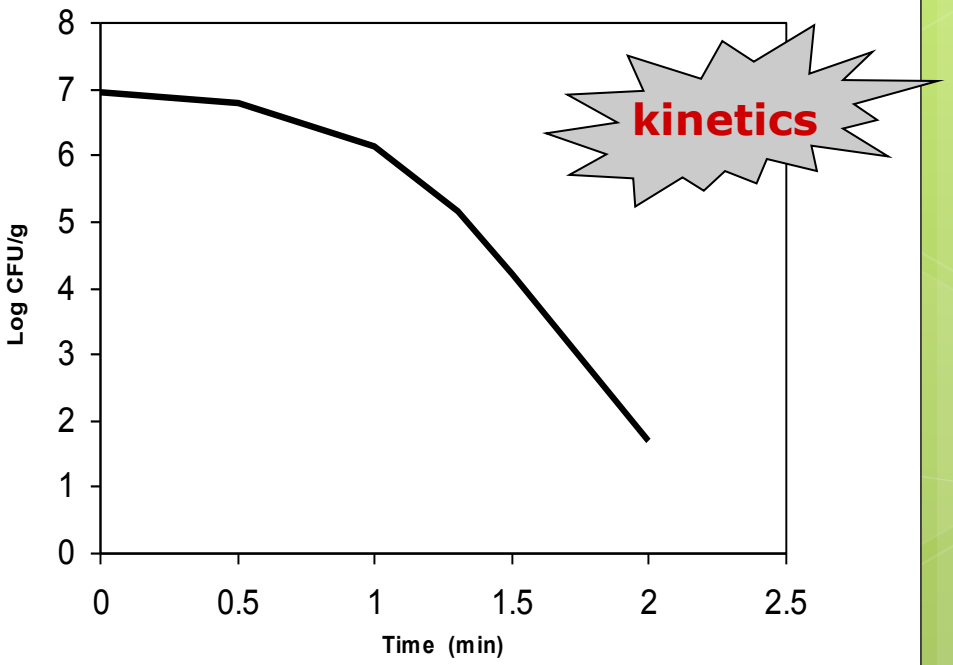
time (min)

Miller (2004)

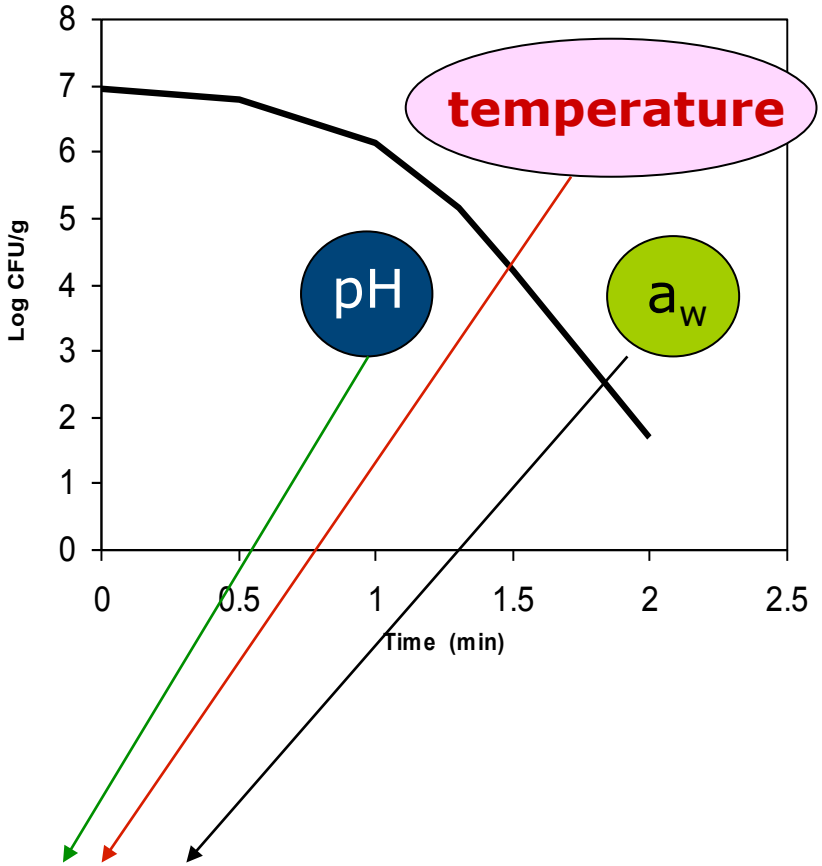
Types of models

- **primary**



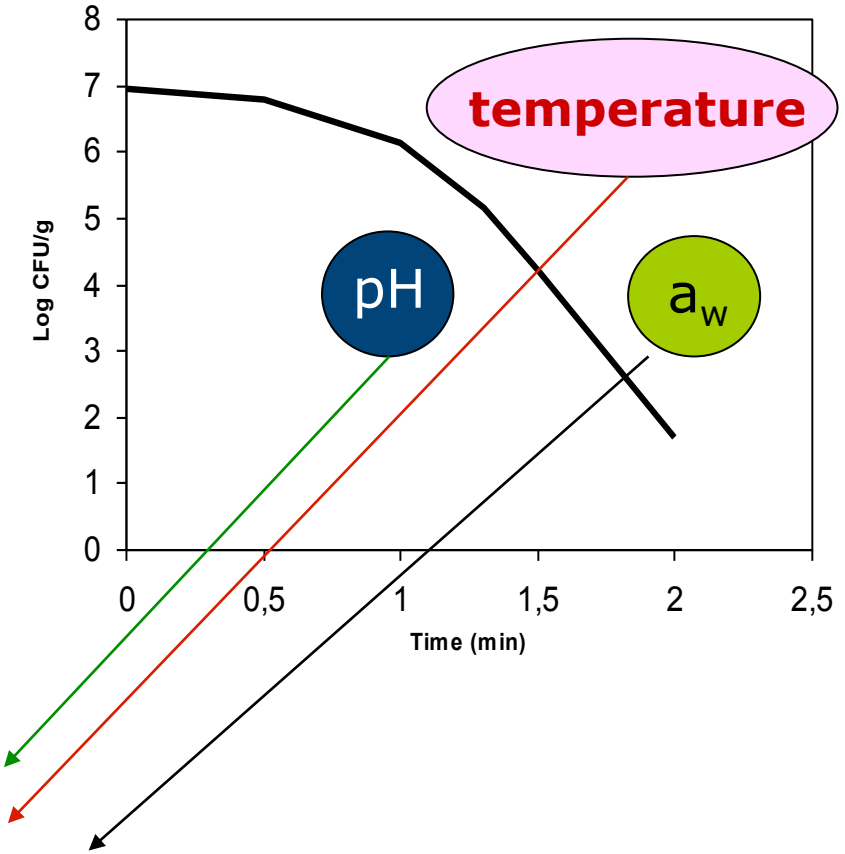
parameters

- **primary**
- **secondary**



parameters

- **primary**
- **secondary**

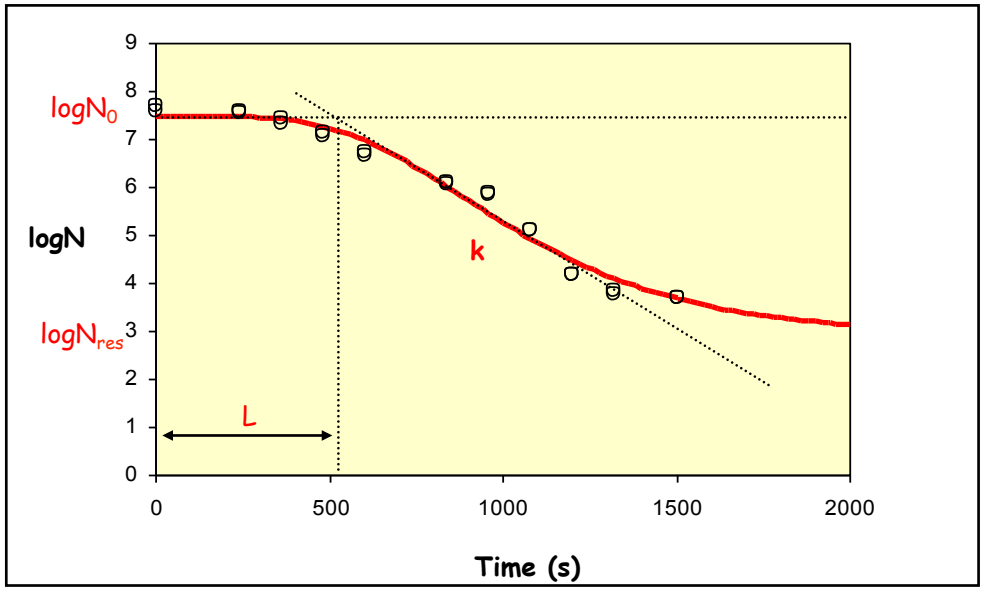


parameters

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

Inactivation models

- **primary**

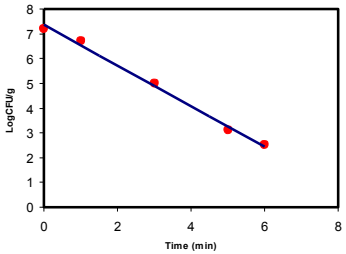


N_0 number of initial viable spore cells
N_{res} number of residual spore cells
k maximum inactivation rate
L lag or shoulder

empirical

fundamental

❖ **primary**



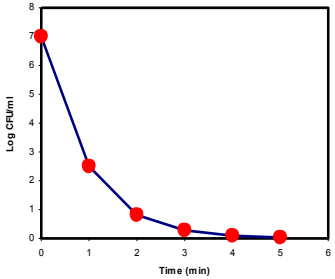
$$N = N_0 \exp(-kt) \quad \Leftrightarrow \quad \log N = \log N_0 - \frac{t}{D}$$

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)



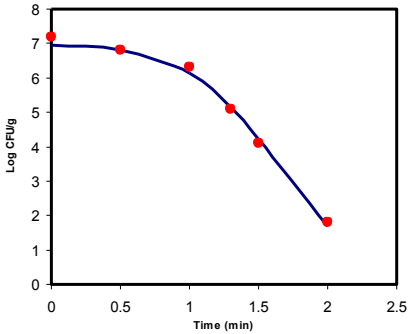
biphasic

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)

- **primary**



Whiting & Buchanan (1992)

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

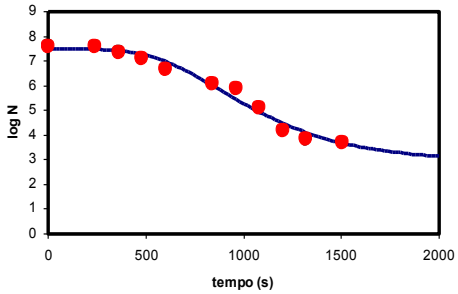
L – lag or shoulder

Cole et al. (1993)

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

distribution of heat sensibility of microbial populations

❖ **primary**



Baranyi et al. (1993)

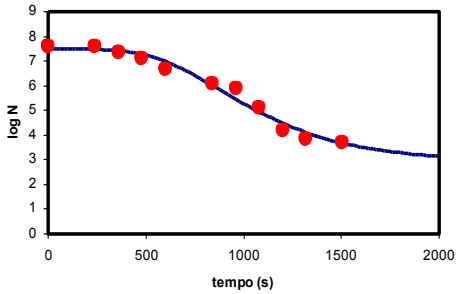
$$\frac{dN}{dt} = -k\,\alpha(t)\,\beta(t)\,N$$

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

'tail' function

'lag' function

❖ **primary**



$$\frac{dN}{dt} = -k\,\alpha(t)\,\beta(t)\,N \qquad N(t=0) = N_0$$

Baranyi et al. (1993)

'tail' function

'lag' function

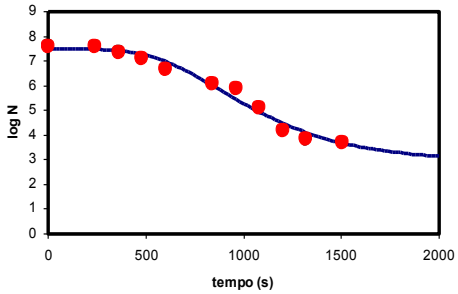
Geeraerd et al. (2000)

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

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

Q – variable related to the physiological state of the cells

❖ **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)

Logistic

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

c – constant

❖ **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

- **tertiary**

softwares

Microbial growth

Shelf life prediction

Microbial inactivation

Difficulties in food processes modelling:

- Dynamic processes
- Complexity and heterogeneity of products
- Structural and physicochemical changes

Gompertz

dynamic situation of temperature

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

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

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

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

Gill (2011)

Linear

dynamic situation of temperature

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

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

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

different temperature histories

approach by Vieira et al. (2002)

Cupuaçu nectar

Case studies:

1

Square-root

$$\sqrt{k_{max}} = \sqrt{c}\,(T - d)$$

c, d constants

2

Arrhenius

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

K_{ref} reaction rate at T_{ref}

Ea activation energy

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

3

Williams-Landel-Ferry

$$L = 10^{\left(\frac{a\,(T - T_{min})}{b + (T - T_{min})}\right)}$$

a, b constants

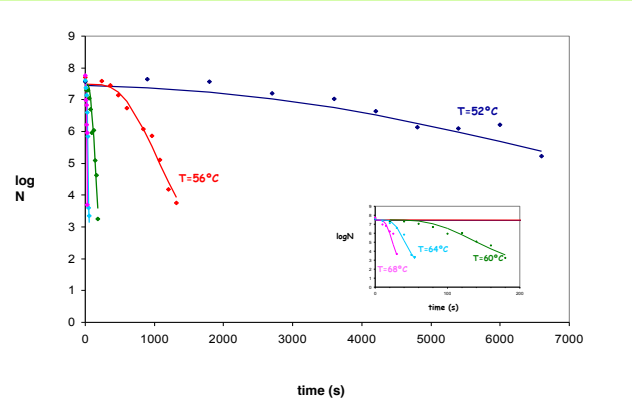
4

Arrhenius

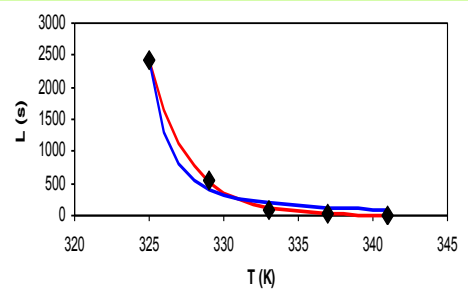
$$L = a\exp\left(b\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)$$

Gill (2011)

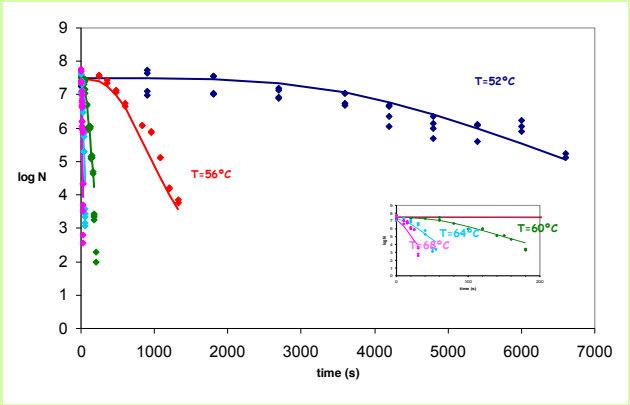
Gompertz



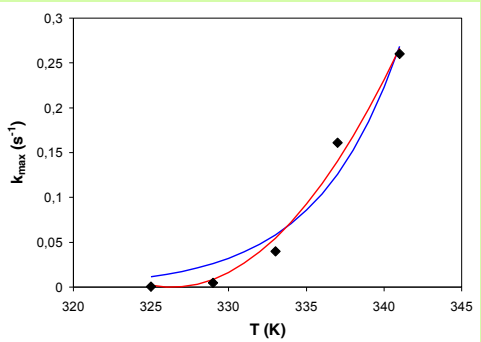
Two-step



L= f(T)



One-step



k_{max}= f(T)

Equations 1 and 4 selected

L. Monocytogenes in half-cream at 52, 56, 60, 64 e 68ºC (Casadei *et al.* 1998)

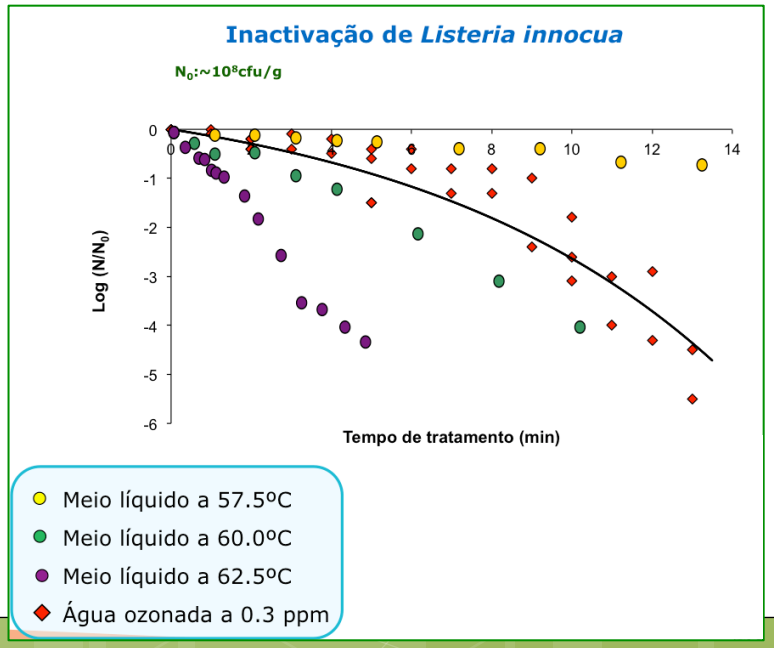
Tank with water
+
L. innocua

15 minutes

Ozonation

Inumeration

Weibull model



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How to obtain the data

- Experiments in broth at various conditions (pH, T, a_w, [growth inhibitors], etc.)
- Inoculation studies in foods under various conditions
- Derive model parameters

Sampling:

- **Heuristic sampling**
- **Experimental design**

Minimize variance of:

- ***predicted response***
- ***parameter estimates***

Data analysis:

- **Regression schemes**

$$SSR = \sum_{i=1}^{n} e_i^2 = \sum_{i=1}^{n} \left[y_i - f\left(x_{ij}, \theta_k\right) \right]^2$$

Least-squares method

- **Analysis of residuals**

Mathematical complexity

Adequate description

quality

model

parameters

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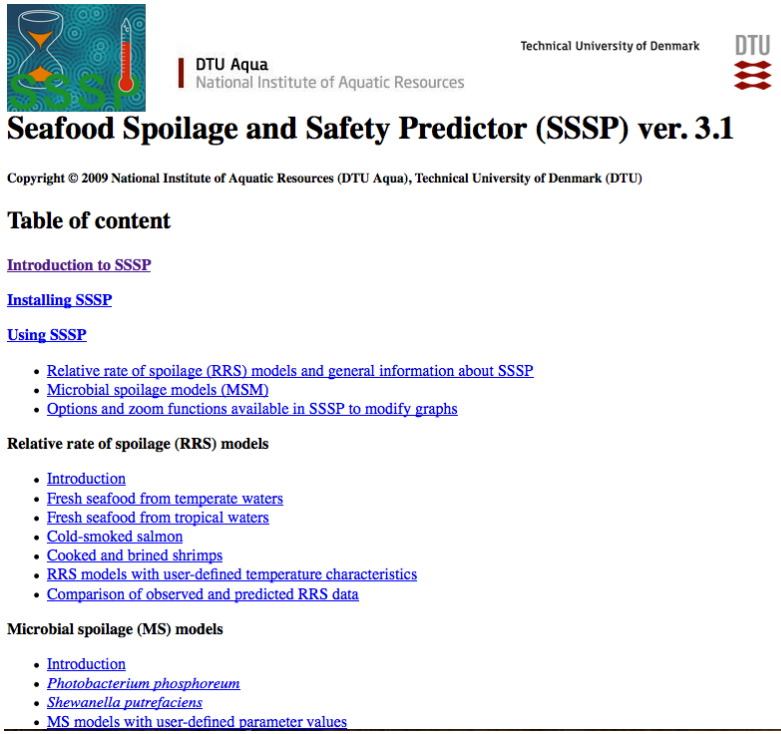
Available software



DTU Aqua
National Institute of Aquatic Resources

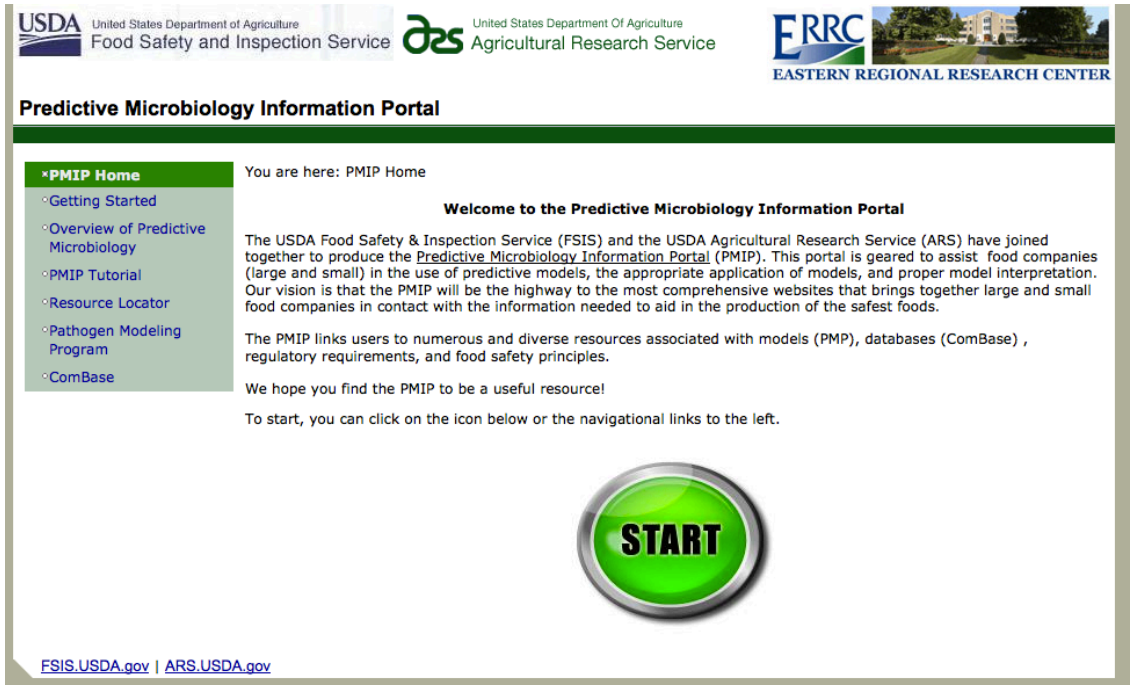
Technical University of Denmark

Seafood Spoilage and Safety Predictor (SSSP) ver. 3.1

Table of content

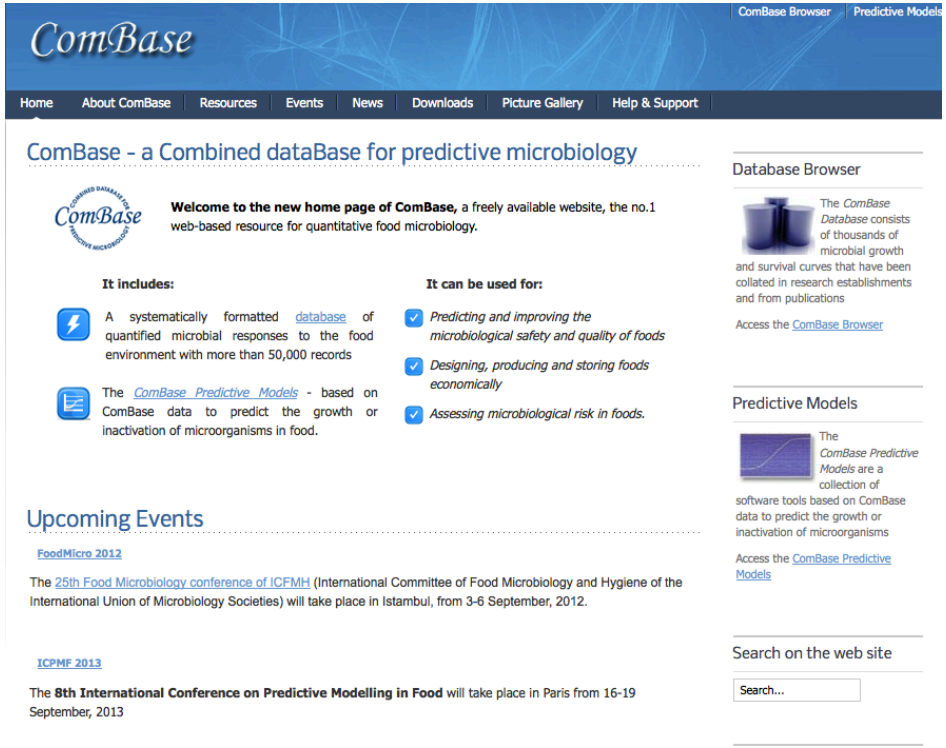
→ predicts shelf-life as well as growth of spoilage and pathogenic bacteria in seafood

→ evaluates the effect of constant or fluctuating temperature storage conditions (Dalgaard et al. 2002, 2003, 2008)



→ more than 40 models for different bacterial pathogens

→ the software allows growth or inactivation of pathogens to be predicted for different combinations of constant temperature, pH, NaCl/a_w and, in some cases, other conditions such as organic acid type and concentration, atmosphere, or nitrate

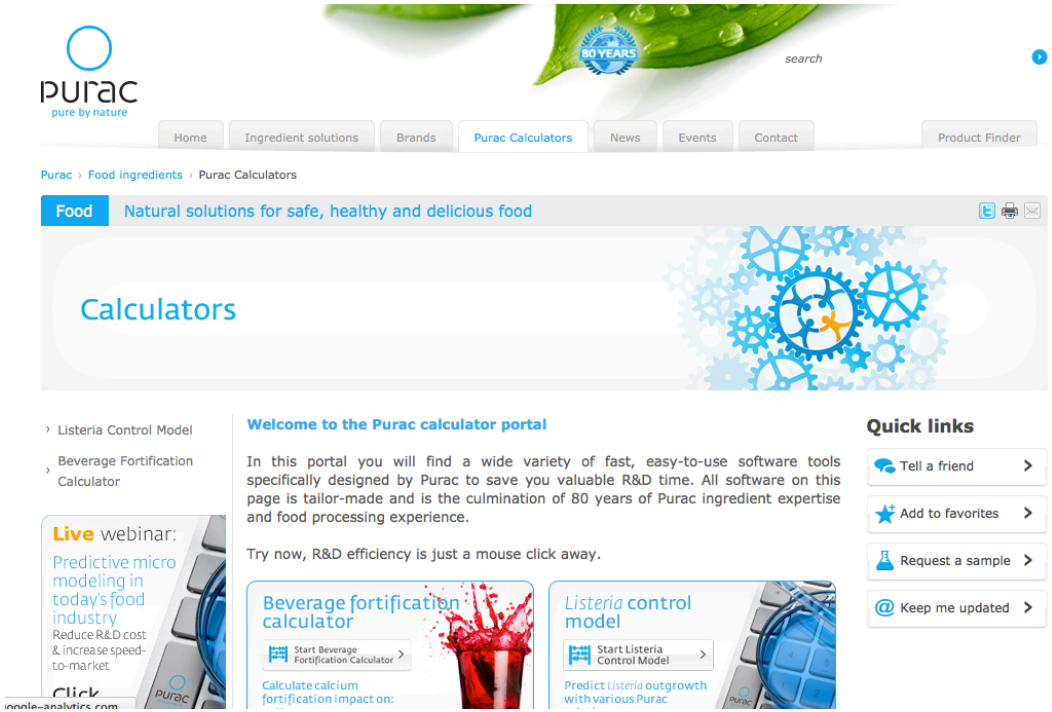


→ includes more than 40.000 curves/data on growth, survival or inactivation of microorganism in foods.

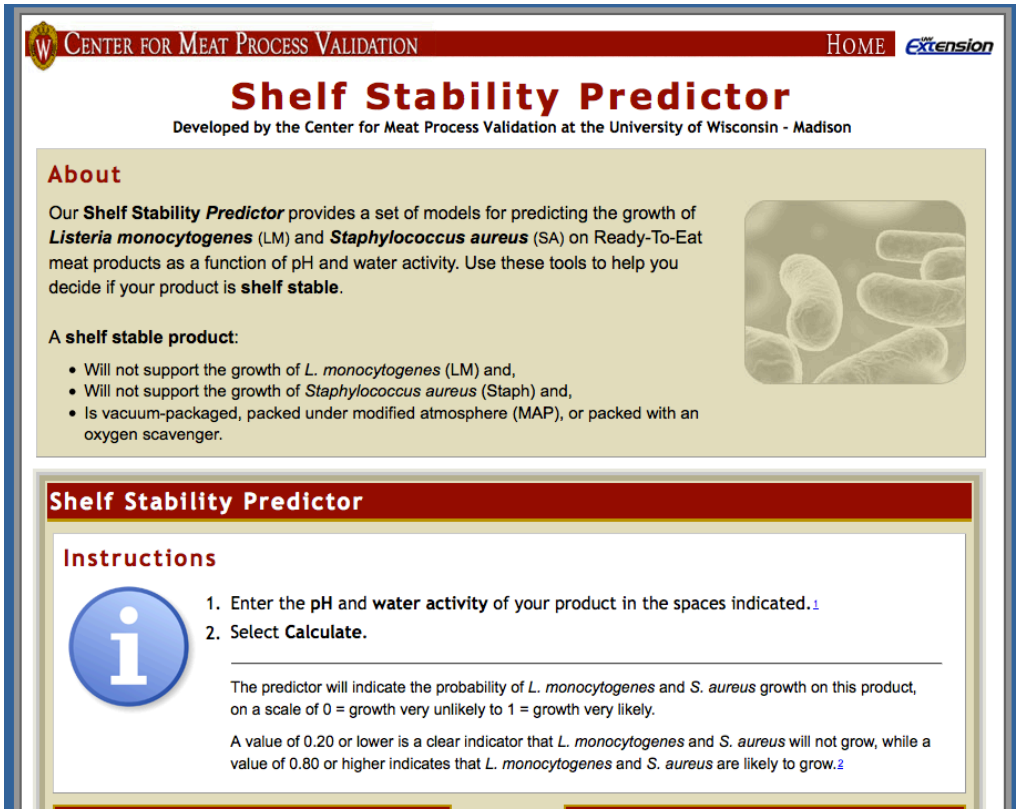
→ data has been obtained from the literature or provided by supporting institutions

→ the modelling toolbox within ComBase includes the Combase Predictor (previously Growth Predictor and Food MicroMoodel).

→ French decision support system that includes (i) a database with growth and inactivation responses of microorganisms in foods and (ii) predictive models for growth and inactivation of pathogenic bacteria and some spoilage microorganisms



→ predicts the effect of organic acids, temperature, pH and moisture on growth of *Listeria monocytogenes* in products



predict the growth of ***Listeria monocytogenes*** and ***Staphylococcus aureus*** on Ready-To-Eat meat products as a function of pH and water activity

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Validation studies

- Before market introduction, **validation** has to be carried out for **new or altered products**

- After the concept/prototype

→ acceptance tests → refinement of models → final formulation → validations in challenge test

have to be performed

Milkowski (2012)

- This is the main difference between free and paied software

- A complete challenge test takes aproximately 3 months + evaluation of process variations + identification of acceptable limits for formulation limits → establish a theoretical shelf life

- Industry saves costs and time when using reliable predictive micro modelling

Milkowski (2012)

- Industry has to perform validation studies, for final verification
- The analytical values for pH, water activity, moisture, etc are crucial
- However, predictive microbiology does not replace hygiene measure or Good Manufacturing Practices

→ <u>models can not be the only hurdle to pathogens</u>

Milkowski (2012)

OUTLINE

- Objectives of food industry
- The challenge
- Predictive microbiology
- How to obtain the data
- Available software
- Validation studies
- Acknowledgement by regulation
- The complexity of dynamic conditions
- Conclusions

Acknowledgement by regulation

- United States:
  - U.S. 9CFR Regulations
  - 2008 USDA Supplementary Guidance
- European Union:
  - 2005 & 2010 EU regulation

Milkowski (2012)

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The complexity of dynamic conditions

Laboratory research

Industrial scale

Laboratory research	Industrial scale
Studies are often carried out at constant temperatures	Time-varying temperature conditions are common

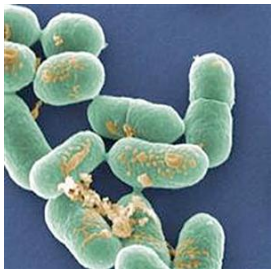
transfer of results is compromised

isothermal **non-isothermal**

- The greatest modeller's effort has been given to data obtained under constant (or static) environmental conditions

- From a realistic point of view this is somehow restrictive, since the majority of thermal processes occur under time-varying environmental conditions, and kinetic parameters obtained under such circumstances may differ from the ones estimated at static conditions, which compromises safety control

Case study



Isothermal conditions

52.5 °C

55.0 °C

57.5 °C

60.0 °C

62.5 °C

65.0 °C

L. innocua NCTC 10528

L. innocua 2030c

culture media

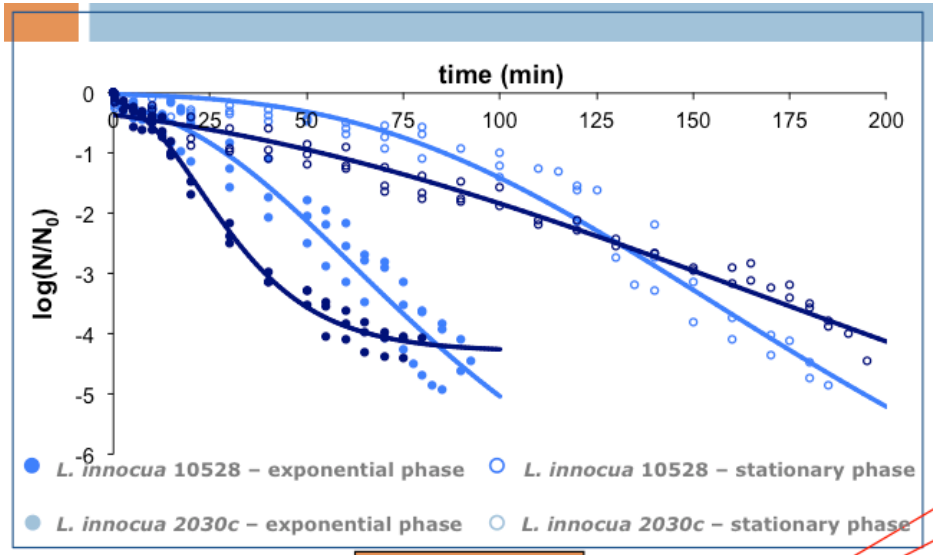
TSBYE

30 ºC/9h

30 ºC/20h

Exponential phase

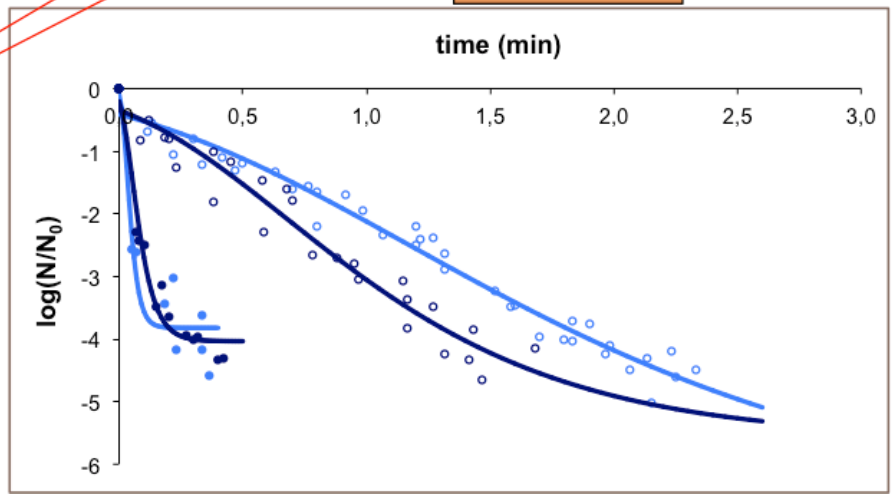
Stationary phase



52.5 °C

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

65.0 °C



Significant temperature effects

$N_0 \cong 10^7$ cfu/mL

Significant temperature effects

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

Shoulder parameter

$$L = c\,(T - d)^2$$

c and d are model parameters

Maximum inactivation rate

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

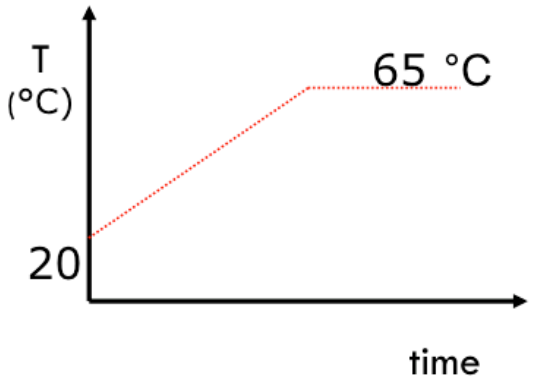
k_{ref} is inactivation rate at temperature T_{ref}
Ea is the inactivation energy
R is the gas constant

Non-isothermal conditions

(P1) 1.5 °C / min

(P2) 1.8 °C / min

(P3) 2.6 °C / min



culture media

TSBYE

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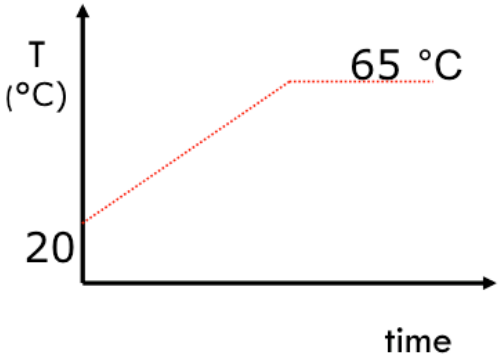
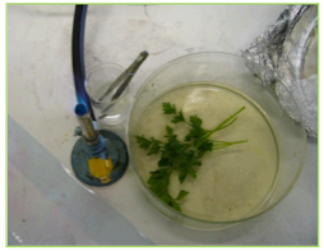


parsley

Petroselinum crispum

parsley artificially inoculated



TSBYE bacterial suspension
~ 10^7 cfu/mL of *L. innocua*

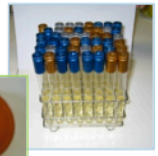
samples vacuum sealed

thermal treatment

sampling

stomaker

Palcam agar



30 °C / 5 days

Gompertz model encompassing the time-temperature effect

T=f(t)

$$\log\left(\frac{N}{N_0}\right)_{non-isothermal} = \int_0^t \frac{d\left(\log\left(\frac{N}{N_0}\right)_{isothermal}\right)}{dt} dt'$$

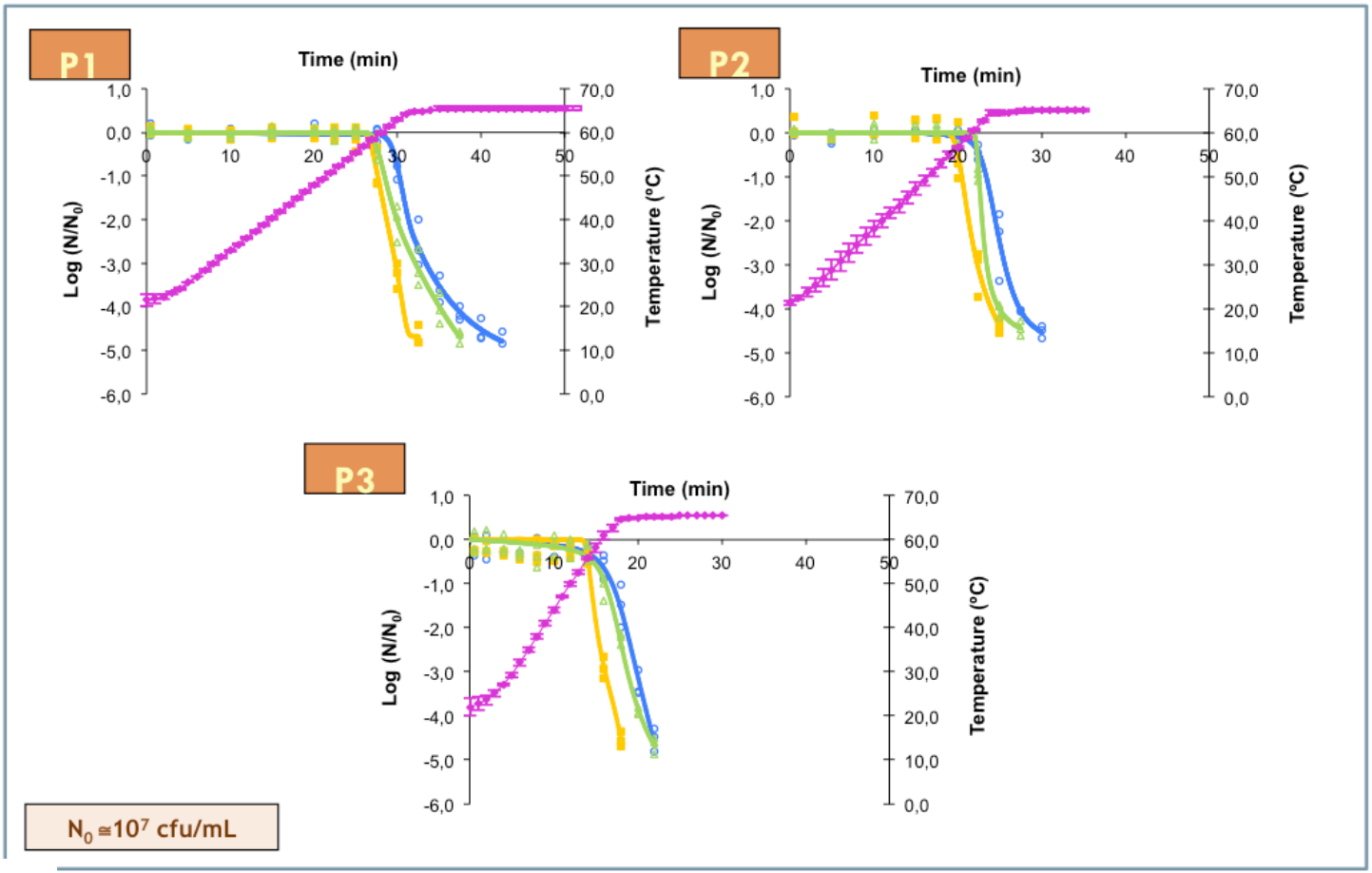
$$k_{max} = k_{ref} \exp\left(-\frac{Ea}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)$$

$$L = c(T-d)^2$$

$$\log\left(\frac{N}{N_0}\right)_{non-isothermal} = \int_0^t \left[-k_{max}(t')e \exp\left(-\frac{k_{max}(t')e}{\log\left(\frac{N_{res}}{N_0}\right)}(L(t')-t')+1\right) \exp\left(-\exp\left(-\frac{k_{max}(t')e}{\log\left(\frac{N_{res}}{N_0}\right)}(L(t')-t')+1\right)\right)\right] dt'$$

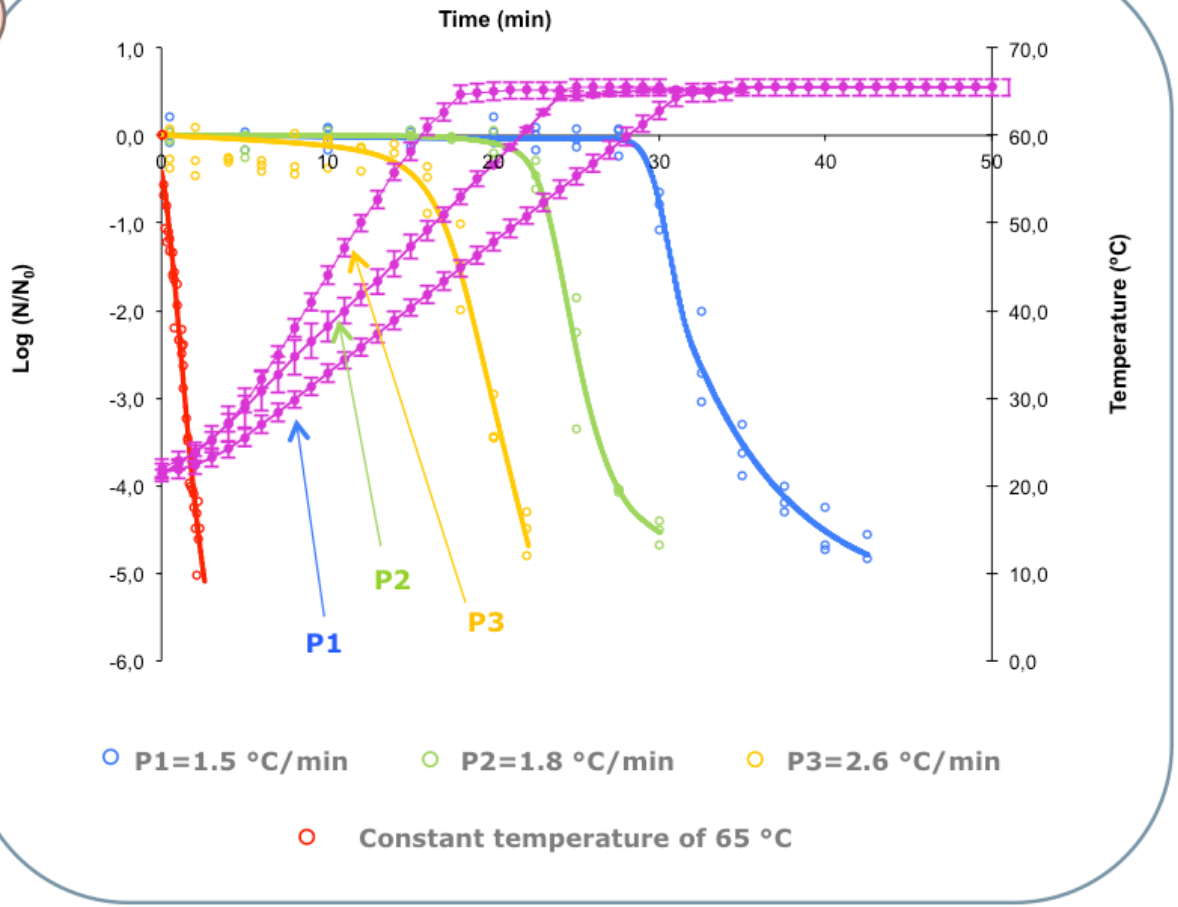
culture media

TSBYE

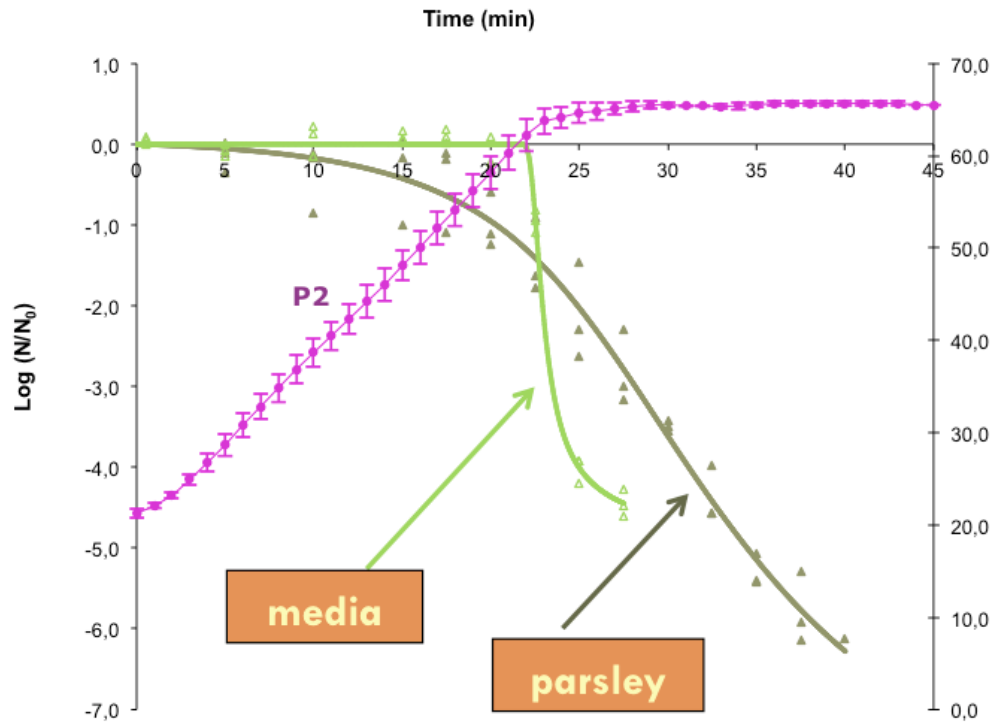


culture media
TSBYE

TSAYE



Palcam Agar



$N_0 \cong 10^7$ cfu/mL

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Conclusions

- Great progresses in the past 20 years
- There are many models available, each with its benefits and limitations
- Yet much work has still to be developed, particularly kinetic studies under dynamic conditions

- Validation studies have to be carried out for new or altered products, due to the complexity of the systems

- Predictive microbiology is a powerful tool, but does not replace hygiene measures or Good Manufacturing Practices

Thank you!

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Mª Manuel Gil
Mª Fátima Miller
Elisabeth Alexandre