Nýsköpun & neytendur
Consumers & Products

Vinnsla & virðisaukning
Value Chain & Processing

Erfðir & eldi
Genetics & Aquaculture

Líftækni & lífefni
Biotechnology & Biomolecules

Mælingar & miðlun
Analysis & Consulting

Öryggi & umhverfi
Food Safety & Environment

matís

SSS Prediction Workshop

Paw Dalgaard
Anna Kristín Daníelsdóttir
Steinar B. Aðalbjörnsson

Öryggi og umhverfi

Skýrsla Matís 12-10
Apríl 2010

ISSN 1670-7192

FINAL REPORT

SSS PREDICTION WORKSHOP on

Seafood shelf-life and safety prediction

Matís project No.: 6025-1966

A one-day workshop

14th January 2010 at Matís, Vínlandsleið 12,

IS-113 Reykjavík, Iceland.

Organised in collaboration between the Aquatic Microbiology and Seafood Hygiene, National Food Institute (DTU Food), Technical University of Denmark and Division of Food Safety & Environment, Matis, Iceland

by

- Dr. Paw Dalgaard, Senior scientist at Aquatic Microbiology and Seafood Hygiene, DTU Food, Denmark
- Dr. Anna Kristín Daníelsdóttir, Dir. Food safety & Environment at Matís, Iceland
- Steinar B. Aðalbjörnsson, Marketing Director at Matís, Iceland

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D

Report summary

ISSN: 1670-7192

Titill / Title	*SSS PREDICTION Námskeið / SSS PREDICTION WORKSHOP*		
Höfundar / Authors	*Paw Dalgaard, Anna Kristín Daníelsdóttir, Steinar B. Aðalbjörnsson*		
Skýrsla / Report no.	12-10	*Útgáfudagur / Date:*	29.04.2010
Verknr. / project no.	6025-1966		
Styrktaraðilar / funding:			
Ágrip á íslensku:	Námskeið í notkun á spáforritum í sjávarútvegi: SSS (Seafood Spoilage and Safety) Prediction version 3.1 2009 (http://sssp.dtuaqua.dk/), Combase (www.combase.cc) and Pathogen Modeling forrit (http://pmp.arserrc.gov/PMPOnline.aspx). Kennari er Dr. Paw Dalgaard frá Tækniháskólanum í Danmörku (DTU) og fer kennslan fram á ensku. Forritið nýtist vísindamönnum, yfirvöldum og iðnaði í sjávarútvegi.		
Lykilorð á íslensku:	*Spáforrit, sjávarútvegur, námskeið, geymsluþol ESB reglugerðir, fæðuöryggi, Listeria monocytogens*		
Summary in English:	Workshop on the practical use of computer software to manage seafood quality and safety. It includes presentations and hands-on computer exercises to demonstrate how available software can be used by industry, authorities and scientists within the seafood sector. Examples with fresh fish, shellfish and ready-to-eat seafood (smoked and marinated products) are included in the workshop. Special attention is given to: (i) the effect of storage temperature and modified atmosphere packing on shelf-life and (ii) management of Listeria monocytogens according to existing EU-regulations (EC 2073/2005 and EC 1441/2007) and new guidelines from the Codex Alimentarius Commission. The presentations included in the workshop are given in English by Paw Dalgaard from the Technical University of Denmark. Participants will use their own laptop computers for the PC-exercises included in the workshop. Instruction for download of freeware will be mailed to the participants prior to the start of the workshop.		
English keywords:	*Prediction software, seafood quality management, food safety, storage, EU regulations, Listeria monocytogens*		

TABLE OF CONTENT

1. INTRODUCTION

Icelandic

Markmiðið var halda námskeið í notkun á spáforritum í sjávarútvegi: SSS (Seafood Spoilage and Safety) Prediction version 3.1 2009 (http://sssp.dtuaqua.dk/), Combase (www.combase.cc) and Pathogen Modeling (http://pmp.arserrc.gov/PMPOnline.aspx) forrit. Kennari var Dr. Paw Dalgaard frá Tækniháskólanum í Danmörku (DTU) og fór kennslan fram á ensku. Forritið nýtist vísindamönnum, yfirvöldum og iðnaði í sjávarútvegi. Alls voru 11 þátttakendur á námskeiðinu.

English

The workshop focused on the practical use of computer software to manage seafood quality and safety. It included presentations and hands-on computer exercises to demonstrate how available software can be used by industry, authorities and scientists within the seafood sector. Examples with fresh fish, shellfish and ready-to-eat seafood (smoked and marinated products) were included in the workshop. Special attention was given to: (i) the effect of storage temperature and modified atmosphere packing on shelf-life and (ii) management of *Listeria monocytogens* according to existing EU-regulations (EC 2073/2005 and EC 1441/2007) and new guidelines from the Codex Alimentarius Commission. The presentations were given by Paw Dalgaard from the Technical University of Denmark. Participants used their own laptop computers for the PC-exercises included in the workshop. Instruction for download of freeware was mailed to the participants prior to the start of the workshop. A total of 11 scientists participated in the workshop.

2. MATERIAL & METHODS

Software and documents

Software used at the SSS PREDICTION WORKSHOP on Seafood shelf-life and safety prediction:

- Seafood Spoilage and Safety Predictor (SSSP) version 3.1 from August 2009.
- Combase (www.combase.cc).
- Pathogen Modelling (http://pmp.arserrc.gov/PMPOnline.aspx).

See also the attached Annex 1 "Workshop Agenda and documents -140110-Reykjavik-Iceland".

Teacher and organizers

- **Teacher:** Dr. Paw Dalgaard, Seafood & Predictive Microbiology (Research group), Section for Aquatic Microbiology & Seafood Hygiene at the Technical University of Denmark (DTU Food).

- **Organisers:** Dr. Anna Kristín Daníelsdóttir and Steinar B. Aðalbjörnsson at Matís, Iceland.

- **Date and location:** 14th January 2010 at Matís ohf., Vínlandsleið 12, IS-113 Reykjavík, Iceland.

Participants

1. Erlingur Brynjúlfsson, erlingur@controlant.com – 38.000.- Greitt
2. Guðrún E. Gunnarsdóttir, gudrune@syni.is – 38.000.- Greitt
3. Guðrún Ólafsdóttir, go@hi.is – 38.000.- Greitt
4. Leó Már Jóhannsson, leo@opseafood.com – 38.000.- Greitt

5. Tómas Hafliðason, tomash@hi.is – 38.000.- Greitt
6. Árni Rafn Rúnarsson, arnir@matis.is – 38.000.- Greitt
7. Helene L. Lauzon, helene@matis.is – 38.000.- Greitt
8. Hrólfur Sigurðsson, hrolfur@matis.is – 38.000.- Greitt
9. Magnea Karlsdóttir, magneag@matis..is – 38.000.- Greitt
10. Nguyen Van Minh, minh@matis.is – 38.000.- Greitt
11. María Guðjónsdóttir, mariag@matis.is – 38.000.- Greitt

Total IKr. 418.000 Thereof DTU IKr. 209.000 and IKr. 209.000 Matís

3. RESULTS

The one day workshop was very successful. Meals and all practical matter were well in place and made it easier to conduct the workshop. The feedback received from the "evaluation" sheets distributed at the end of the workshop was positive. The participants found the workshop well organized, relevant and practical.

4. DISCUSSION & CONCLUSIONS

The workshop was very successful and as a result, more workshops will be organized in Iceland in the near future. Also, further cooperation opportunities were identified between Matis and DTU Food on joint national, Nordic and European projects.

5. ACKNOWLEDGEMENTS

Thanks to the administrative staff of Matis ohf. for a good job on the practical matters.

6. REFERENCES

See Annex 1

Seafood safety and shelf-life prediction – a one-day workshop

Time	Topic
8.45 - 9.00	Registration
9.00 - 9.10	Welcome and opening
9.10 - 10.30	Shelf-life prediction – effect of temperature. Presentation and PC exercises using the SSSP software
10.30 - 10.45	Coffee break
10.45 - 12.00	Predicting growth and inactivation of bacteria in seafood. Presentation and PC exercises using SSSP and other freeware
12.00 - 13.00	Lunch
13.00 - 14.00	Seafood safety prediction 1. Presentation and PC exercises concerning histamine formation and histamine fish poisoning
14.00 - 14.15	Coffee break
14.15 - 15.45	Seafood safety prediction 2. Presentation and PC exercises concerning *Listeria monocytogenes* in ready-to-eat seafood
15.45 - 16.00	Evaluation and close of the workshop

Technical University of Denmark

Shelf-life prediction – effect of temperature

Paw Dalgaard

Seafood & Predictive Microbiology (Research group)

Section for Aquatic Microbiology & Seafood Hygiene

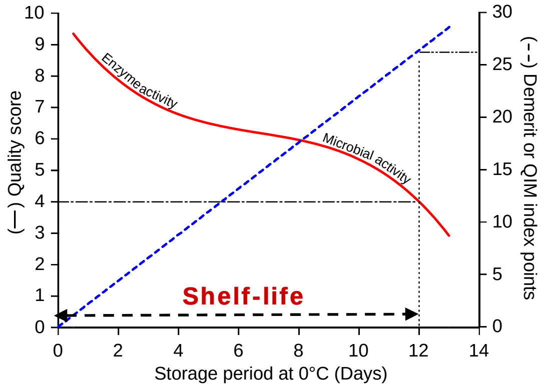
pad@aqua.dtu.dk

DTU Food
National Food Institute

Shelf-life prediction – effect of temperature

- Shelf-life of food – determination by sensory evaluation
- Storage temperature – effect on shelf-life
- Relative rate of spoilage (RRS)
    - Definition
    - RRS-models for different types of food
- Shelf-life prediction and time-temperature integration
    - Examples using the SSSP software
- Seafood Spoilage and Safety Predictor (SSSP) software
    - PC Exercises

Sensory changes and shelf-life –
an example with fresh fish



Shelf-life of seafood is always determined by sensory evaluation:

- Torry method: Scale from 10 to 1
- Quality index method(QIM): Several attributes are evaluated

  Sum of points from 0 to e.g. 30

Sensory changes and shelf-life –
an example with fresh fish

Simplified Torry scheme

		Grade		Score
Acceptable	No off-odour/flavour	**I**	Odour/flavour characteristic of species, very fresh, seaweedy	10
				9
				8
	Slight off-odours/flavour	**II**	Loss of odour/flavour	7
			Neutral	6
			Slight off-odours/flavours such as mousy, garlic, bready, sour, fruity, rancid	5
				4
		Limit of acceptability		
Reject	Severe off-odour/flavour	**III**	Strong off-odours/flavours such as stale cabbage, NH_3, H_2S or sulphides	3
				2
				1

Shewan et al. 1953

Sensory changes and shelf-life
an example with fresh fish

Quality index method (QIM) – simple scheme

Quality parameter		Point
General appearance	Surface appearance	0 - 3
	Skin	0 – 1
	Slime	0 – 3
	Stiffness	0 – 1
Eyes	Clairity	0 – 2
	Shape or pupil	0 – 2
Gills	Colour	0 – 2
	Smell	0 – 3
	Slime	0 – 2
Flesh colour	Open surfaces	0 – 2
Blood	In throat cut	0 – 2
Sum of demerit points		**0 – 23**

Bremner 1985; www.qim-eurofish.com/

Storage temperature – effect on shelf-life

	% of samples or refrigerators			
Temp. (^{0}C)	Denmark[a]	Portugal[b]	Sweden[c]	USA[d]
< 2	20	?	?	5-41
2 - 5	37	22	40	40-56
5 - 10	36	66	50	8-54
> 10	7	12	10	< 2

a) Olsen (1996). Regional Veterinary and Food Control Authority, Copenhagen
b) Azevedo (2005). Food Control, 16, 121-124
c) Lindblad & Boysen (2004). National Food Administration, Rapport 14
d) Godwin et al. (2007). Food Prot. Trends. 27, 168-173

Temperature of food can vary substantially during distribution and it is important to determine the effect of variable storage temperatures

Storage temperature – effect on shelf-life

Example with fresh fish from cold water

Temperature (°C)	Shelf-life (days)	Relative rate of spoilage (RRS)
-3.0	25	0.48
-1.5	17	0.72
0	12	1.0
5	5	2.3
10	3	4.0
15	2	6.3

- Important effect of superchilling below 0°C
- Regular refrigerators often operate at +5°C (or higher) – at 0°C shelf-life of fresh fish is more than twice as long
- The overall effect of a chill chain from harvest/processing to the consumer must be considered

Relative rate of spoilage (RRS)

- RRS: Shelf-life at T_{ref} (°C) divided by shelf-life at T °C

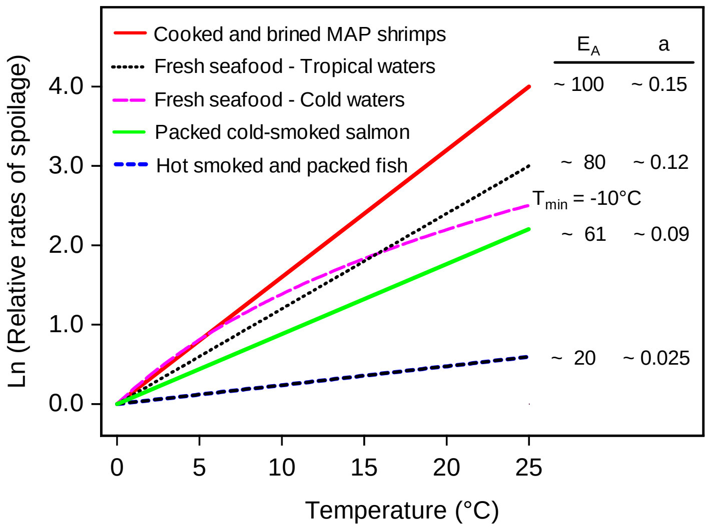
$$RRS(T\,^{\circ}C) = \frac{\text{Shelf-life}(T_{ref}\,^{\circ}C)}{\text{Shelf-life}(T\,^{\circ}C)} \leftrightarrow \text{Shelf-life}(T\,^{\circ}C) = \frac{\text{Shelf-life}(T_{ref}\,^{\circ}C)}{RRS(T\,^{\circ}C)}$$

Shelf-life can be predicted at different temperatures when:

1. Shelf-life at a single constant temperature is known
2. RRS at different temperatures are known (RRS model)

Spencer & Baines (1964), Olley & Ratkowsky (1973)

Storage temperature – effect on RRS



Empirical models for relative rates of spoilage

Exponential RRS model:

$$RRS = \frac{Shelf-life\ at\ T_{ref}}{Shelf-life\ at\ T} = Exp\left[a \times (T - T_{ref})\right]$$

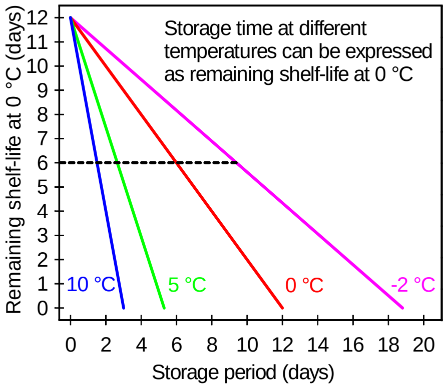
Arrhenius RRS model:

$$RRS = Exp\left[\frac{E_A}{R} \times \left(\frac{1}{(T+273)} - \frac{1}{T_{ref}+273}\right)\right]$$

Square-root RRS model:

$$RRS = \left(\frac{T - T_{min}}{T_{ref} - T_{min}}\right)^2;\ T_{min} = -10°C -> \sqrt{RRS} = 1 + 0.1 \times T°C$$

Storage temperature – effect on shelf-life



Example with fresh fish

Temperature (°C)	Shelf-life (days)
-2	19
0	12
5	5
10	3

$$\mathrm{RRS}\,(10°\mathrm{C}) = (1 + 0.1 \times \mathrm{T°C})^2 = 4$$

$$\text{Shelf-life}\,(10^{o}\mathrm{C}) = \frac{\text{Shelf-life}\,(\mathrm{T_{ref}}\ °\mathrm{C})}{\mathrm{RRS}(\mathrm{T}\,°\mathrm{C})} = \frac{12}{4} = 3\ \text{days}$$

Shelf-life at variable storage temperatures

Example: Fresh fish with shelf-life of 12 days at 0°C

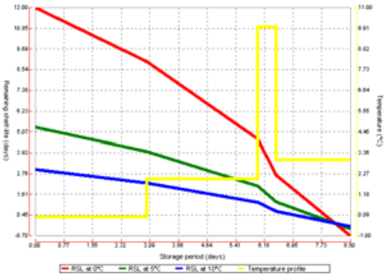
Time	Temperature profile and remaining shelf-life	
	Example 1	Example 2
3 days	0°C	- 2°C
3 days	+ 2°C	+ 2°C
12 hours	+10°C	+ 4°C
2 days	+ 3°C	+ 3°C
Total 8.5 days	Remaining shelf-life at 0°C	
	? days	? days

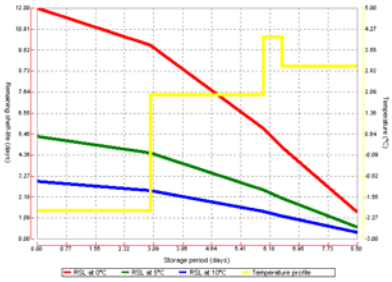
- Is it possible to store the products one more day at 2°C ?
- Is it possible to store the products three more days 2°C ?

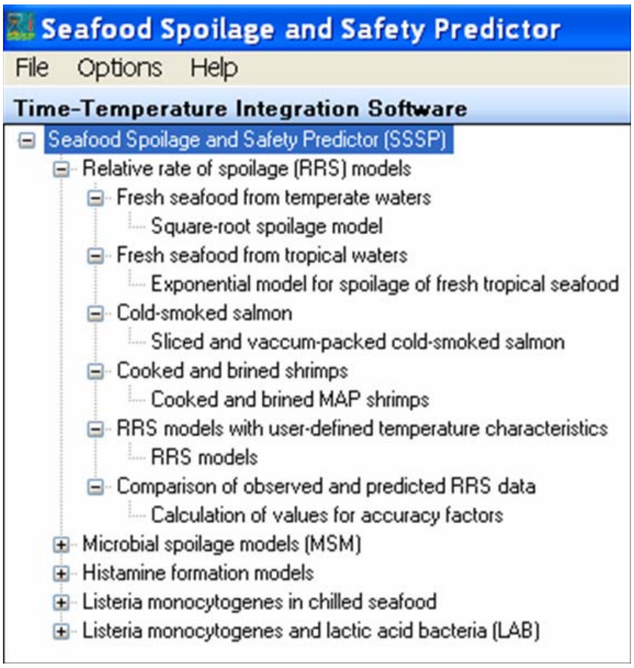
Shelf-life at variable storage temperature

Example: Fresh fish with shelf-life of 12 days at 0°C

Time	Temperature profile and remaining shelf-life	
	Example 1	Example 2
3 days	0°C	- 2°C
3 days	+ 2°C	+ 2°C
12 hours	+10°C	+ 4°C
2 days	+ 3°C	+ 3°C
	Remaining shelf-life at 0°C	
Total 8.5 days	None	1-2 days

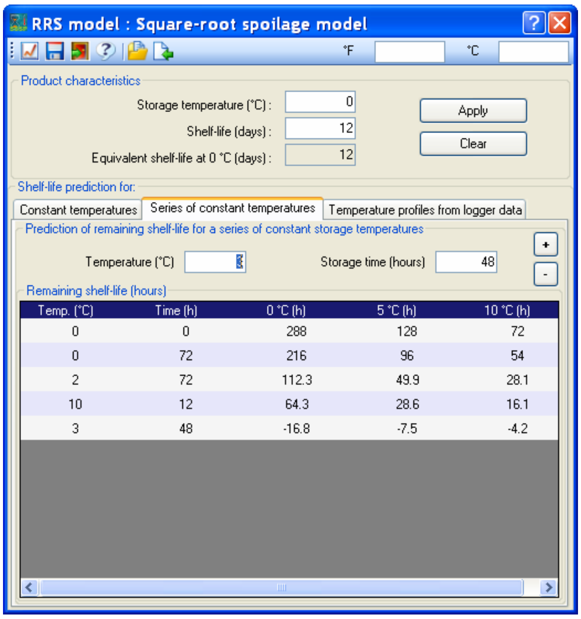






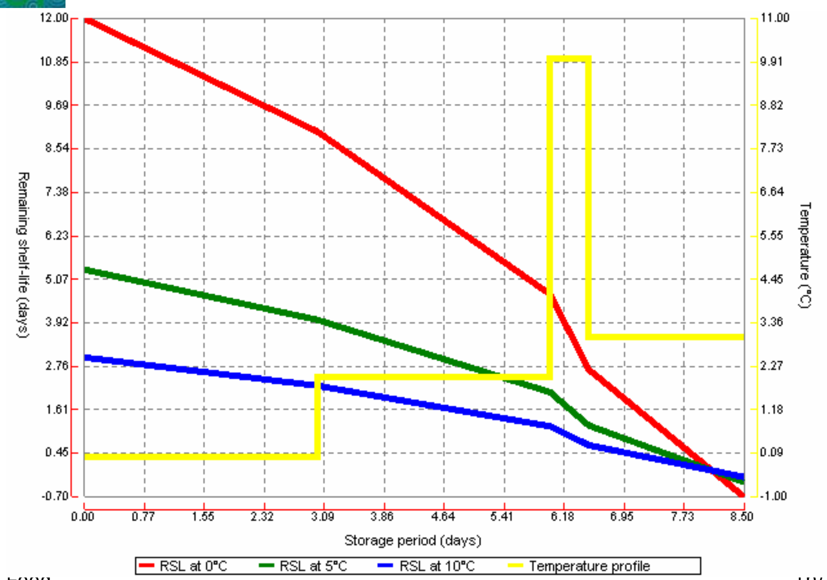
http://sssp.dtuaqua.dk

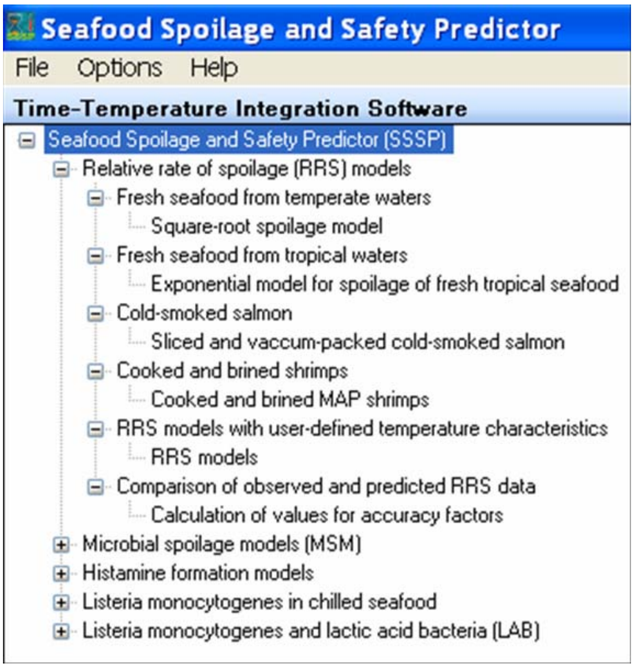
Seafood Spoilage and Safety Predictor (SSSP)



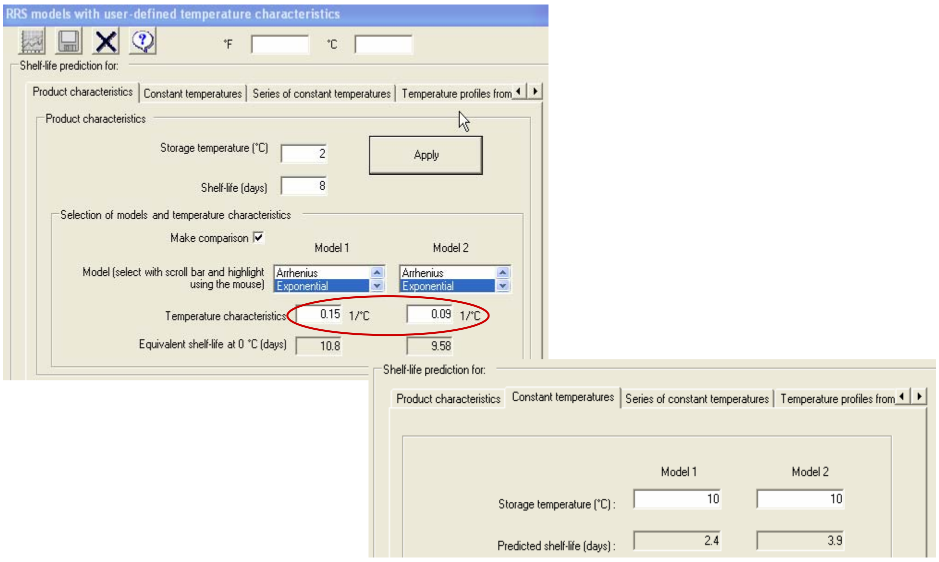
Temp. (°C)	Time (h)	0 °C (h)	5 °C (h)	10 °C (h)
0	0	288	128	72
0	72	216	96	54
2	72	112.3	49.9	28.1
10	12	64.3	28.6	16.1
3	48	-16.8	-7.5	-4.2

Seafood Spoilage and Safety Predictor (SSSP)

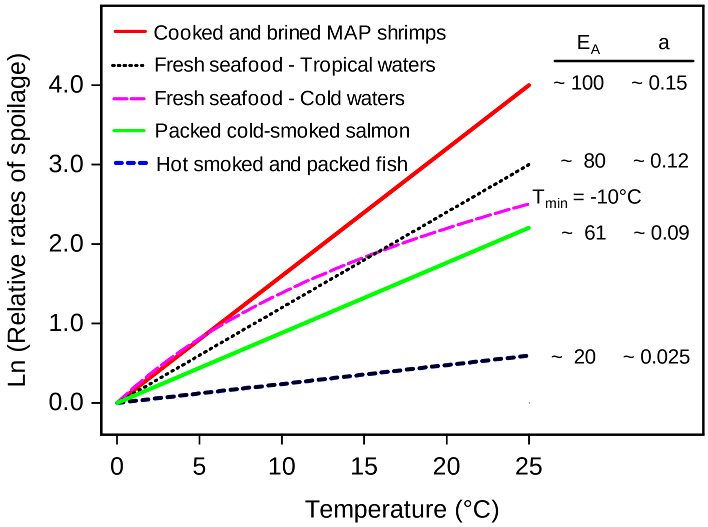




Shelf-life prediction for foods with known temperature sensitivity (RRS models)

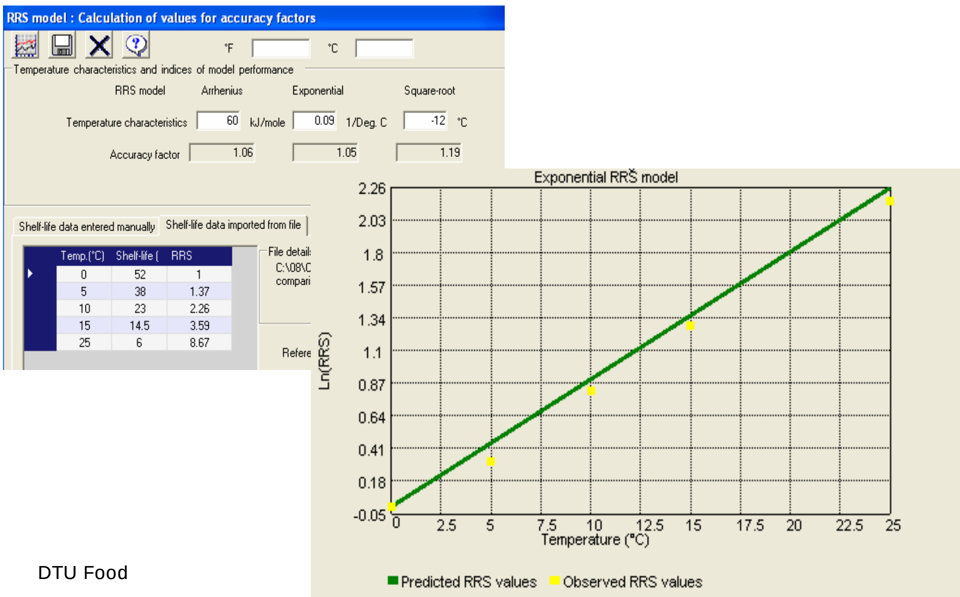


Storage temperature – effect on RRS

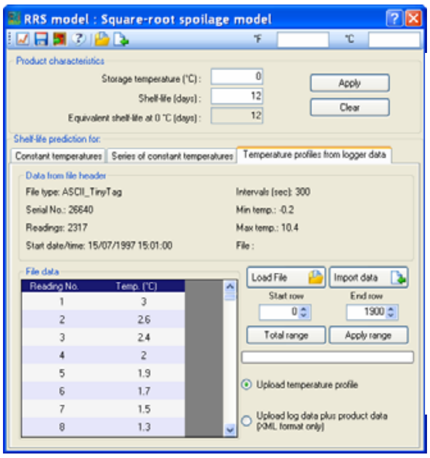


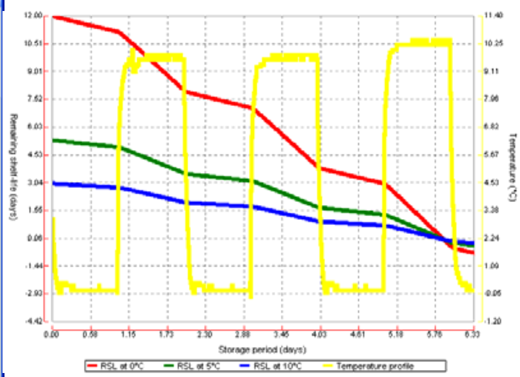
Dalgaard & Jørgensen (2000)

Comparison of observed and predicted RRS data – case for cold smoked salmon



The effect of temperature profiles recorded by data loggers can be predicted using SSSP

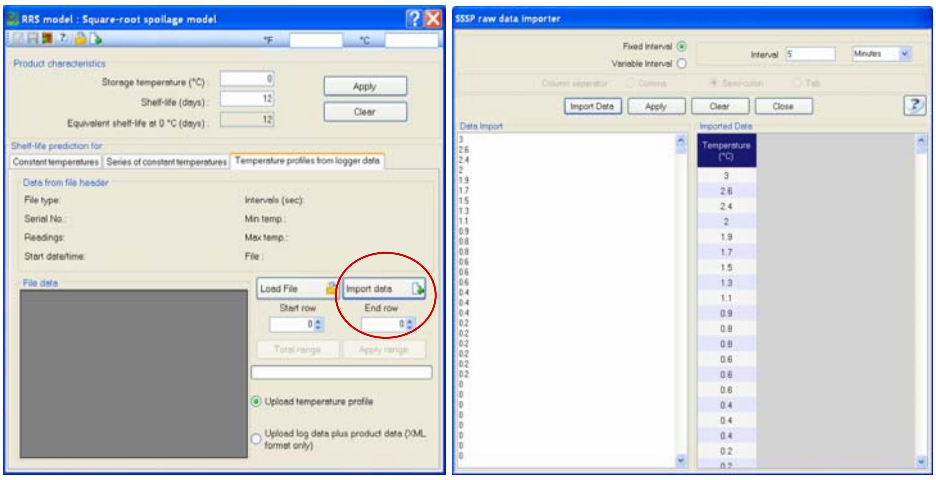




Numerous dataloggers are available to record the temperature of food during storage and distribution

- A challenge for handling of temperature data

To facilitate evaluation of product temperature profiles SSSP includes a module that allow data to be imported by copy and paste from spreadsheets (like MS Excel)



SSSP – Help menu

Table of content

Relative rate of spoilage (RRS) models

Seafood Spoilage and Safety Predictor (SSSP)

- SSSP has been available since January 1999
  - New versions in 2004, 2005, 2008 and 2009 (v. 3.1 in August)

- SSSP is used by more than 4000 people/institutions from 105 different countries:
  - Production and distribution of seafood : 30 %
  - Seafood inspection : 20 %
  - Research : 20 %
  - Teaching : 15 %

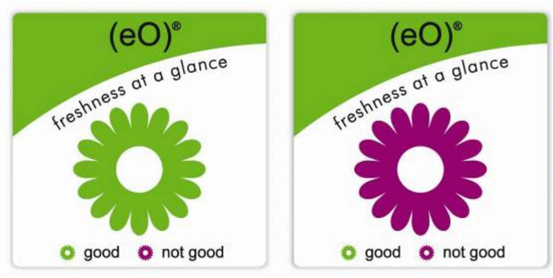
- SSSP is available for free and in different languages
  - SSSP v. 3.1 from 2009: 15 languages

Shellf-life prediction and time-temperature integration

- Various systems are available to evaluate the effect of temperature (chill chains) on the shelf-life of food

Shellf-life prediction and time-temperature integration

- Various systems are available to evaluate the effect of temperature(chill chains) on the shelf-life of food



http://www.cryolog.com/en/

http://www.vitsab.com/

Shellf-life prediction and time-temperature integration

- Various systems are available to evaluate the effect of temperature (chill chains) on shelf-life of food

AVANT

TRACEO® est transparent,
le code-barres est lisible,
le produit est frais

APRÈS

TRACEO® est rose,
le code-barres est voilé,
le produit n'est plus consommable

References

FRESHNESS, QUALITY AND SAFETY IN SEAFOODS

FLAIR-FLOW EUROPE
TECHNICAL MANUAL
F-FE 380A/00

May 2000

…/workshop-140110/shelf-life prediction/Dalgaard 2000.pdf

http://flairflow4.vscht.cz/seafood00.pdf

Shelf-life prediction – effect of temperature

- Shelf-life of food – determination by sensory evaluation
- Storage temperature – effect on shelf-life
- Relative rate of spoilage (RRS)
    - Definition
    - RRS-models for different types of food
- Shelf-life prediction and time-temperature integration
    - Examples using the SSSP software
- Seafood Spoilage and Safety Predictor (SSSP) software
    - PC Exercises

Seafood Spoilage and Safety Predictor (SSSP)

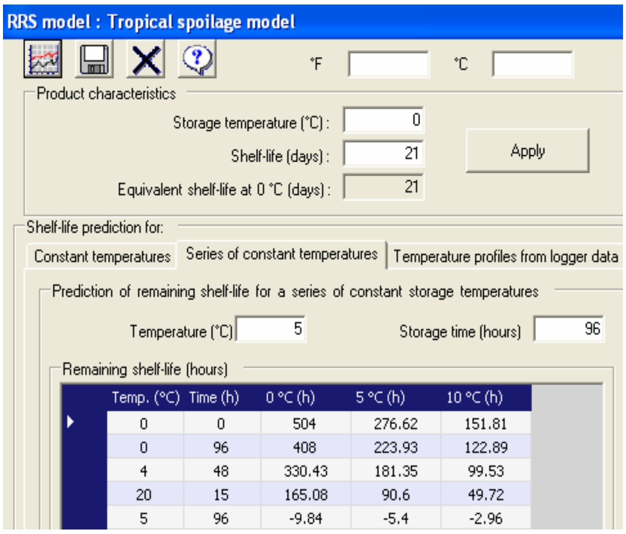
Exercise 1: RRS model with fixed temperature sensitivity

Tropical fresh fish can have a shelf-life of 21 days at 0°C. To evaluate shelf-life at other temperatures start the SSSP software and activate the RRS model ”Fresh seafood from tropical waters” (’double click’):

- Determine shelf-life for a temp. profile including: (i) 4 days at 0°C, (ii) 2 days at 4°C, (iii) 15 hours at 20°C and (iv) 4 day at 5°C
  (Use e.g. the zoom function to facilitate reading of shelf-life from graph – activate zoom by holding down the left mouse button)
  Answer: The shelf-life is _____ days. Thus ____ days of shelf-life is lost compared to storage at 0°C.
- Save data and predictions as C:\workshop-140110\shelf-life prediction\Ex1.xml and relevant graph as C:\workshop-140110\shelf-life prediction\Ex1.png. Prediction can then easily be used later and send to other with interest in the chill chain
- Try e.g. to save graph/predictions in a different language

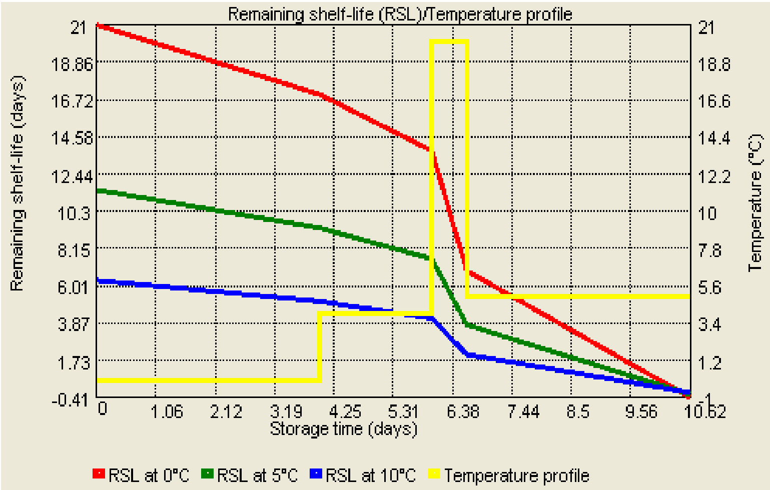
Seafood Spoilage and Safety Predictor (SSSP)

Exercise 1: RRS model with fixed temperature sensitivity



Seafood Spoilage and Safety Predictor (SSSP)

Exercise 1: RRS model with fixed temperature sensitivity



Seafood Spoilage and Safety Predictor (SSSP)

Exercise 2: RRS models with user defined temperature characteristics

The temperature characteristic (the parameter ’a’) in the exponential RRS-model used for ’Fresh fish from tropical waters’ is 0.12 (°C^{-1}).

What is the effect of the temperature profile evaluated in exercise 1 on another product with a shelf-life of 21 days at 0°C but with a more pronounced temperature sensitivity corresponding to a temperature characteristics ’a’ of 0.15 (°C^{-1}) ?

- Use ’RRS models with user defined temperature characteristics’ to compare shelf-life for the two products with temperature characteristics of respectively 0.12 and 0.15 (°C^{-1}).
  Answer: Shelf-life with a temperature characteristic of 0.15 (°C^{-1}) in the exponential RRS model is ___ days.

(You do not have to type the temperature profile again – activate ’Temperature profile from logger data’ to read the data you saved in Ex1.xml)

Seafood Spoilage and Safety Predictor (SSSP)

Exercise 2 (Cont.):

- The 15 hours at 20°C (in the evaluated temperature profile, Ex1.xml) influence shelf-life very differently for the two products with temperature characteristics of 0.12 and 0.15 (°C^{-1}). How many days of remaining shelf-life at 0°C is used in this step of the temperature profile for each of the two products ?
  Answer:

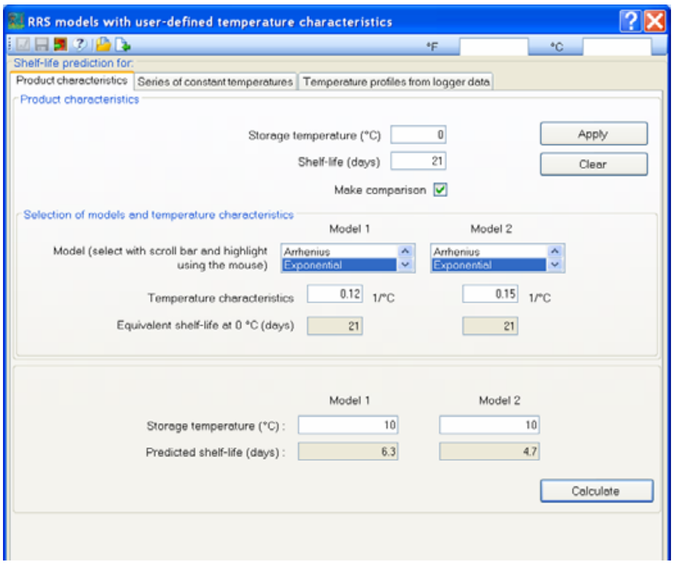
  - ___ days for product with temperature characteristic of 0.12 (°C^{-1})

  - ___ days for product with temperature characteristic of 0.15 (°C^{-1})

The models included in SSSP under 'RRS models with user defined temperature characteristics' allow shelf-life to be predicted for any food where the temperature characteristic and shelf-life (at a single constant temperature) are known

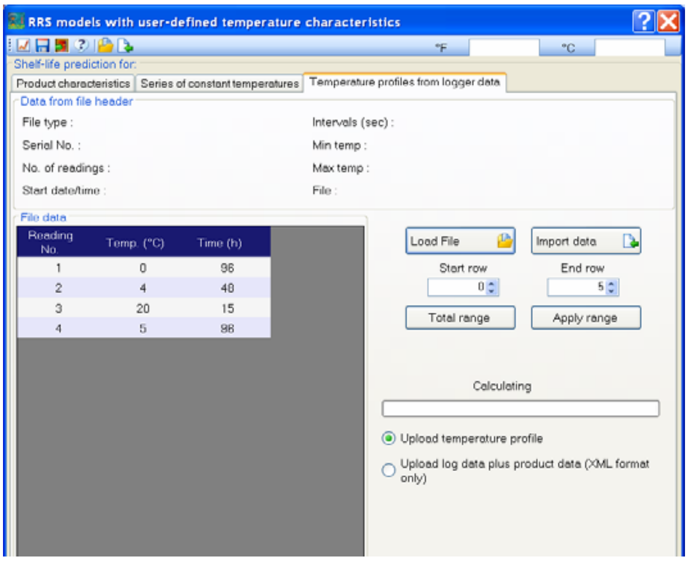
Seafood Spoilage and Safety Predictor (SSSP)

Exercise 2: RRS models with user defined temperature characteristics



Seafood Spoilage and Safety Predictor (SSSP)

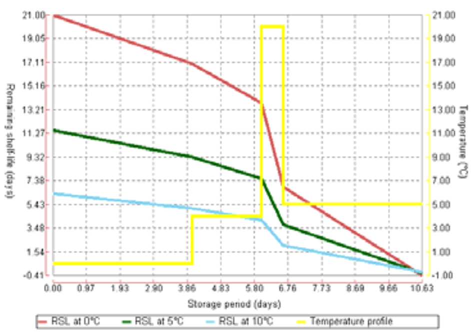
Exercise 2: RRS models with user defined temperature characteristics



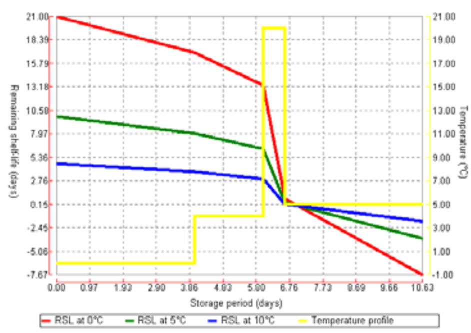
Seafood Spoilage and Safety Predictor (SSSP)

Exercise 2: RRS models with user defined temperature characteristics

Temperature characteristic 'a' = 0.12 °C^{-1}



Temperature characteristic 'a' = 0.15 °C^{-1}



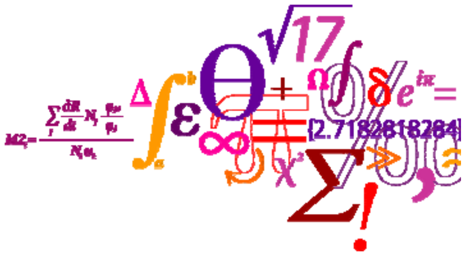
Predicting the growth and inactivation of bacteria in seafood

Paw Dalgaard

Seafood & Predictive Microbiology (Research group)

Section for Aquatic Microbiology and Seafood Hygiene

pad@aqua.dtu.dk

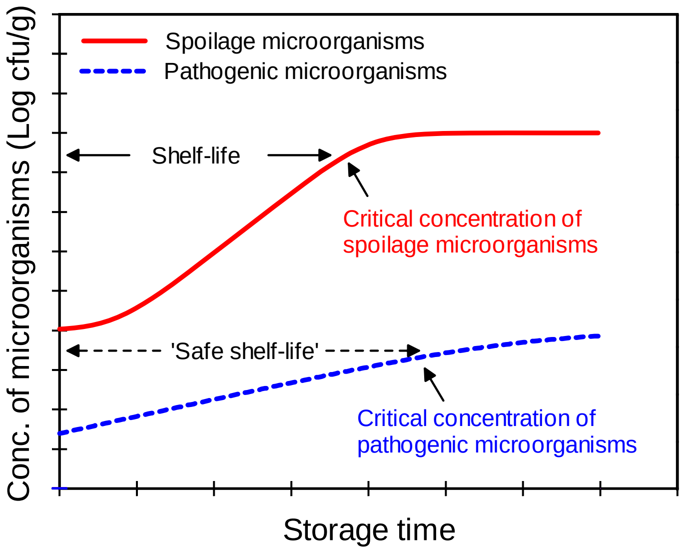


DTU Food
National Food Institute

Predicting the growth and inactivation of bacteria in seafood

- Predictive microbiology - concept
- Primary growth and inactivation models
- Secondary models and product evaluation/validation
- Predictive microbiology – applications and software
- PC Exercises

Predicting the growth of bacteria in food



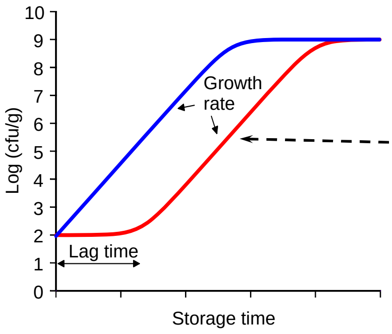
Predictive microbiology – the concept

- Growth, survival and inactivation of microorganisms in foods are reproducible responses
- A limited number of environmental parameters in foods determine the kinetic responses of microorganisms
  - Temperature
  - Water activity/water phase salt
  - pH
  - Food preservatives (organic acids, nitrite, ...)
- A mathematical model that quantitatively describes the combined effect of the environmental parameters can be used to predict growth, survival or inactivation of a microorganism and thereby contribute important information about product shelf-life

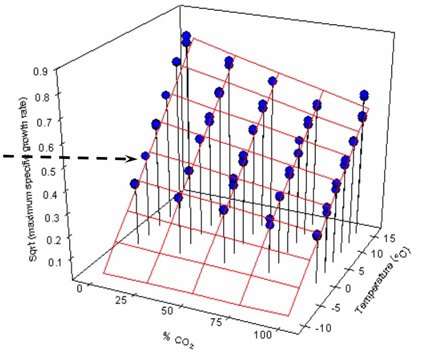
Roberts & Jarvis (1983)

Development of predictive microbiology models

Models are usually developed in two steps from large experiments including the effect of several environmental parameters



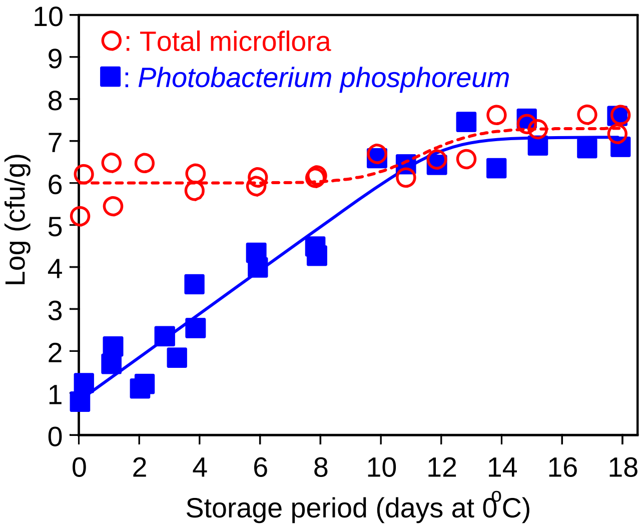
Primary model

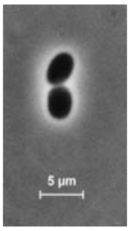


Secondary model

Models allow microbial responses to be predicted at conditions that have not been specifically studied

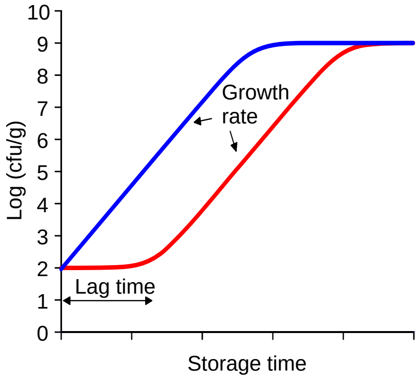
Growth of spoilage bacteria in fresh MAP cod fillets





Dalgaard (1998)

Primary models

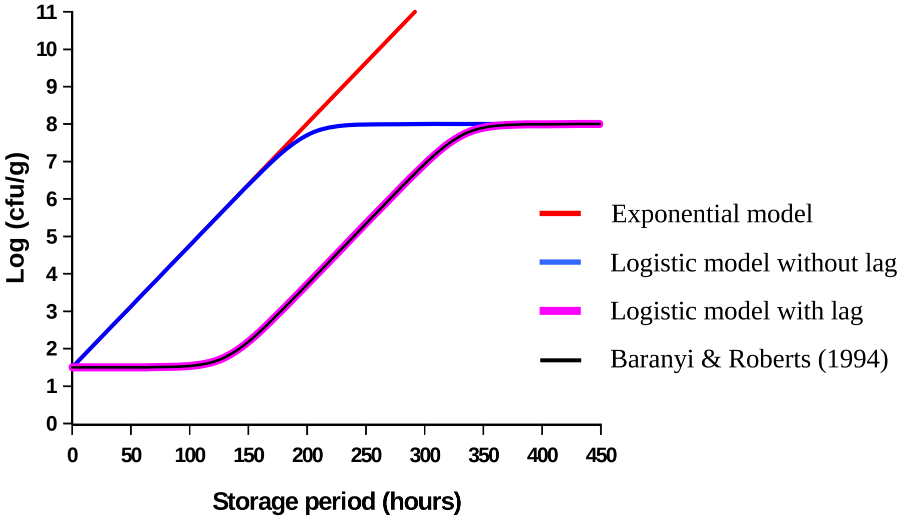


Curve fitting software:

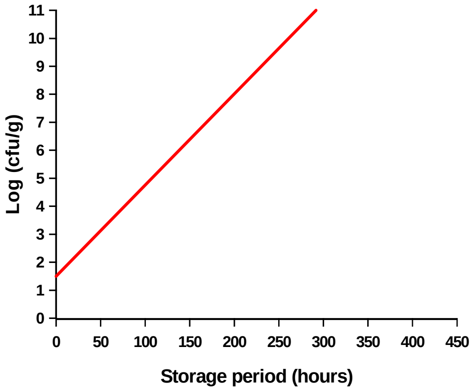
- Numerous stasticstis programmes
- MS Excel with solver add-in
- Combase/DMFit (www.combase.cc)
- MicroFit (www.ifr.bbsrc.ac.uk/MicroFit)
- GInaFit (cit.kuleuven.be/biotec/downloads/GInaFit/get_tool.php)

N	Cell concentration (cfu/g)
dN/dt	Absolute growth rate (cfu/g/hour)
(dN/dt)/N = μ	Specific growth rate (1/hour)

Primary growth models



Exponential growth model



$$\mu_{max} = \text{Slope} \times \text{Ln}(10) = \frac{\text{Log}(N_2) - \text{Log}(N_1)}{\text{time}_2 - \text{time}_1} \times \text{Ln}(10)$$

Differential form:

$$\frac{dN}{dt} = N \times \mu_{max}$$

Integrated form:

$$N_t = N_o \times \exp(\mu_{max} \times time)$$

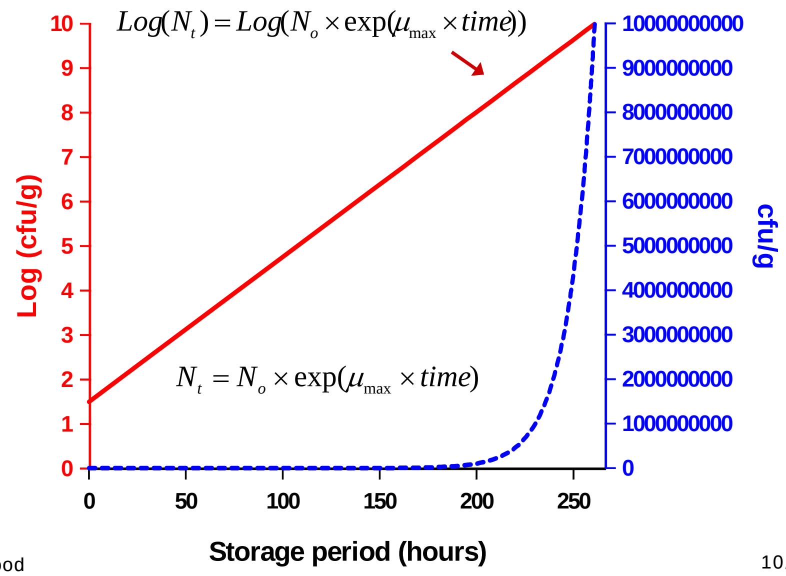
Integrated and transformed:

$$Log(N_t) = Log(N_o \times \exp(\mu_{max} \times time))$$

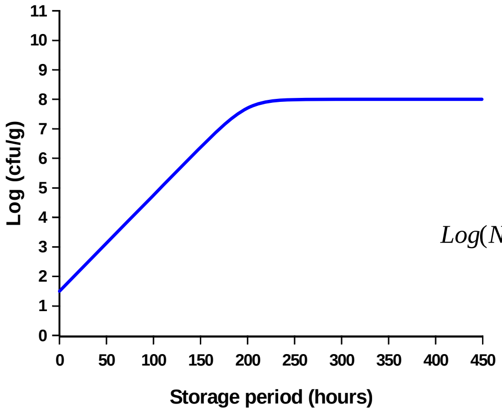
or

$$Log(N_t) = Log(N_o) + (\mu_{max} \times time) / Ln(10)$$

Exponential growth model



Logistic growth model



Differential form:

$$\frac{dN}{dt} = N \times \mu_{max} \left[1 - \frac{N_t}{N_{max}} \right]$$

Integrated form:

$$Log(N_t) = Log\left(\frac{N_{max}}{1 + \left[\frac{N_{max}}{N_0} - 1 \right] \times \exp(-\mu_{max} \times time)} \right)$$

or

$$Log(N_t) = Log\left(\frac{N_0 \times N_{max}}{N_0 + [N_{max} - N_0] \times \exp(-\mu_{max} \times time)} \right)$$

Logistic growth model with delay

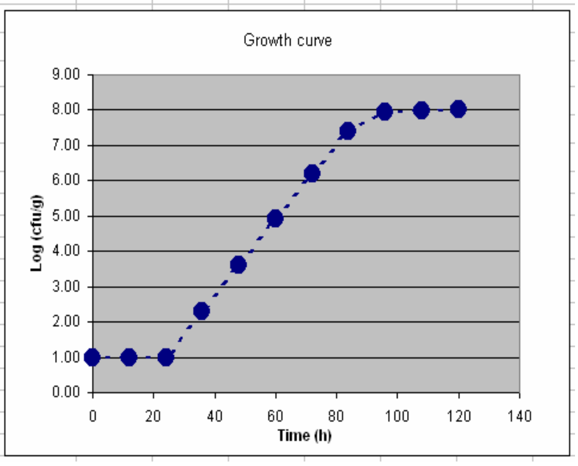
\Logistic-with-delay.xls

$t < t_{lag}$: $N_t = N_0$

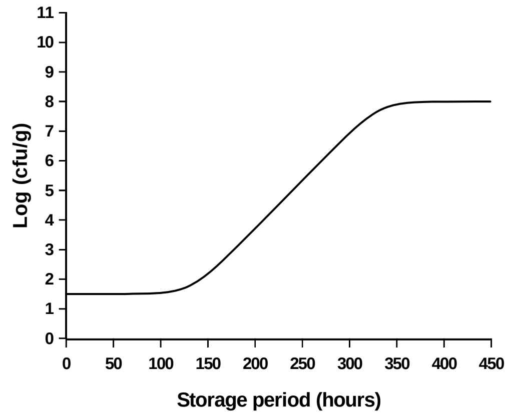
$t \geq t_{lag}$: $N_t = Nmax/[1+((N_{max}/N_0)-1)*exp(-\mu*(t-t_{lag}))]$

N_0	1.0E+01
$Log(N_0)$	1.00
Lag time (h)	24
μ_{max} (1/h)	0.25
N_{max}	1.0E+08
$Log(N_{max})$	8.00

Time, h	Log cfu/g	cfu/g
0	1.000	10
12	1.000	10
24	1.000	10
36	2.303	201
48	3.606	4034
60	4.908	80965
72	6.205	1601483
84	7.392	24636471
96	7.938	86782972
108	7.997	99247450
120	8.000	99962263



Baranyi and Roberts model



Differential form (simplified):

$$\frac{dN}{dt} = N \times \mu_{max} \left(\frac{q_t}{q_t + 1} \right) \times \left[1 - \frac{N_t}{N_{max}} \right]$$

Integrated form:

$$Log(N_t) = Log(N_0) + \frac{1}{\mu_{max}} \times \left[time + \frac{1}{\mu_{max}} \times Ln\left(\frac{\exp(-\mu_{max} \times time) + q_0}{1 + q_0} \right) \right] - \frac{1}{Log(10)} \times Ln\left(1 + \frac{\exp\left(\mu_{max} \times \left[time + \frac{1}{\mu_{max}} \times Ln\left(\frac{\exp(-\mu_{max} \times time) + q_0}{1 + q_0} \right) \right] \right) - 1}{\exp(Log(N_{max}) - Log(N_0))} \right)$$

DMFit / ComBase includes the Baranyi and Roberts model

http://ifrsvwwwdev.ifrn.bbsrc.ac.uk/CombasePMP/GP/Login.aspx?ReturnUrl=%2fCombasePMP%2fGP%2fDefault.aspx

Example:

- Data from Logistic model with delay
- Data input by copy and paste

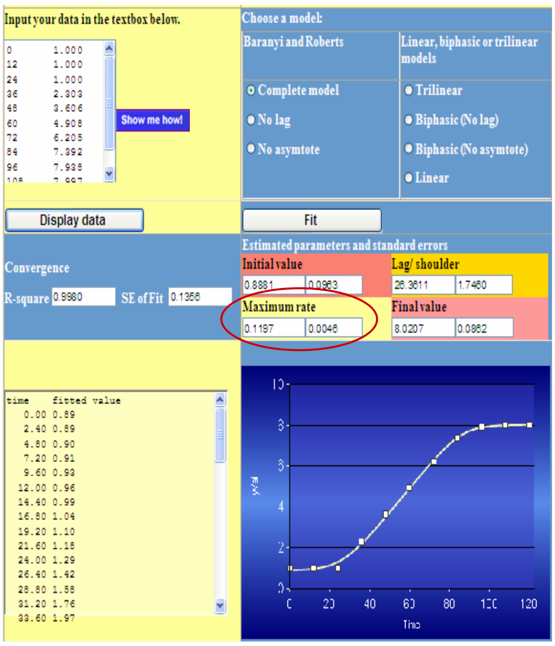
- Estimated growth rate depends on the unit of the data

-Ln(cfu/g):

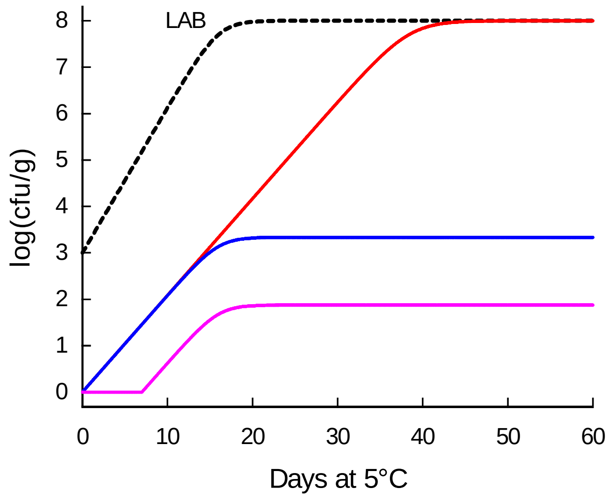
Maximum rate = μ_{max} (1/h)

-Log10(cfu/g):

Maxumum rate*Ln(10) = μ_{max} (1/h)



Primary model for microbial interaction

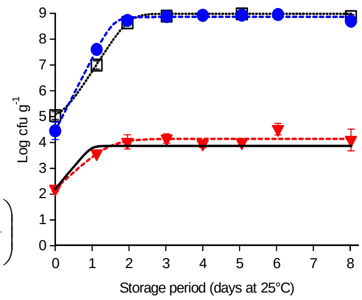


Primary model for microbial interaction

- Jameson effect (Simplifying assumption/hypothesis):
  All microorganisms in a food stop growing when the dominating microflora reaches its maximum population density
- Differential form of Logistic model for growth of LAB (Intra-species competition)

$$\frac{dLAB/dt}{LAB_t} = \mu_{\max}^{LAB} \times \left(1 - \frac{LAB_t}{LAB_{\max}}\right)$$

$$\frac{dLm/dt}{Lm_t} = \mu_{\max}^{Lm} \times \left(1 - \frac{Lm_t}{Lm_{\max}}\right) \times \left(1 - \frac{LAB_t}{LAB_{\max}}\right)$$



- Logistic model for growth and interaction between LAB and *L. monocytogens* (Lm)

Primary inactivation models

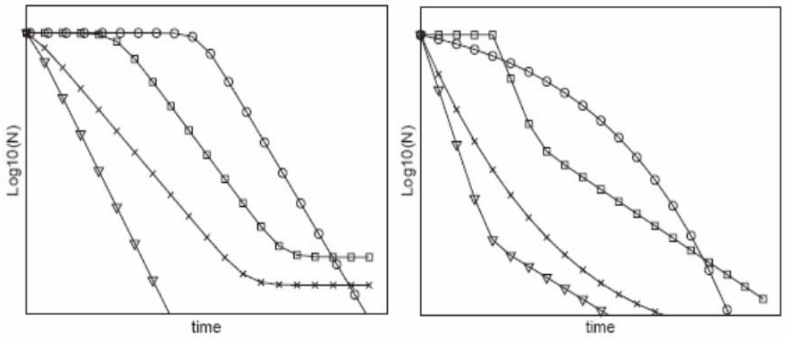


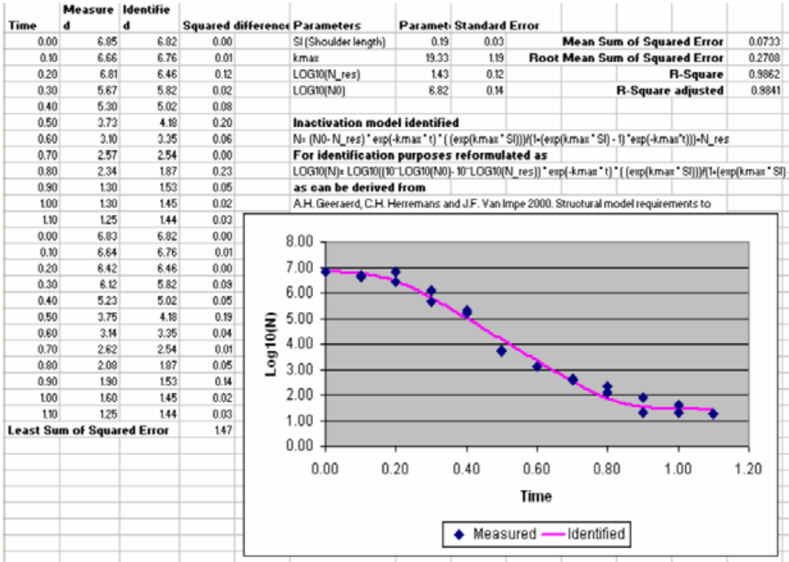
Fig. 1. Commonly observed types of inactivation curves. Left plot: linear (▽, shape I), linear with tailing (×, shape II), sigmoidal-like (□, shape III), linear with a preceding shoulder (○, shape IV). Right plot: biphasic (▽, shape V), concave (×, shape VI), biphasic with a shoulder (□, shape VII), and convex (○, shape VIII).

N	Cell concentration (cfu/g)
dN/dt	Absolute inactivation rate (cfu/g/h)
(dN/dt)/N = k	Specific inactivation rate (1/h)

Primary inactivation models

Model	Differential form	Integrated form
Log-linear:	$\frac{dN}{dt} = N \times -k_{max}$	$Log(N_t) = Log(N_0 \times exp(-k_{max} \times time))$
Log-linear with shoulder (S) and/or tailing: S_1 (time)	$\frac{dN}{dt} = N \times -k_{max} \times \left(\frac{1}{1+C_c}\right) \times \left[1-\frac{N_{res}}{N_t}\right]$	$Log(N_t) = Log\left[(N_0 - N_{res}) \times e^{-k_{max} \times t} \times \left(\frac{e^{k_{max} \times S_1}}{1+(e^{k_{max} \times S_1}-1) \times e^{-k_{max} \times t}}\right) + N_{nes}\right]$
Weibull model : (concave, convex)		$Log(N_t) = Log\left[(N_0 - N_{res}) \times 10^{(-(\frac{t}{\delta})^p)} + N_{res}\right]$
Biphasic models:		$Log(N_t) = Log(N_0) + Log(f \times e^{-k_{max1} \times t} + (1-f) \times e^{-k_{max2} \times t})$

Primary inactivation model fitting - GInaFit



Time	Measured	Identified	Squared difference
0.00	6.85	6.82	0.00
0.10	6.66	6.76	0.01
0.20	6.81	6.46	0.12
0.30	5.67	5.82	0.02
0.40	5.30	5.02	0.08
0.50	3.73	4.18	0.20
0.60	3.10	3.35	0.06
0.70	2.57	2.54	0.00
0.80	2.34	1.87	0.23
0.90	1.30	1.53	0.05
1.00	1.30	1.45	0.02
1.10	1.25	1.44	0.03
0.00	6.83	6.82	0.00
0.10	6.64	6.76	0.01
0.20	6.42	6.46	0.00
0.30	6.12	5.82	0.09
0.40	5.23	5.02	0.05
0.50	3.75	4.18	0.19
0.60	3.14	3.35	0.04
0.70	2.62	2.54	0.01
0.80	2.08	1.87	0.05
0.90	1.90	1.53	0.14
1.00	1.60	1.45	0.02
1.10	1.25	1.44	0.03
Least Sum of Squared Error			1.47

Parameters	Parameter	Standard Error
Sl (Shoulder length)	0.19	0.03
kmax	19.33	1.19
LOG10(N_res)	1.43	0.12
LOG10(N0)	6.82	0.14

Mean Sum of Squared Error	0.0733
Root Mean Sum of Squared Error	0.2708
R-Square	0.9862
R-Square adjusted	0.9841

Inactivation model identified

N= (N0- N_res) * exp(-kmax * t) * ((exp(kmax * Sl)) / (1+(exp(kmax * Sl) - 1) * exp(-kmax*t))) +N_res

For identification purposes reformulated as

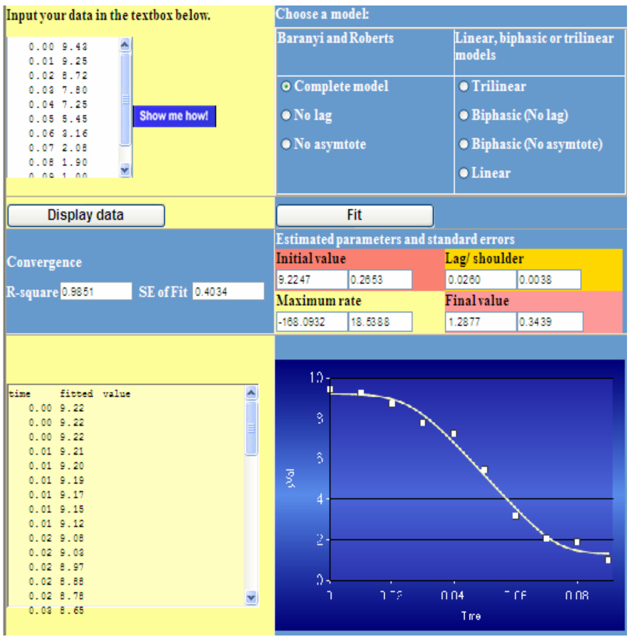
LOG10(N)= LOG10((10^LOG10(N0)- 10^LOG10(N_res)) * exp(-kmax * t) * ((exp(kmax * Sl)) / (1+(exp(kmax * Sl) ...

as can be derived from

A.H. Geeraerd, C.H. Herremans and J.F. Van Impe 2000. Structural model requirements to

cit.kuleuven.be/biotec/downloads/GInaFit/get_tool.php

Primary inactivation model fitting – Combase/DMFit

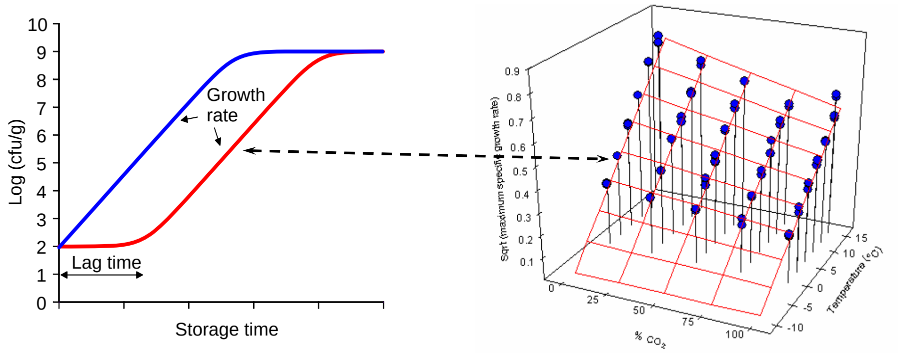


Predicting the growth and inactivation of bacteria in seafood

- Predictive microbiology - concept
- Primary growth and inactivation models
- Secondary models and product evaluation/validation
- Predictive microbiology – applications and software
- PC Exercises

Development of predictive microbiology models

Models are usually developed in two steps from large experiments including the effect of several environmental parameters



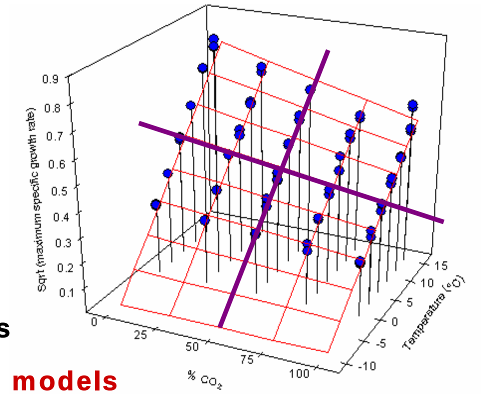
Primary model **Secondary model**

Models allow microbial responses to be predicted at conditions that have not been specifically studied

Secondary growth or inactivation models

Kinetic growth models

- Lag time (λ)
- Growth rate (μ_{max})
- Maximum cell density (N_{max})



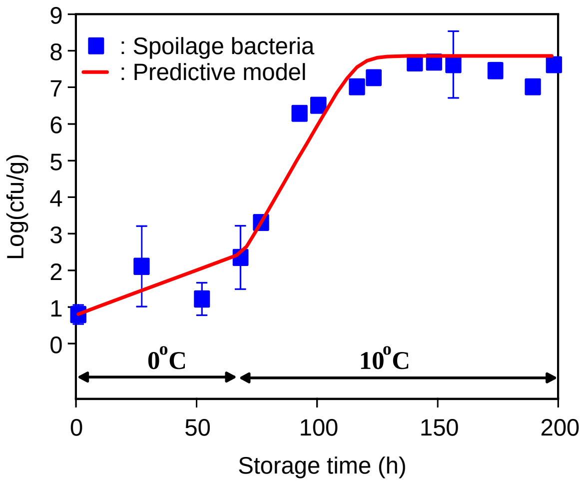
Probability of growth models

Growth / no growth interface models

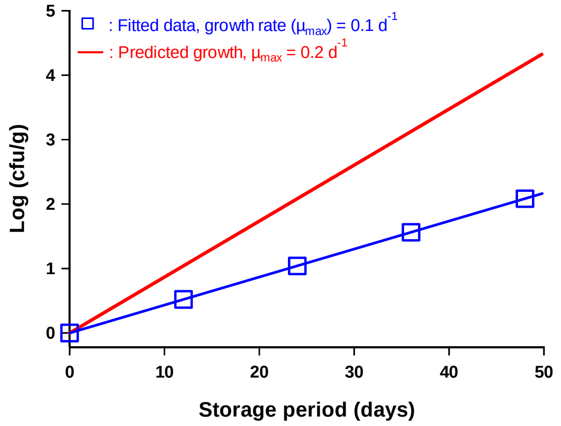
Kinetic inactivation models

Evaluation / validation of growth models

A *P. phosphoreum* growth model has been successfully validated by comparison of predictions and data from naturally contaminated fresh MAP fish at constant and changing storage temperatures



Evaluation / validation of growth models



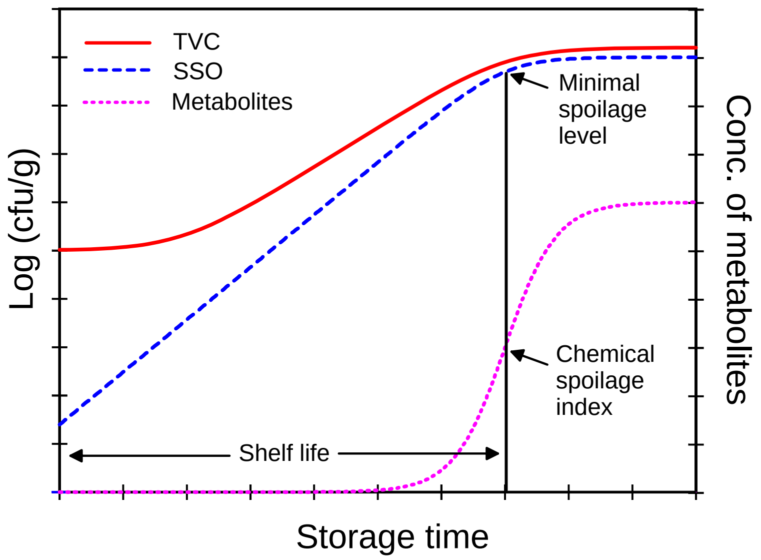
$$Bias\ factor = \frac{Predicted\ growth\ rate}{Observed\ growth\ rate} = \frac{0.2\ d^{-1}}{0.1\ d^{-1}} = 2.0$$

Acceptable model: 0.75 < Bias factor < 1.25

Predicting the growth of bacteria in food

- Predictive microbiology - concept
- Primary growth models
- Secondary models and product evaluation/validation
- Predictive microbiology – applications and software
- PC Exercises

Specific spoilage organisms (SSO) and shelf-life prediction



Dalgaard (1993)

Application of predictive microbiology models

1. Determine product characteristics and storage conditions of food

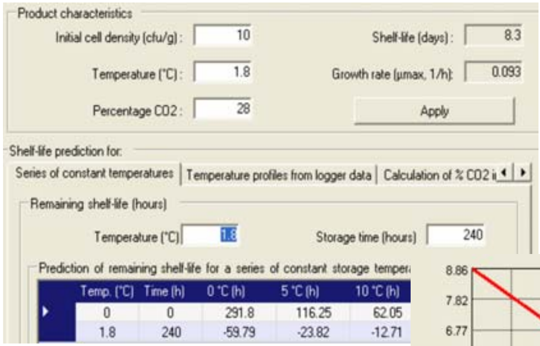
   Temperature, a_w/NaCl, pH, organic acids, nitrit,

   smoke components, inhibting microflora

2. Secondary model → lag time, growth rate, etc.
3. Primary model → Growth curve (Concentration over time)

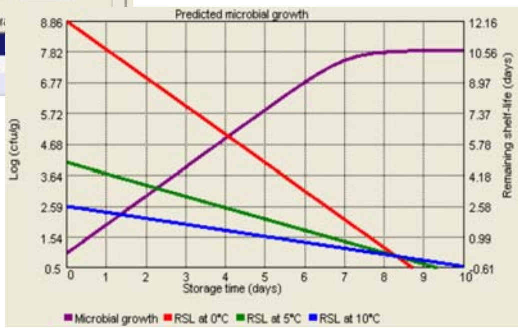
- Application software facilitates step 2 and 3
- Predictions can be useful or misleading depending on:
  - Successful product validation and correct use of models
  - Appropriate information about food and storage conditions

Application of a predictive model –
Example with fresh fish in modified atmosphere packaging



Seafood Spoilage and Safety Predictor

http://sssp.dtuaqua.dk

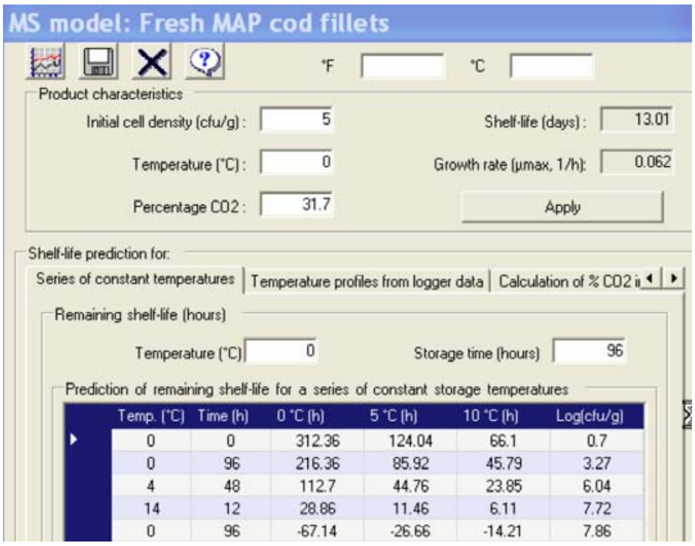


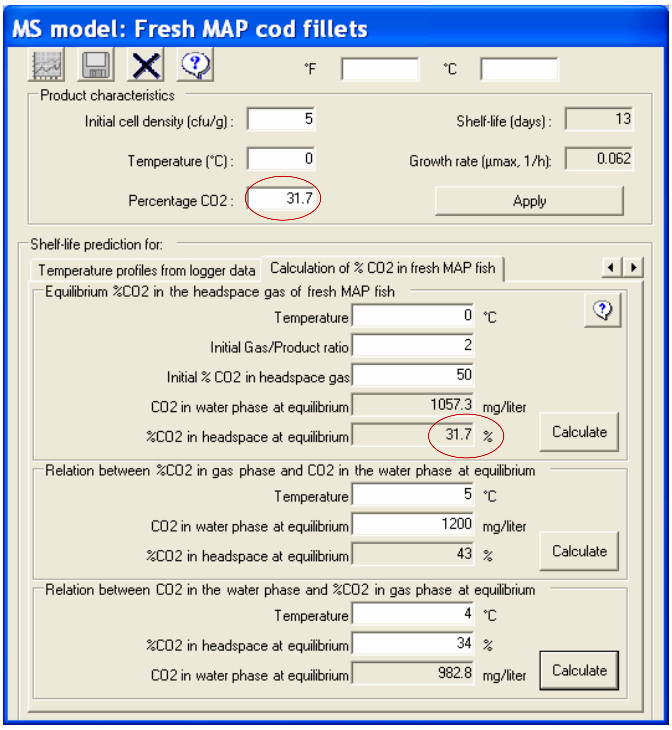
Predicting growth of spoilage bacteria – example with fresh MAP fish

Application of SSSP - effect of atmosphere, hygiene and temperature on shelf-life of e.g. fresh MAP cod

Temperature (° C)	*P. phosphoreum* (cfu/g)	CO_2 (%)	Shelf-life (days)
0	10	30	12,4
0	10	50	14,4
2	10	50	9,3
2	1000	50	7,0
15	1000	50	1,4
15	1000	30	1,2

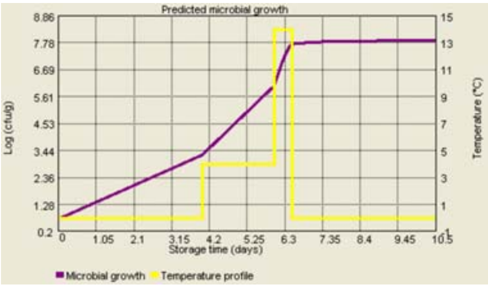
Seafood Spoilage and Safety Predictor (SSSP)

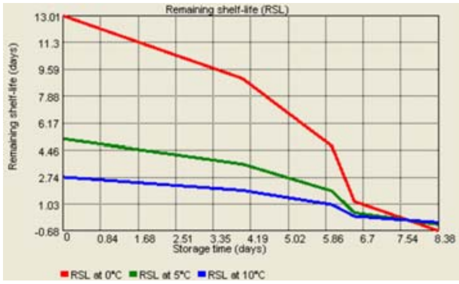




Seafood Spoilage and Safety Predictor (SSSP)

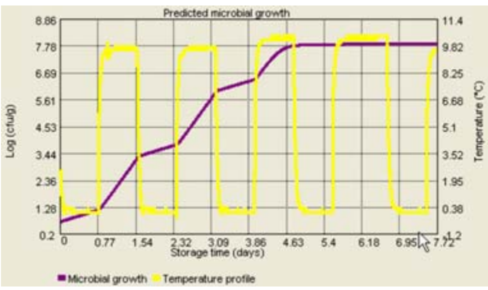
Effect of a simple temperature profile on growth of *P. phosphoreum* (SSO) and on shelf-life of fresh MAP fish

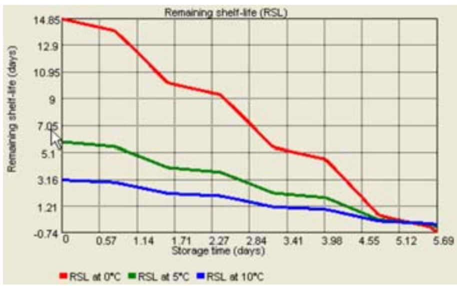




Seafood Spoilage and Safety Predictor (SSSP)

Effect of temperature profile recorded by a data logger on growth of *P. phosphoreum* (SSO) and on shelf-life of fresh MAP fish





Shelf-life prediction - models and freeware

SSO	Product	Freeware
H_2S-producing *Shewanella*	Fresh seafood	- Seafood Spoilage and Safety Predictor
Pseudomonas spp.	Fresh seafood	- Combase Predictor - Fish Shelf Life Prediction
Photobacterium phosphoreum	Fresh marine MAP fish and shell-fish	- Seafood Spoilage and Safety Predictor
Lactic acid bacteria	Fresh and lightly preserved products	- Seafood Spoilage and Safety Predictor
Brochothrix thermosphacta	Fresh and lightly preserved products	- Combase Predictor

- Seafood Spoilage and Safety Predictor (http://sssp.dtuaqua.dk)
- Combase Predictor (http://www.combase.cc)
- Fish Shelf Life Prediction (http://www.azti.es/...)

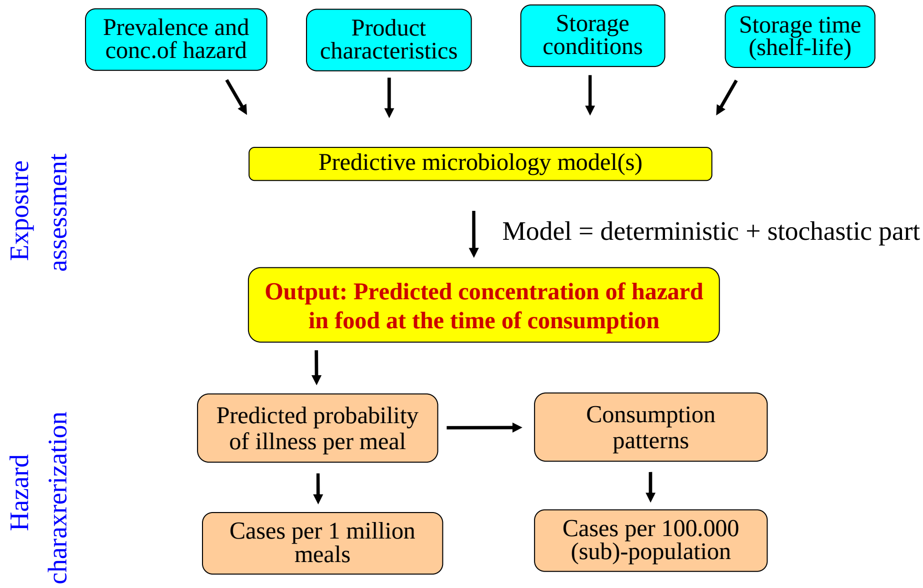
Application of <u>successfully validated</u> predictive microbiology models

- Predict the effect of product characteristics and storage conditions on growth, survival of inactivation of microorganisms
  - Development or reformulation of products
- HACCP plans – establish limits for CCP
- Food safety objectives – equivalence of processes
- Education – easy access to information
- Quantitative microbiological risk assessment (QMRA)

The concentration of microbial hazards in foods may increase or decrease substantially (millions of folds) during processing and distribution

McMeekin et al. (2006)

Application of predictive microbiology in QMRA



Predictive microbiology software (freeware)

- Predictive Microbiology Information Portal (PMIP; portal.arserrc.gov) and Pathogen Modeling Programme (PMP; pmp.arserrc.gov/PMPOnline.aspx) (USA)
  - > 40 models of growth, survival and inactivation
  - Reqularly updated (7 versions of PMP)
  - Available free of charge during the last 15 years
  - Models and tutorials available online
- ComBase (UK, USA) – www.combase.cc

  ComBase Predictor (previously Growth Predictor and Food MicroMoodel)
  - Online models for growth or inactivation of 12 foodborne pathogens
  - Model for growth of *Brochothrix thermosphacta*

  ComBase Browser
  - Data for growth, survival or inactivation of food-related microorganisms
  - >45000 growth/inactivation curves

Predictive microbiology software (freeware)

- Seafood Spoilage and Safety Predictor (DK) – http://sssp.dtuaqua.dk
  - Time-temperature integration
  - 15 models for shelf-life, specific spoilage organisms, histamine formation and growth of *Listeria monmocytogenes*
- Refrigeration index calculator (Australien) – www.mla.com.au/publications
  - Growth of *E. coli* during chilling of meat e.g. in relation to portioning
- Perfringens Predictor (UK) - www.ifr.ac.uk/Safety/GrowthPredictor/
  - Growth of *Clostridium perfringens* during chilling of food
- Process Lethality Determination spreadsheet (AMI Foundation, USA)
  - www.amif.org/FactsandFigures/AMIF-Process-ProcessLethality.htm
  - Calculation of heat inactivation for time-temperature profile

Predictive microbiology software (freeware)

- Opti-Form *Listeria* control model 2007 (PURAC)
  - http://www.purac.com/purac_com/d9ed26800a03c246d4e0ff0f6b74dc1b.php
  - Effect of organic acids, temperature, pH and moisture on growth of *Listeria*

Curve fitting software:

- *DMFit* (UK) – www.combase.cc
  - Estimation of growth kinetic parameters from growth curve data
- MicroFit (UK) – www.ifr.bbsrc.ac.uk/MicroFit/
  - Estimation of growth kinetic parameters (lag time, maximum specific growth rate and maximum population density) from growth curve data
- GInaFit (Belgium) - http://cit.kuleuven.be/biotec/downloads/GInaFit/get_tool.php
  - Estimation of kinetic parameters from inactivation curves of various shapes (Log-linear, shoulders, tails, concave and convex)

Predictive microbiology software

Commercially available

- Sym'Previus (France) - www.symprevius.net
  - Extensive database with predictive software/expert system

- Food Spoilage Predictor (Australien)
  - ~500 AUD, 1 model for growth of *Pseudomonas* spp. in meat
  - Prediction of shelf-life, time-temperature integration

Predicting the growth of bacteria in food

- Predictive microbiology - concept
- Primary growth models
- Secondary models and product evaluation/validation
- Predictive microbiology – applications and software
- PC Exercises

Seafood Spoilage and Safety Predictor (SSSP)

Predicting growth of spoilage bacteria (*Shewanella*)

H_2S-producing *Shewanella* bacteria are well known spoilage micro-organisms in fresh fish and in some fresh meat products with high pH above ~6. *Shewanella* bacteria are primarily important for spoilage of products when stored in air but they can also contrinute to spoilage of vacuum-pakked food. Use the SSSP model 'H_2S-producing Shewanella-Fresh seafood stored in air' to predict the effect of growth of this spoilage bacterium on product shelf-life:

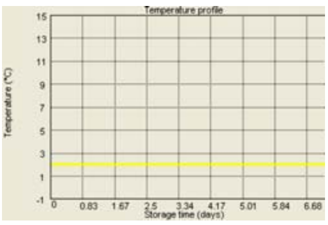
- With and initial concentration of 10 *Shewanella*/g the predicted shelf-life of fresh fish at 0°C is 12.8 days.
- What is the shelf-life at 0°C with and an initial concentration of 1000 *Shewanella*/g? Answer: ____ days.
- At what temperature is this shelf-life obtained for a product with only 10 *Shewanella*/g? Answer: ____ °C (Use a trial and error approach).

Seafood Spoilage and Safety Predictor (SSSP)

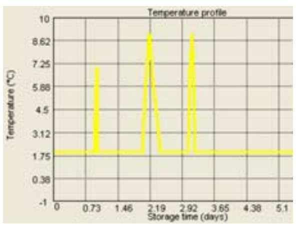
Predicting growth of spoilage bacteria (*Shewanella*)

High storage temperatures reduce the shelf-life of food markedly. Variable storage temperatures can also have a sever effect on growth of spoilage bacteria and on shelf-life but increased product temperatures during short periods may excede critical temperature limits without having an important effect on shelf—life.

- How much is the concentration of *Shewanella* increasing during 120 hours of storage at a constant temeperature of 2.0°C – when the initial cell concentration is 10 cfu/g? Answer: ____ log(cfu/g)



.../Growth of bacteria/ASCII-2.txt



.../Growth of bacteria/ASCII-2-7-9-9.txt

Seafood Spoilage and Safety Predictor (SSSP)

Predicting growth of spoilage bacteria (*Shewanella*)

- How much is the concentration of *Shewanella* increasing during 120 hours of storage with the temperature profile shown on the previous slide (and included in the file …/ASCII-2-7-9-9.txt) as compared to storage at 2°C?
  (Use e.g. the zoom-function to obtain information from graphs)
  Answer: ____ log (cfu/g).
- How much is shelf-life of the product reduced by the temperature profile (…/ASCII-2-7-9-9.txt) as compared the storage at 2°C?
  Answer: ____ days.

  (Is this an important reduction of shelf-life?)

Seafood Spoilage and Safety Predictor (SSSP)

Growth of *Shewanella* and shelf-life – **fishmonger example**

Some fishmongers expose whole gutted fish in their shop window. These fish are not entierly covered with ice and during a working day the temperature of the fish may increase to 5-10°C. Is this important for shelf-life and concentrations of bacteria on these fish?

- With and initial concentration of 1000 *Shewanella*/g the predicted shelf-life of fresh fish at 0°C is 8.6 days.
- Let us assume the fishmonger keeps this fish at 2°C during 48 hours before it is sold and that in addition some fish are displayed during 5 hours in the shop window at 7.5°C.
- Let us also assume that a consumer, after buying the fish, keep it in a refrigerator at 5°C.

(The questions to be answered are on the next slide)

Seafood Spoilage and Safety Predictor (SSSP)

Growth of *Shewanella* and shelf-life – **fishmonger example**

Use the SSSP model ‘H_2S-producing Shewanella-Fresh seafood stored in air’ to predict remaining shelf-life of the fish in the consumer refrigerator at 5°C after:

1. The fishmonger has keept the fish at 2°C during 48 hours.
   Answer: ____ days.

2. The fishmonger has keept the fish at 2°C during 48 hours and it has then been displayed during 5 hours in the shop window at 7.5°C.
   Answer: ____ days.

   How much is the concentration of *Shewanella* increasing during the display in the shop window (5 hours at 7.5°C)?
   Answer: ____ log (cfu/g) = _____fold.
3. Is this storage of fish in the show window important for the overall product shelf-life? Answer: ______.

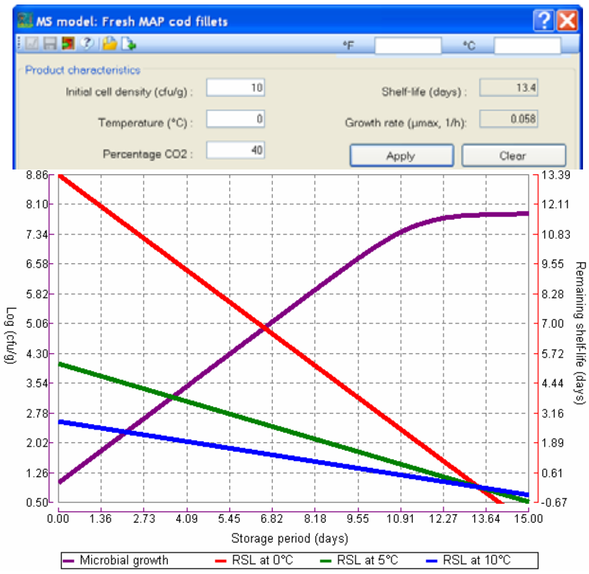
Seafood Spoilage and Safety Predictor (SSSP)

Predicting growth of spoilage bacteria (*Photobacterium*)

Photobacterium phosphoreum is responsible for spoilage of fresh marine fish when stored in modified athosphere packing (MAP). Fresh MAP white fish like cod and plaice with 10 *P. phosphoreum*/g have shelf-life of 11-12 days when stored in MAP with 25% CO_2/75% N_2 at 0°C. Use the SSSP model ‘*Photobacterium phosphoreum’* to predict the effect of storage temperatue and atmosphere on growth of this spoilage bacterium and on product shelf-life:

- How much is shelf-life extended (and growth *P.phosphoreum* delayed) by increading the concentration of CO_2 from 25% to 40%?
  Answer: ____ days.
- How much is shelf-life reduced by using vacuum-packing (corresponding to 0% CO_2) compared to MAP with 40% CO2 and 60% N_2?
  Answer: ____ days.

Predicting growth of spoilage bacteria (*Photobacterium*)



Seafood safety prediction 1.

Presentation and PC exercises concerning histamine formation and histamine fish poisining

Paw Dalgaard

Seafood & Predictive Microbiology (Research group)

Section for Aquatic Microbiology and Seafood Hygiene

pad@aqua.dtu.dk

DTU Food
National Food Institute

Food safety prediction

- Histamine formation and histamine fish poisoning
- Modelling growth and histamine (metabolite) formation
- Prediction of histamine formation by *Morganella* bacteria
- PC exercises

Histamine formation in marine finfish

- Histamine fish poisoning is responsible for more foodborne incidents of disease than any other hazard in fish and shell-fish

Free histidine → Histidine decarboxylase → **Histamine**

- Significant growth is required → more than 1-10 million bacteria/g
- Toxic histamine concentrations (> 500 mg/kg) can be formed by:
  - Mesophilic bacteria at above 7–10˚C
  - Psychrotolerant bacteria at above ~0˚C
- Toxic histamine concentrations can be formed in marine finfish when these are chilled in agreement with regulations for EU or USA

Histamine and histamine fish poisoning (HFP)
Existing legislation and controls

Critical concentrations of histamine:

EU : 100-200 mg/kg and 200-400 mg/kg if maturated in brine (EC 2073/2005)

USA : 50 mg/kg (Defect action level, FDA/CFSAN 2001)

Critical temperatures for storage and distribution fish:

EU : Fresh and thawed fish (0-2˚C) and lightly preserved seafood (5˚C) (EU 853/2004)

USA : Fersh fish (4.4˚C) with demands for rates of chilling (FDA/CFSAN 2001)

Histamine fish poisoning (HFP) - occurrence

Country	Year	Incidents	Cases	
			Total	per year/million
Hawaii, USA	1990-2003	111	526	31
Denmark	1986-2005	64	489	4.9
New Zealand	2001-2005	11	62	3.1
Japan	1970-1980	42	4122	3.2
	1994-2005	68	1523	1.1
France	1987-2005	123	2635	2.5
Finland	1998-2005	15	89	2.1
Taiwan	1986-2001	8	535	1.5
UK	1976-2004	515	1300	0.8
Switzerland	1966-1991	76	111	0.7
South Africa	1992/2004	10/3	22/21	0.4
Australia	1995-2000	7	34	0.4
USA	1990-2003	341	1651	0.3
Canada	1975-1995	39	109	0.2

Dalgaard et al. (2008)

Examples of marine finfish that cause histamine fish poisoning

Tuna (bluefin)/tun
(*Thunnus thynnus*)

Mahi-mahi/guldmakrel
(*Coryphaena hippurus*)

Escolar/escolar
(*Lepidocybium flavobrunneum*)

Garfish/hornfisk
(*Belone belone*)

HFP and bacteria responsible for histamine formation

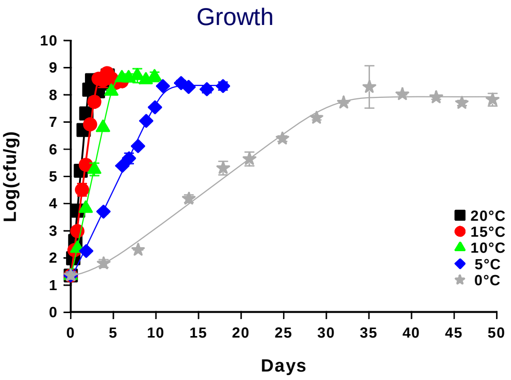
Both mesophilic and psychrotolerant bacteria can be responsible for histamine formation and thereby HFP

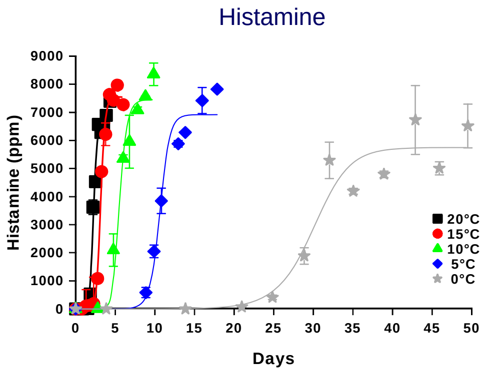
Seafood	Bacteria	Place and time
Fresh tuna	*Morganella morganii*	Japan, 1955
Fresh tuna	*Morganella morganii*	Japan, 1965
Fresh tuna	*Hafnia sp.*	Praque, 1967
Fresh tuna	*Raoultella planticola (Klebsiella pneumoniae)*	California, 1977
Dried Sardine	*Photobacterium phsophoreum*	Japan, 2002
Tuna in chilisauce	*Morganella psychrotolerans* or *Photobacterium phosphoreum*	Denmark, 2003
Cold smoked tuna	*Photobacterium phosphoreum*	Denmark, 2004
Cold smoked tuna	*Morganella psychrotolerans*	Denmark, 2004
Tuna (packed in film)	*Morganella morganii*	Denmark, 2004
Fresh tuna	*Photobacterium phosphoreum*	Denmark, 2006
Dried milkfish	*Raoultella ornithinolytica*	Taiwan, 2006

Modified from Dalgaard and Emborg (2009) in 'Foodborne Pathogens'

Histamine formation in marine finfish

Morganella psychrotolerans can grow and is able to produce toxic concentrations of histamine at 0°C



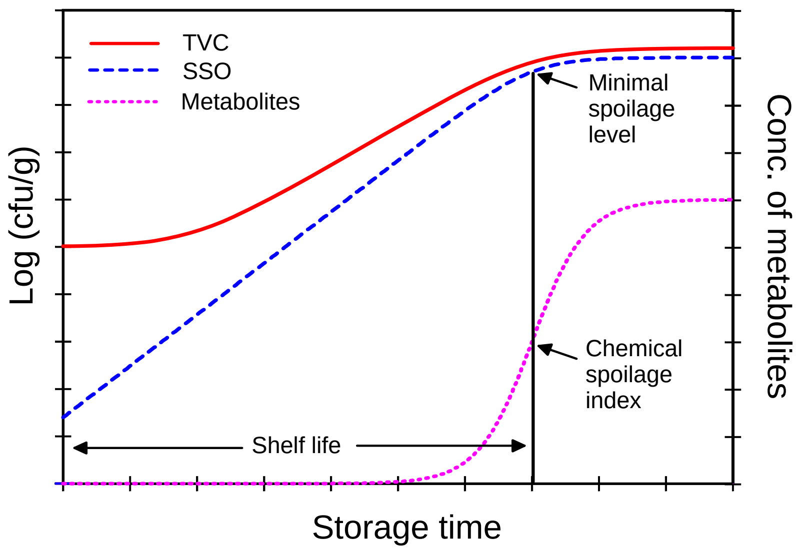


Emborg & Dalgaard (2008a)

Food safety prediction

- Histamine formation and histamine fish poisoning
- Modelling growth and histamine (metabolite) formation
- Prediction of histamine formation by *Morganella* bacteria
- PC exercises

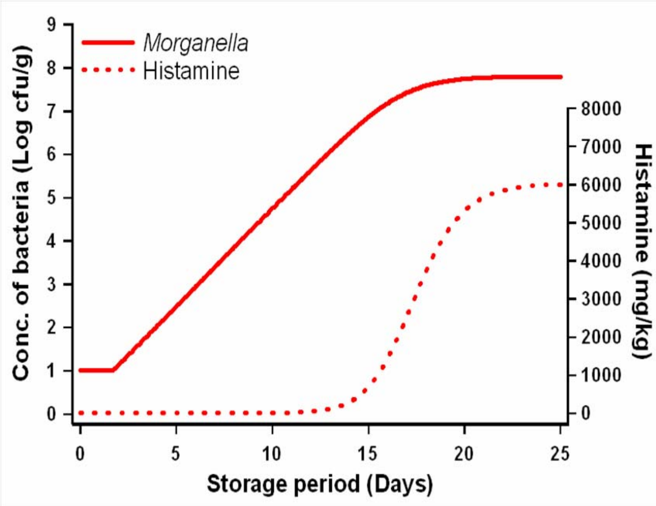
Specific spoilage organisms (SSO) and indices of quality / spoilage



Dalgaard, 1993

Prediction of histamine formation

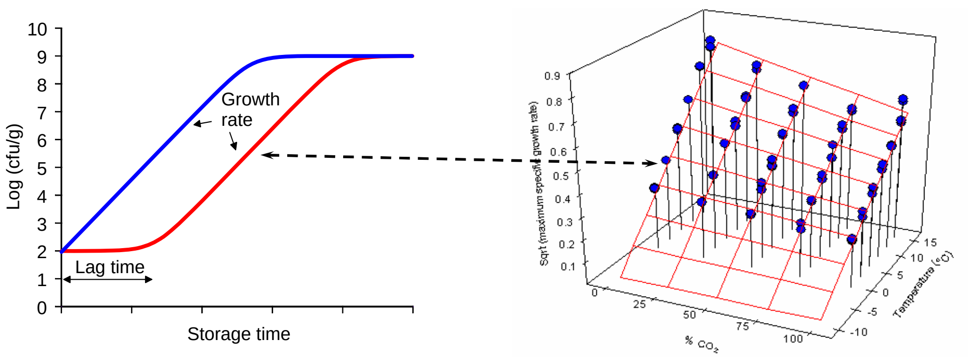
Growth of the histamine producing bacteria must be related to histamine formation in relevant fish products



Emborg and Dalgaard (2008a)

Development of predictive microbiology models

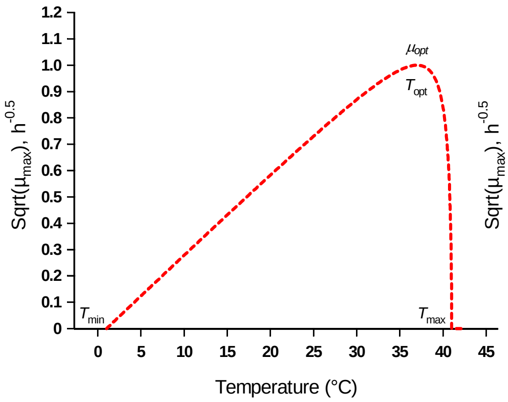
Models are usually developed in two steps from large experiments including the effect of several environmental parameters

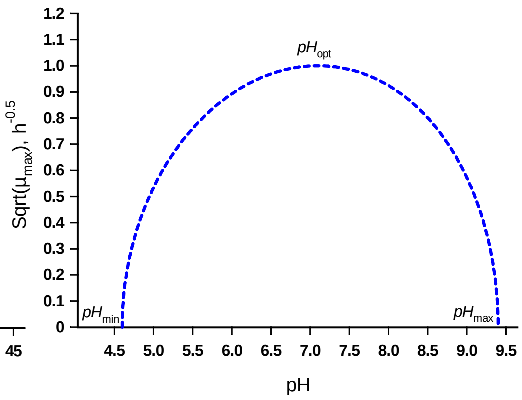


Primary model **Secondary model**

Models allow microbial responses to be predicted at conditions that have not been specifically studied

Secondary models: Cardinal parameter models

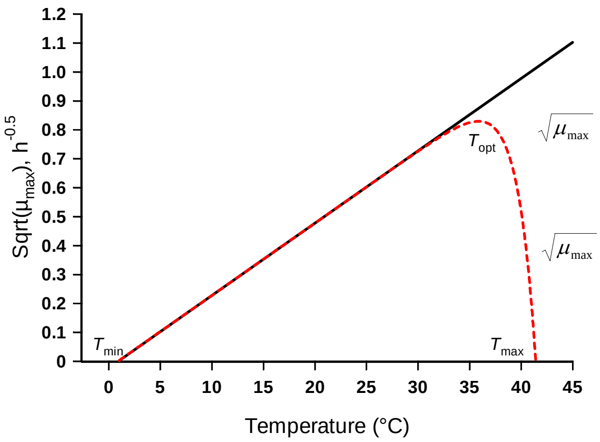




Rosso et al. 1995; Augustin & Carlier 2000; Le Marc et al. 2002

Secondary square-root type model

Effect of storage temperature on growth rate



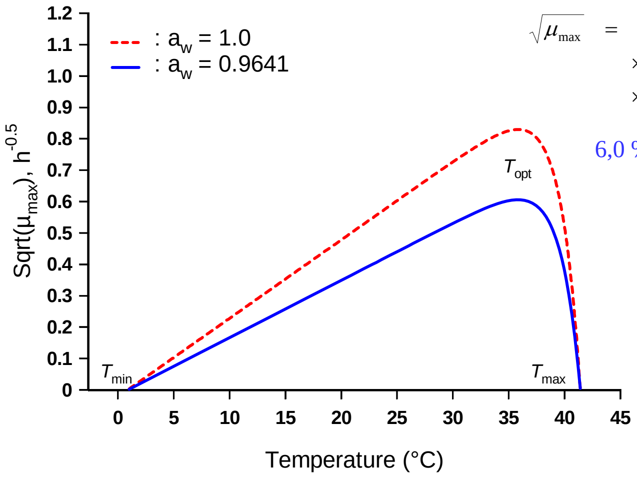
$$\sqrt{\mu_{max}} = b \times (T - T_{min}) \times (1 - \exp(c(T - T_{max})))$$

$$\sqrt{\mu_{max}} = b \times (T - 0.88) \times (1 - \exp(0.536(T - 41.4)))$$

Ratkowsky et al. (1983)

Secondary square-root type model

Effect of temperature and NaCl/water activity



$$\sqrt{\mu_{max}} = b \times (T - 0.88) \times (1 - \exp(0.536(T - 41.4))) \times \sqrt{(0.9641 - 0.923)/(1.0000 - 0.923)}$$

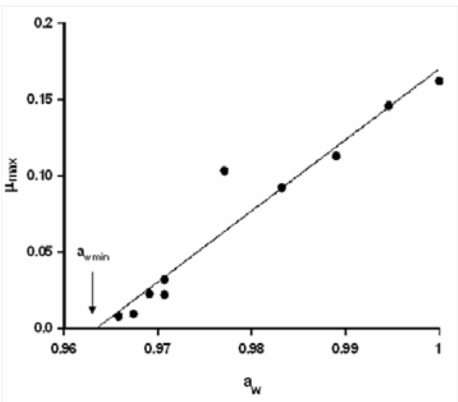
6,0 % NaCl in water phase ~ a_w 0.9641

term for water activity

$$\sqrt{(a_w - a_{w\ min})/(a_{w\ opt} - a_{w\ min})}$$

Secondary square-root type model

Simplified cardinal parameter models for sub-optimum environmental conditions



Effect of water activity (a_w) on the maximum specific growth (μ_{max}) of the histamine producing bacterium *Morganella psychrotolerans*

Emborg & Dalgaard (2008a)

Simplified cardinal parameter model for sub-optimum environmental conditions (*M. psychrotolerans*)

$$\mu_{max} = 0.535 \cdot \left(\frac{T+6.22}{20+6.22}\right)^2 \cdot \frac{a_w - 0.963}{1-0.963} \cdot 1-10^{5.12-pH} \cdot \left(\frac{266-CO_2}{266}\right)^2 \cdot \xi$$

- Few parameters with (at least some) biological significance
- Include terms without dimension and with values between 0 og 1

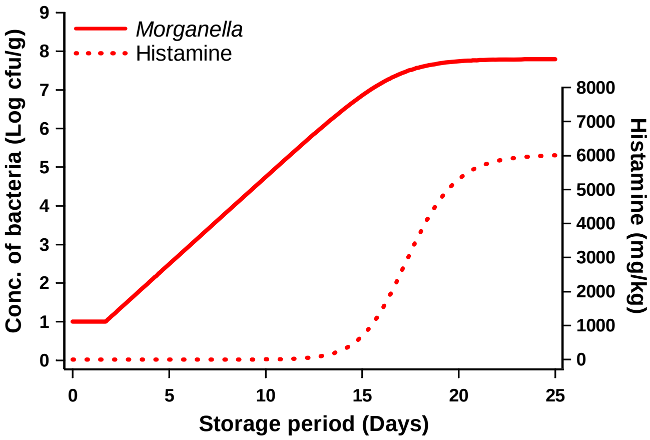
Secondary lag time models

- Secondary lag time models can be developed in the same way as growth rate models (1/lag time = lag rate)
- Lag time of microorganisms depend not only on environmental parameters but also on the physiological state of the microorganisms
- Lag time data is more variable than growth rate data
- 'Relative lag time' (RLT) = Lag time/generation time (t_{gen}) is used to predict lag time from μ_{max}

$$Lag\ time = RLT \cdot t_{gen} = RLT \cdot Ln(2) / \mu_{\max}$$

Ross and Dalgaard 2004

Modelling of growth and histamine formation



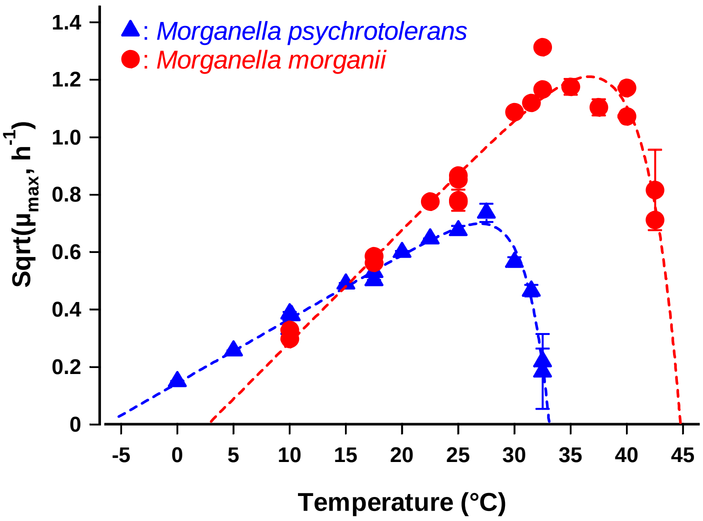
Growth model

$$\frac{dN_t}{dt} = N_t \times \mu_{max} \times \left(1 - \left(\frac{N_t}{N_{max}}\right)^m\right)$$

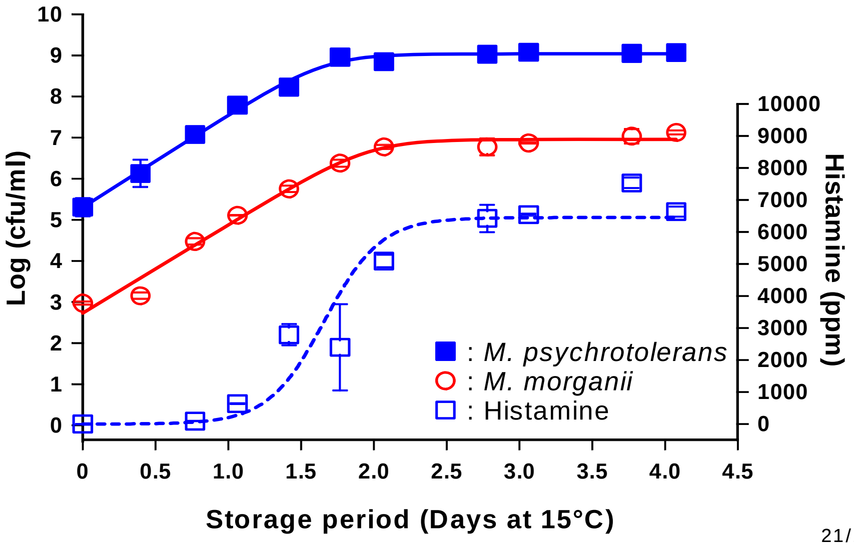
Histamine formation model

$$\frac{dHist}{dt} = Y_{\frac{Hist}{cfu}} \times \frac{dN_t}{dt}$$

Models for growth and histamine formation by both *M. psychrotolerans* and *M. morganii* have been developed and validated



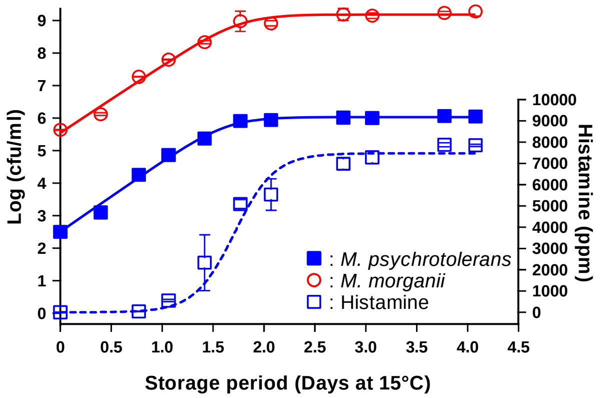
High concentrations of *M. psychrotolerans* inhibit growth of *M. morganii* (Jameson effect)



High concentrations of *M. morganii* inhibit growth of *M. psychrotolerans* (Jameson effect)

Growth model: Example for *M. psychrotolerans*

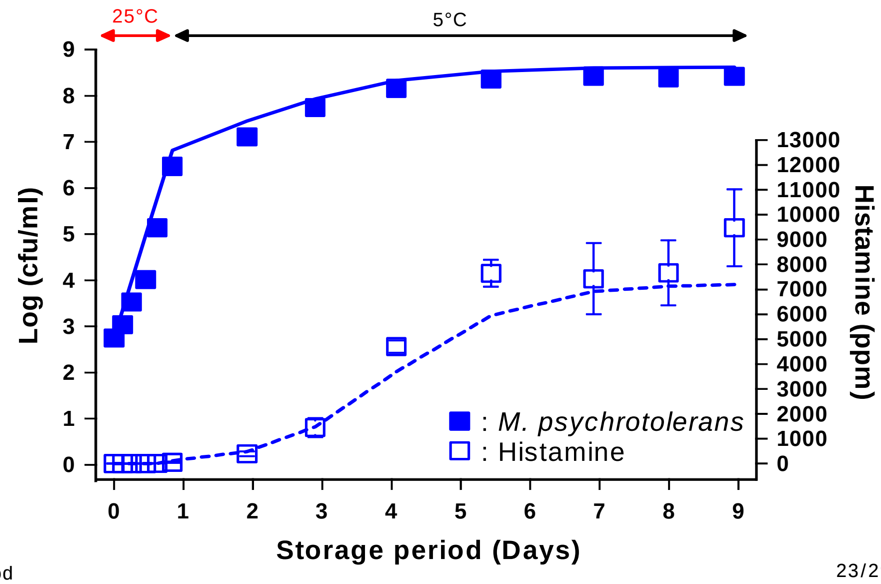
$$\frac{dMp_t}{dt} = Mp_t \times \mu_{max}^{Mp} \times \left(1 - \left(\frac{Mp_t}{Mp_{max}}\right)^m\right) \times \left(1 - \left(\frac{Mm_t}{Mm_{max}}\right)^m\right)$$



Histamine formation model

$$\frac{dHist}{dt} = Y_{\frac{Hist}{cfu}}^{Mp} \times \frac{dMp_t}{dt} + Y_{\frac{Hist}{cfu}}^{Mm} \times \frac{dMm_t}{dt}$$

New models allow growth and histamine formation to be predicted at changing temperatures

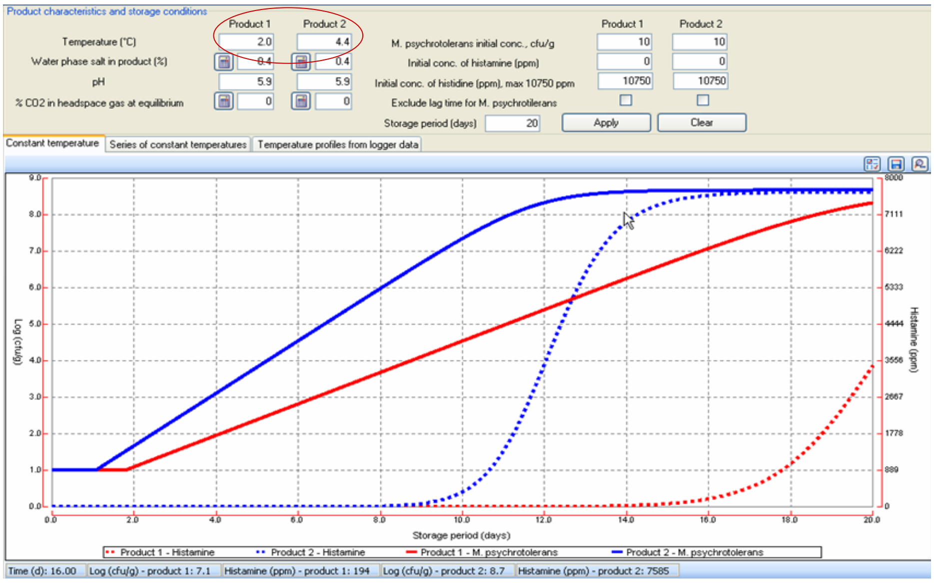


Food safety prediction

- Histamine formation and histamine fish poisoning
- Modelling growth and histamine (metabolite) formation
- Prediction of histamine formation by *Morganella* bacteria
- PC exercises

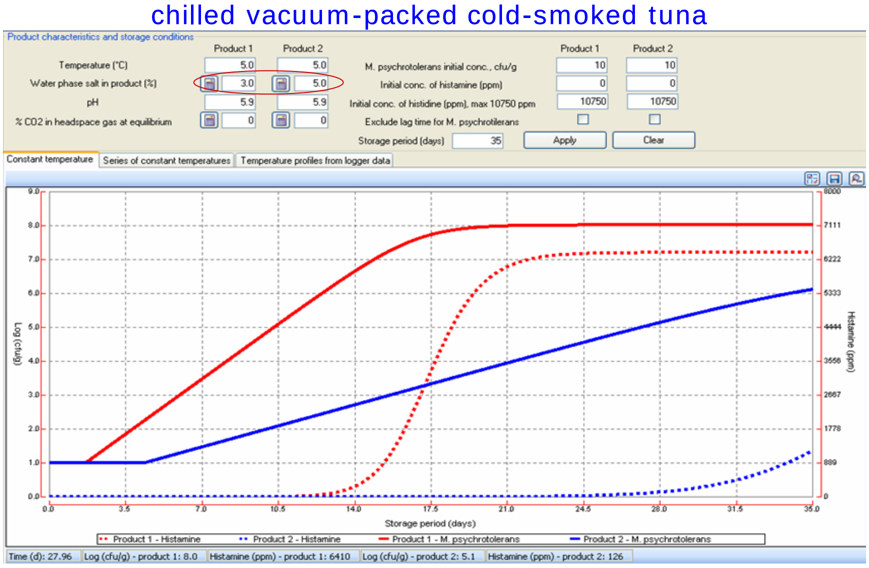
Prediction of histamine formation

Histamine formation by *M. psychrotolerans* can be predicted for vacuum packed fresh tuna and it is markedly faster at 4.4 °C compared to 2.0 °C



Prediction of histamine formation

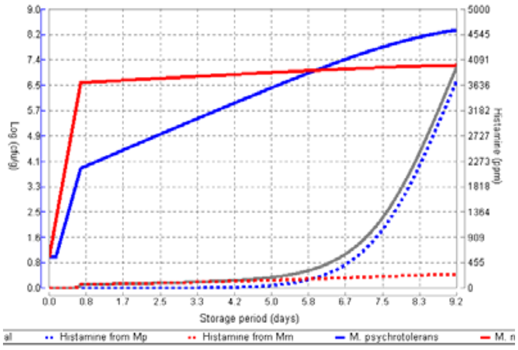
Salt is essential to prevent toxic concentrations of histamine in chilled vacuum-packed cold-smoked tuna

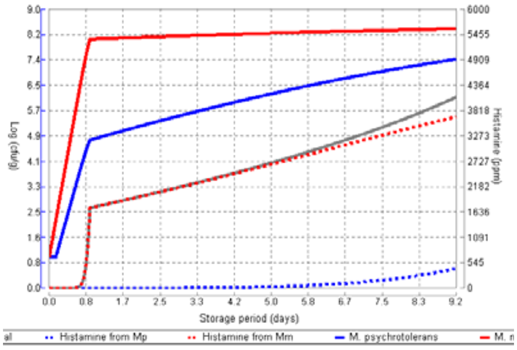


Prediction of histamine formation in marine finfish

- New combined model for *M. psychrotolerans* and *M. morganii* predicts histamine formation for a wide range of storage temperatures
- The model allows the effect of delayed chilling to be predicted

Delayed chilling: **25°C for 17 h** | **25°C for 22 h**
Then chilled storage at: **5°C** | **5°C**





Emborg & Dalgaard (2008b) – http://sssp.dtuaqua.dk

Seafood safety prediction – histamine formation

Exercise 1: *Morganella* – effect of storage temperature

Histamine formation in fish can be due to both psychrotlerant and mesophilic bacteria. Use the SSSP model '*Morganella morganii* and *M. psychrotolerans* – growth and histamine formation' to predict the effect of storage temperatures between 0°C and 25°C on the time to toxic histamine formation:

- With an initial concentrations of 1 cfu/g for both *M. morganii* and *M. psychrotolerans* predict the time to formation of 500 mg histamine/kg:

Temp. (°C)	Time to 500 mg/kg	Most important bacterium
0°C		
5°C		
10°C		
15°C		
20°C		
25°C		

Seafood safety prediction – histamine formation

Exercise 2: *Morganella psychrotolerans* – effect of NaCl and CO_2

Histamine formation in chilled cold-smoked tuna can be due to *Morganella psychrotlerant*. Use the SSSP model '*Morganella psychrotolerans* – growth and histamine formation' to predict the effect of salt (NaCl) and storage atmosphere (% CO_2 in MAP) on histamine formation at 5°C:

- With an initial concentrations of 1 *M. psychrotolerans/g* predict the time to formation of 500 mg histamine/kg in a product with pH 5.9:

% NaCl in water phase	% CO_2	Time to 500 mg/kg
3%	0 %	
3%	30 %	
5%	0 %	
5%	30 %	

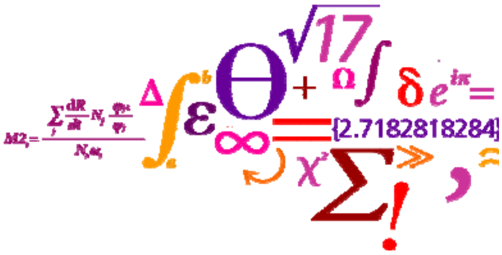
(Info. can help evaluate the effect of uneven salt distribution in smoked tuna)

Seafood safety prediction 2

Presentation and PC exercises concerning *Listeria monocytogenes* in ready-to-eat seafood

Paw Dalgaard

(pad@aqua.dtu.dk)



DTU Food
National Food Institute

Outline

- Predictive models for *Listeria monocytogenes*
  - Why – predictive models
  - Available predictive models for *L. monocytogenes*
  - International validation study
- Application of models
  - Examples
  - Exercises

Why – predictive models

- The EU-regulation (EC 2073/2005) differentiates between ready-to-eat foods that are able or unable to support growth of *L. monocytogenes*

Ready-to-eat foods	Critical limit	Comment
Able to support growth	None in 25 g (n = 5)	When produced
Able to support growth	100 CFU/g	It must be documented that 100 CFU/g is not exceeded within the storage period
Unable to support growth	100 CFU/g	It must be documented that growth is prevented

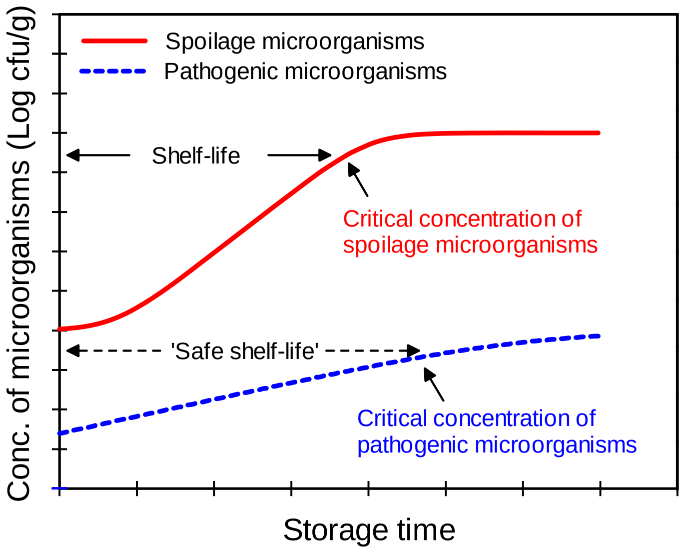
- Documentation → product characteristics, challenge tests, predictive models
- Similar criteria has been approved by the Codex Alimentarius

Why – predictive models

- More people becomes sick from listeriosis
- Complex products → several parameters affects growth of bacteria
- Increased assortment of products
- Wish/demand for products with reduced content of preservation
- Regulations → documentation
- Fast answer
- Flexible
- Easy to use

- Knowledge about products characteristics and storage conditions are needed

Predicting the growth of bacteria in food



Predictive models for *L. monocytogenes*

- Growth and growth boundary model for *L. monocytogenes* in lightly preserved seafood (Mejlholm and Dalgaard, 2009)
  - Temperature
  - pH
  - NaCl/water activity
  - Smoke components (phenol)
  - Nitrite
  - CO_2
  - Acetic acid
  - Benzoic acid
  - Citric acid
  - Diacetat
  - Lactic acid
  - Sorbic acid

  } 12 parameters

  - Interactions between all these parameters



Predictive models for *L. monocytogenes*

Growth model of Giménez and Dalgaard (2004) including the effect of temperature, NaCl/water activity, pH, lactic acid, nitrite and smoke components

↓

Expanded with terms for the effect of diacetate and CO_2 as well as interactions between all the environmental parameters

↓

Calibration of model to data for growth of *L. monocytogenes* in well-characterised lightly preserved seafood (n = 41)

↓

Growth and growth boundary model of Mejlholm and Dalgaard (2007) including the effect of 8 parameters + interactions between all these parameters

↓

Expanded with terms for the effect of acetic, benzoic, citric and sorbic acid as well as their contribution to interactions between the environmental parameters

↓

Growth and growth boundary model of Mejlholm and Dalgaard (2009) including the effect of 12 parameters + interactions between all these parameters

Predictive models for *L. monocytogenes*

Model of Mejlholm and Dalgaard (2009)

$$\mu_{max} = \mu_{ref} \cdot \left[\frac{(T - T_{min})}{T_{ref} - T_{min})}\right]^2 \cdot \frac{(a_w - a_{w\,min})}{(a_{w\,opt} - a_{w\,min})} \cdot \left[1 - 10^{(pH_{min} - pH)}\right] \cdot \left(1 - \frac{[LAC_U]}{[MIC_{U\,lactic\ acid}]}\right) \cdot \frac{(P_{max} - P)}{P_{max}}$$

$$\cdot \left[\frac{(NIT_{max} - NIT)}{NIT_{max}}\right]^2 \cdot \frac{(CO_{2\,max} - CO_{2\,equilibrium})}{CO_{2\,max}} \cdot \left(1 - \sqrt{\frac{[DAC_U]}{[MIC_{U\,diacetate}]}}\right) \cdot \left(1 - \sqrt{\frac{[AAC_U]}{[MIC_{U\,acetic\ acid}]}}\right)$$

$$\cdot \left(1 - \frac{[BAC_U]}{[MIC_{U\,benzoic\ acid}]}\right) \cdot \left(1 - \frac{[CAC_U]}{[MIC_{U\,citric\ acid}]}\right) \cdot \left(1 - \frac{[SAC_U]}{[MIC_{U\,sorbic\ acid}]}\right) \cdot \xi$$

$$\xi(\varphi(e_i)) = \begin{cases} 1 & , \quad \psi \le 0.5 \\ 2(1-\psi), & 0.5 < \psi < 1 \\ 0 & , \quad \psi \ge 1 \end{cases}$$

$$\psi = \sum_i \frac{\varphi_{e_i}}{2\prod_{j \ne i}(1 - \varphi_{e_j})}$$

Interactions between the environmental parameters (Le Marc et al. 2002)

Predictive models for *L. monocytogenes*

- Validated for a wide range of lightly preserved and ready-to-eat seafood
- Validation → comparison of predicted and observed growth
  - Growth rates
  - Growth/no-growth

- Cooked and peeled shrimp
- Cold-smoked and marinated seafood
- Brined shrimp
  - Benzoic, citric and sorbic acid
  - Acetic and lactic acid

Increasing complexity





Predictive models for *L. monocytogenes*

Journal of Food Protection, Vol. 72, No. 10, 2009, Pages 2132–2143
Copyright ©, International Association for Food Protection

Development and Validation of an Extensive Growth and Growth Boundary Model for *Listeria monocytogenes* in Lightly Preserved and Ready-to-Eat Shrimp

OLE MEJLHOLM* AND PAW DALGAARD

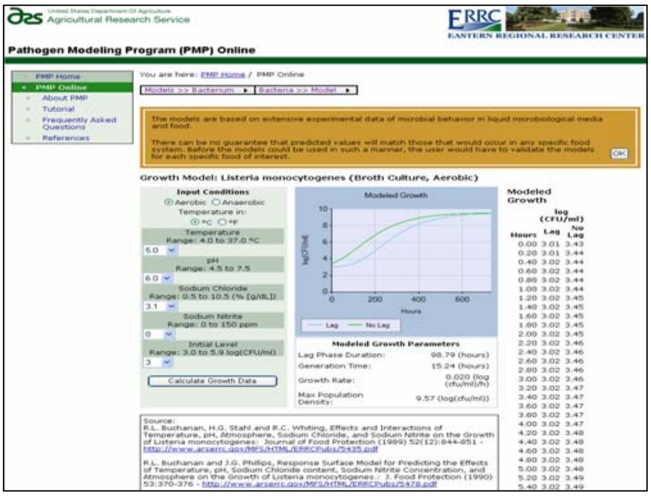
Seafood and Predictive Microbiology, Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources (DTU Aqua), Technical University of Denmark, Søltofts Plads, Building 221, DK-2800, Kgs. Lyngby, Denmark

MS 09-054: Received 4 February 2009/Accepted 27 April 2009

http://sssp.dtuaqua.dk/

Predictive models for *L. monocytogenes*

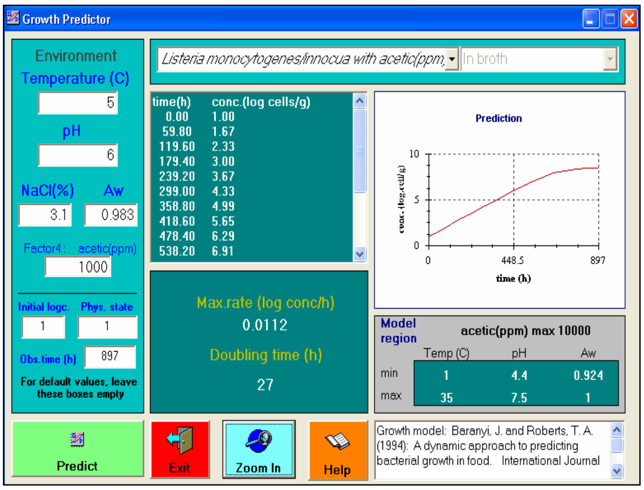
- Other predictive models for *L. monocytogenes*
  - Pathogen Modeling Program (http://pmp.arserrc.gov/)



- Temperature
- pH
- NaCl
- Nitrite

Predictive models for *L. monocytogenes*

- Other predictive models for *L. monocytogenes*
  - Combase predictor (http://www.combase.cc/)



- Temperature
- pH
- NaCl/a_w
- Acetic acid

Predictive models for *L. monocytogenes*

- Other predictive models for *L. monocytogenes*
  - PURAC

- Temperature
- pH
- NaCl
- Nitrite
- Mixtures of organic acids
  - Acetic acid
  - Diacetate
  - Lactic acid

Outline

- Predictive models for *Listeria monocytogenes*
  - Why – predictive models
  - Available predictive models for *L. monocytogenes*
  - International validation study
- Application of models
  - Examples
  - Exercises

International validation study

- Objective → to evaluate and compare the performance of existing predictive models for *L. monocytogenes* on
  - A large number of data from different ready-to-eat foods
  - Data from different laboratories and countries



International validation study

Predictive Models	Parameters included in the models								
	Temp.	NaCl/ a_w	pH	Smoke comp.	CO_2	Nitrite	Acetic acid/ diacetate	Lactic Acid	Inter-actions
Delignette-Muller et al. (2006)	+	-	-	-	-	-	-	-	-
Augustin et al. (2005)	+	+	+	+	+	+	-	-	+
Zuliani et al. (2007)	+	+	+	-	-	-	+	+	+
PURAC (2007)	+	+	+	-	-	+	+	+	-
DMRI (2007)[a]	+	+	+	-	+	+	+	+	+
Mejlholm and Dalgaard (2009)	+	+	+	+	+	+	+	+	+

[a] Danish Meat Research Institute

International validation study

Products	Number of growth responses for *L. monocytogenes* Growth	No-growth	Total
Meat	442	260	702
Seafood	160	33	193
Poultry	50	14	64
Dairy	55	0	55
	707	307	1014

- Collected from 37 independent sources (published and unpublished data)
- More than 20 different types of products
- 50% of the products were added acetic acid/diacetate and/or lactic acid
- More than 100 different isolates of *L. monocytogenes*

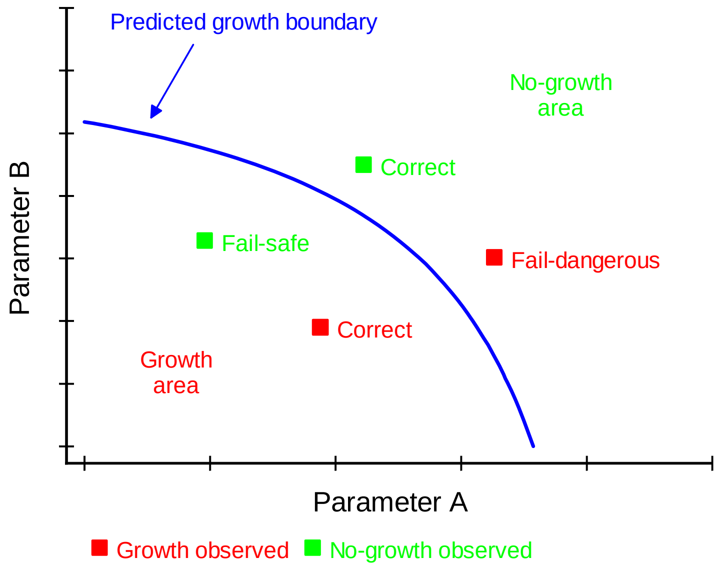
International validation study

- Growth rates (μ_{max})
  - Calculation of bias and accuracy factors
  - Bias factor = 1.0 → predicted growth is equal to observed growth
  - Bias factor > 1.0 → predicted growth is faster than observed growth
  - Bias factor < 1.0 → predicted growth is slower than observed growth

  - Bias factor → to graduate the performance of models (Ross, 1999)
    - 0.95-1.11 → Good
    - 0.87-0.95 or 1.11-1.43 → Acceptable
    - < 0.87 or > 1.43 → Unacceptable

- Growth/no-growth responses
  - Correct predictions
  - Fail-dangerous predictions
  - Fail-safe predictions

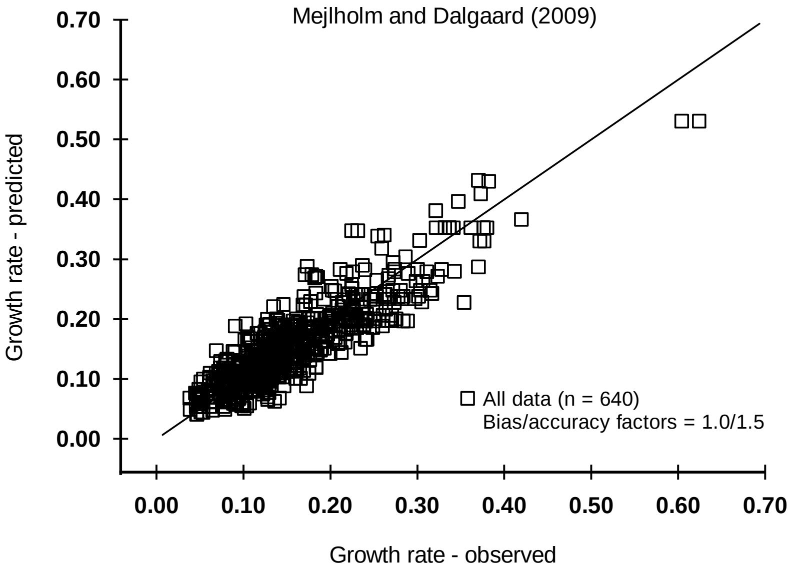
International validation study



International validation study

		Bias/accuracy factors					
Products	n	Delignette-Muller et al. (2006)	Augustin et al. (2005)	Zuliani et al. (2007)	PURAC (2007)	DMRI (2007)	Mejlholm & Dalgaard (2009)
Meat	702	2.3/2.4	2.1/2.5	1.3/2.1	1.4/1.8	1.1/1.5	1.0/1.5
Seafood	193	1.7/1.8	0.7/1.9	1.2/1.6	1.3/1.5	1.4/1.6	1.0/1.4
Poultry	64	1.5/1.9	2.0/2.1	1.0/1.5	1.0/1.5	1.2/1.5	0.9/1.5
Dairy	55	0.7/1.6	0.9/1.3	1.0/1.3	0.9/1.3	1.3/1.6	0.9/1.3
Total	1014	2.0/2.2	1.8/2.3	1.3/1.9	1.3/1.7	1.2/1.6	1.0/1.5
		Uacceptable	Uacceptable	Acceptable	Acceptable	Acceptable	Good

International validation study

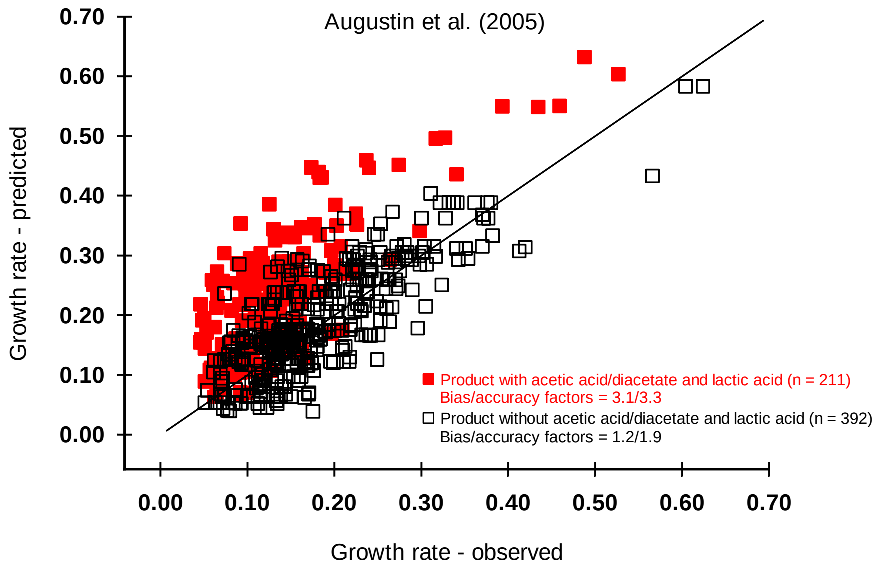


International validation study

Products	n	Delignette-Muller et al. (2006)	Augustin et al. (2005)	Zuliani et al. (2007)	PURAC (2007)	DMRI (2007)	Mejlholm & Dalgaard (2009)
		Bias/accuracy factors					
Meat	702	2.3/2.4	2.1/2.5	1.3/2.1	1.4/1.8	1.1/1.5	1.0/1.5
Seafood	193	1.7/1.8	0.7/1.9	1.2/1.6	1.3/1.5	1.4/1.6	1.0/1.4
Poultry	64	1.5/1.9	2.0/2.1	1.0/1.5	1.0/1.5	1.2/1.5	0.9/1.5
Dairy	55	0.7/1.6	0.9/1.3	1.0/1.3	0.9/1.3	1.3/1.6	0.9/1.3
Total	1014	2.0/2.2	1.8/2.3	1.3/1.9	1.3/1.7	1.2/1.6	1.0/1.5

Without the effect of acetic and lactic acid

International validation study



International validation study

		Percentage of correct growth/no-growth predictions					
Products	n	Delignette-Muller et al. (2006)	Augustin et al. (2005)	Zuliani et al. (2007)	PURAC (2007)	DMRI (2007)	Mejlholm & Dalgaard (2009)
Meat	702	63	76	82	65	81	86
Seafood	193	83	70	89	83	86	96
Poultry	64	78	78	84	78	95	97
Dairy	55	100	100	100	100	100	100
Total	**1014**	**70**	**76**	**85**	**71**	**83**	**89**
Fail-dangerous (%)		0	9	10	0	4	5
Fail-safe (%)		30	15	5	29	13	6
Interaction (+/-)		-	+	+	-	+	+

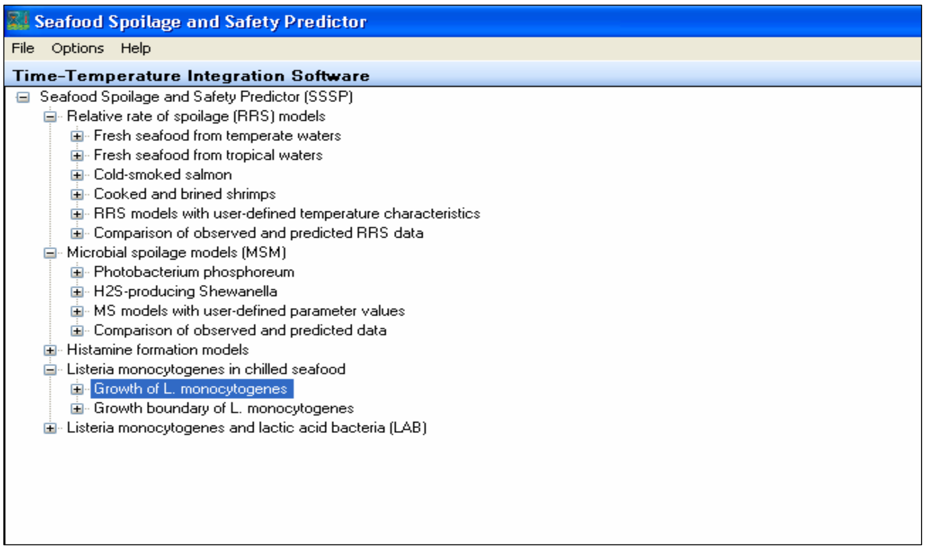
International validation study

- The performance of six predictive models for *L. monocytogenes* was evaluated on more than 1000 data sets from ready-to-eat foods
- To predict growth in complex foods → predictive models with a corresponding degree of complexity are needed
- Predictive models can be generally applicable → product specific models are not necessarily needed
- Ready to be used for assessment and management of food safety

Outline

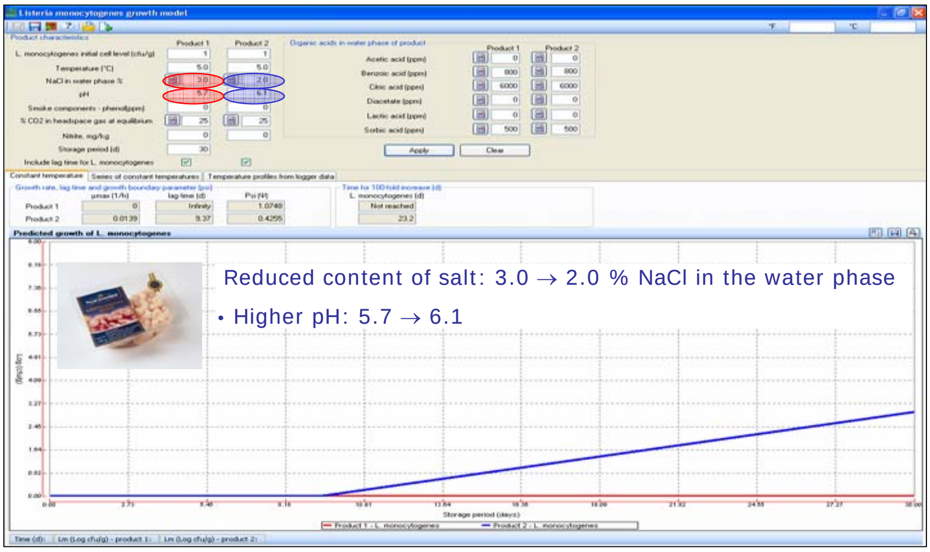
- Predictive models for *Listeria monocytogenes*
  - Why – predictive models
  - Available predictive models for *L. monocytogenes*
  - International validation study
- Application of models
  - Examples
  - Exercises

Application of models - examples



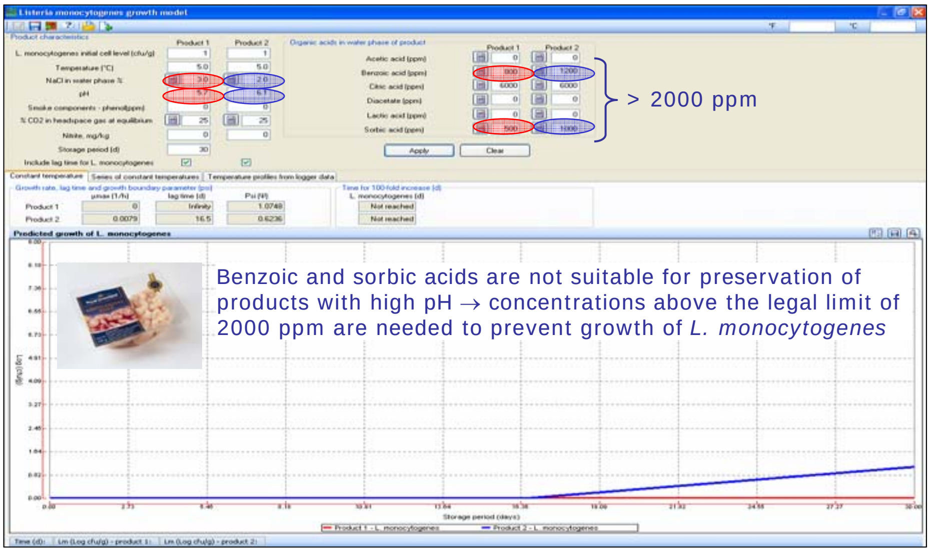
Application of models - examples

Product development/reformulation



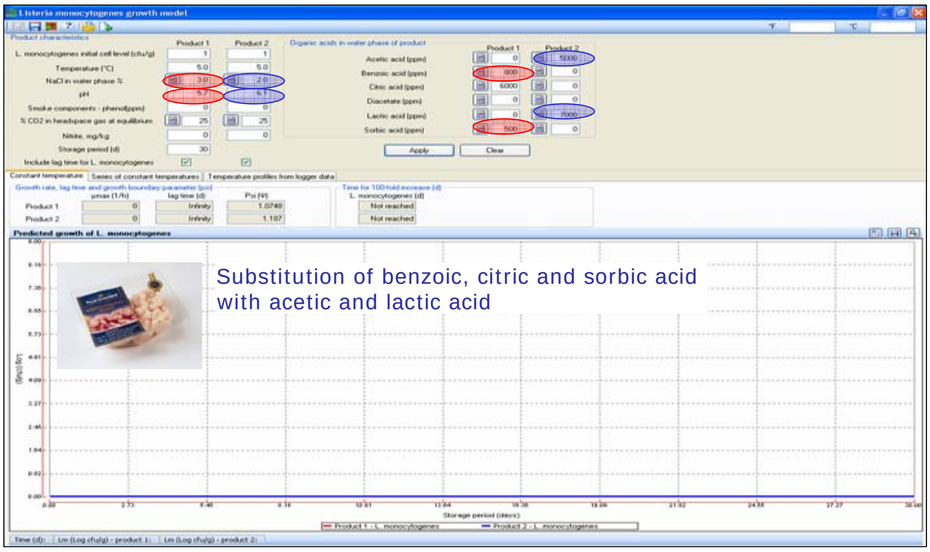
Application of models - examples

Product development/reformulation

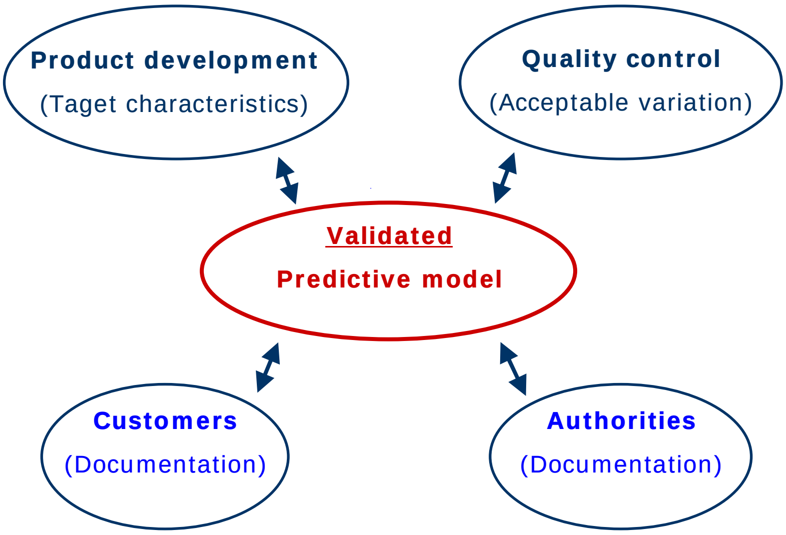


Application of models - examples

Product development/reformulation



Application of predictive microbiology models



Outline

- Predictive models for *Listeria monocytogenes*
  - Why – predictive models
  - Available predictive models for *L. monocytogenes*
  - International validation study
- Application of models
  - Examples
  - Exercises

Application of models - exercises

Exercise 1: Growth of *L. monocytogenes*

Model: *Listeria monocytogenes* in chilled seafood → growth of *L. monocytogenes*

A ready-to-eat food has the following characteristics:

- Temperature: 5 °C
- 2.5% NaCl in the water phase
- pH 6.1
- Smoke components: 8 ppm phenol
- 25% CO_2 at equilibrium
- 500 ppm acetic acid in the water phase
- 8000 ppm lactic acid in the water phase

- Initial concentration of *L. monocytogenes* = 1 CFU/g
- Storage period (shelf life) = 21 days
- No lag time for *L. monocytogenes*

Application of models - exercises

Exercise 1: Growth of *L. monocytogenes* - continued

a) Is growth of *L. monocytogenes* prevented in this product? Yes/no. If no – what is the concentration of *L. monocytogenes* following storage for 21 days at 5 °C
Answer: (CFU/g)

b) How much should the concentration of acetic acid be increased to prevent growth of *L. monocytogenes* at 5 °C
Answer: From 500 ppm acetic acid to ppm acetic acid

c) How much should the concentration of acetic acid be increased to prevent growth of *L. monocytogenes* at 5 °C if the concentration of smoke components is 15 ppm phenol instead of 8 ppm phenol
Answer: From 500 ppm acetic acid to ppm acetic acid

Application of models - exercises

Exercise 1: Growth of *L. monocytogenes* - continued

d) Use the initial characteristics from question a) and predict the concentration of *L. monocytogenes* at the end of the following storage period: 14 days (336 hours) at 5 °C (retail) + 2 hours at 15 °C (transport) + 7 days (168 hours) at 8 °C (home storage)
Answer: log (CFU/g)

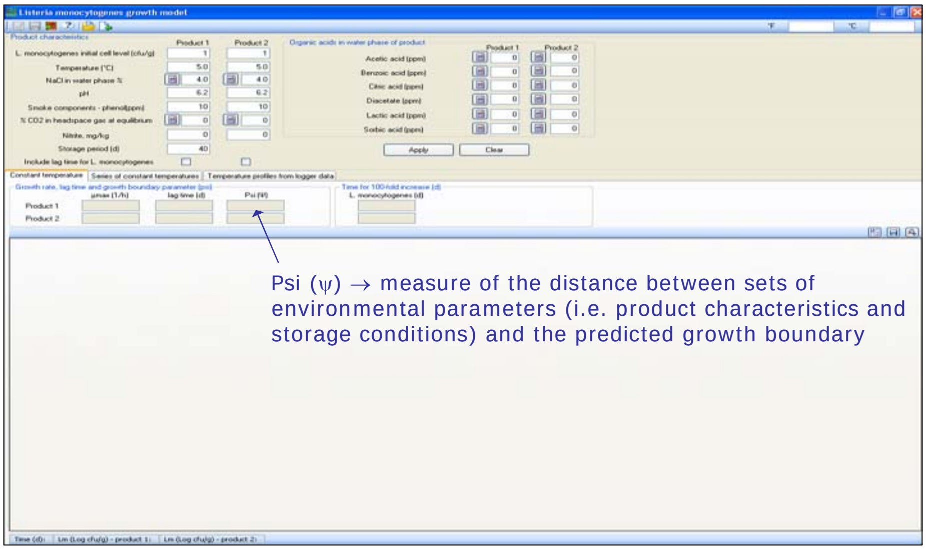
e) After how many days will the product reach the critical limit of 100 CFU/g (= 2 log CFU/g)
Answer: days

Outline

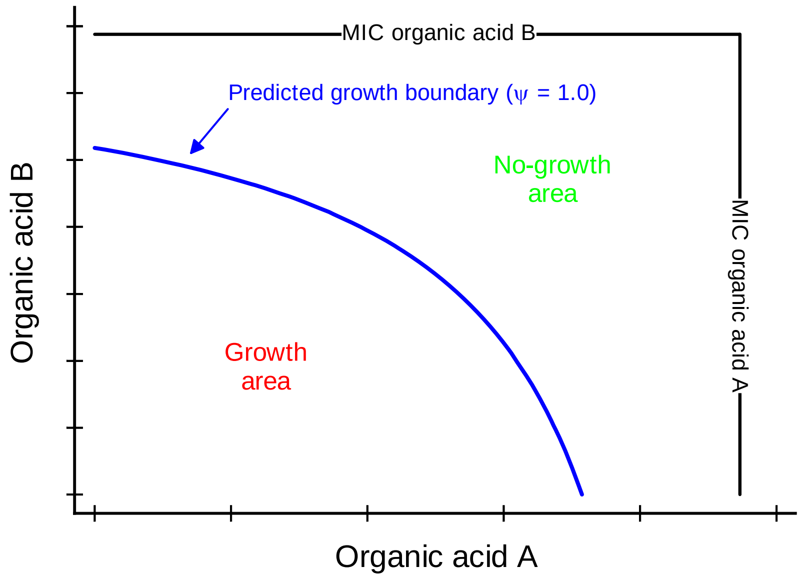
- Predictive models for *Listeria monocytogenes*
  - Why – predictive models
  - Available predictive models for *L. monocytogenes*
  - International validation study
- Application of models
  - Examples
  - Exercises

Application of models - examples

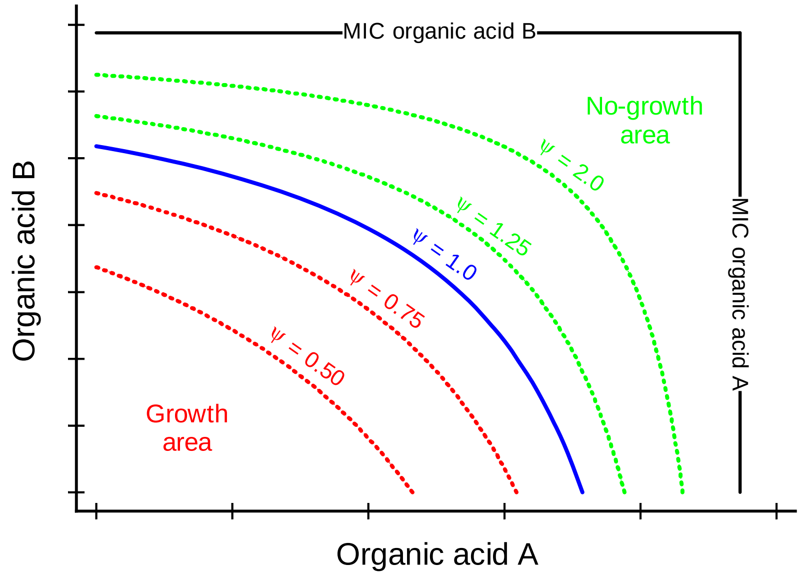
Distance to the growth boundary (psi-value)



Application of models - examples

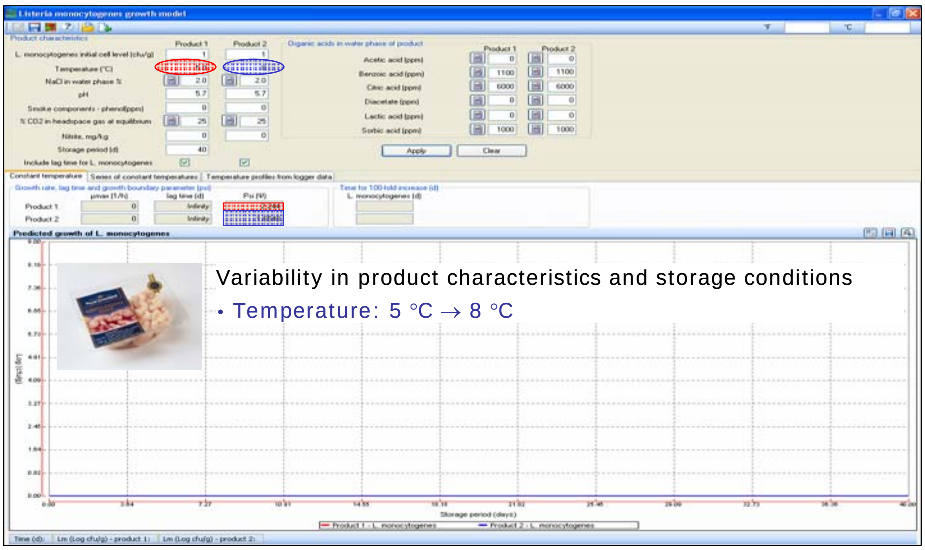


Application of models - examples

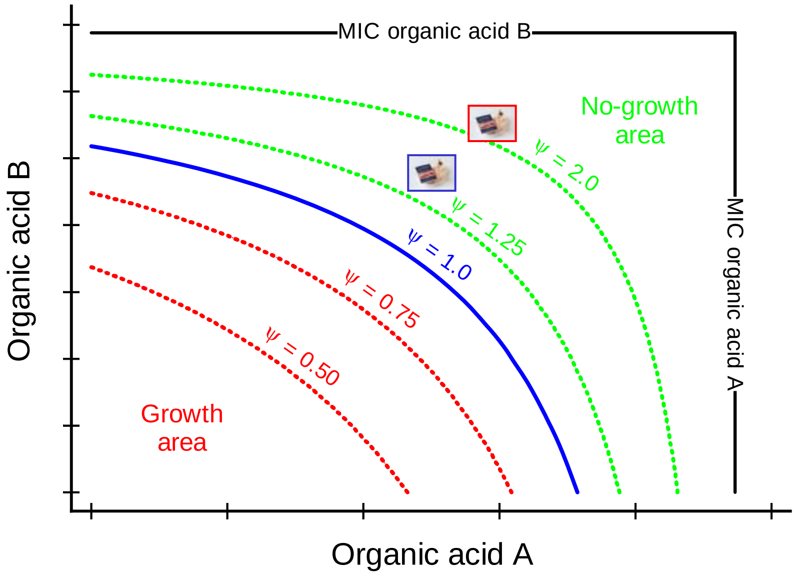


Application of models - examples

Distance to the growth boundary (psi-value)

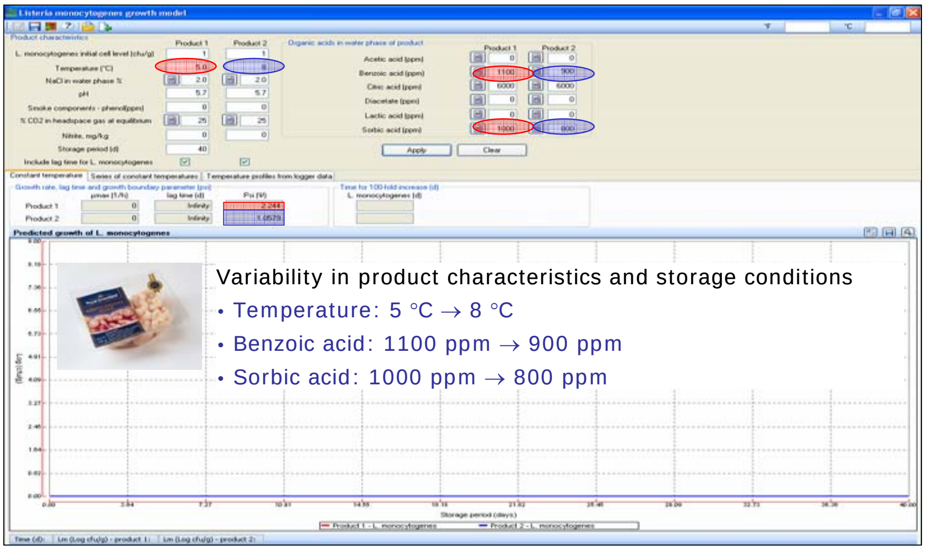


Application of models - examples

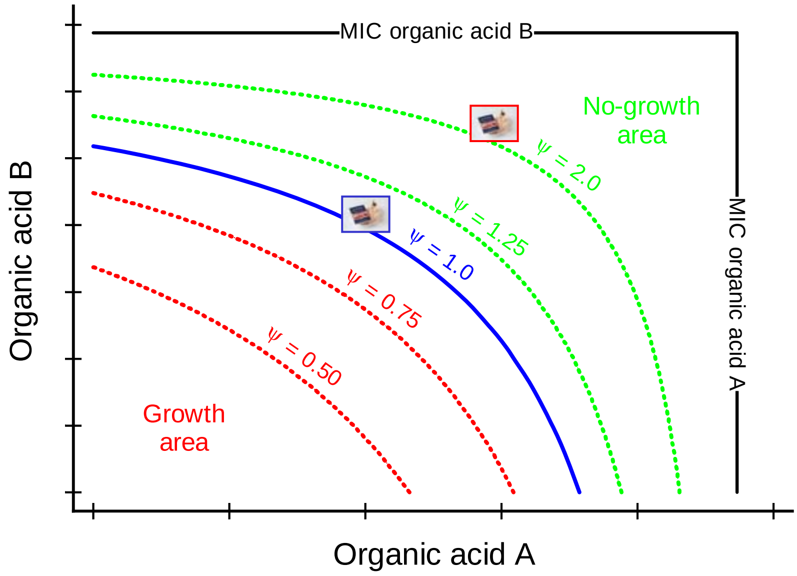


Application of models - examples

Distance to the growth boundary (psi-value)

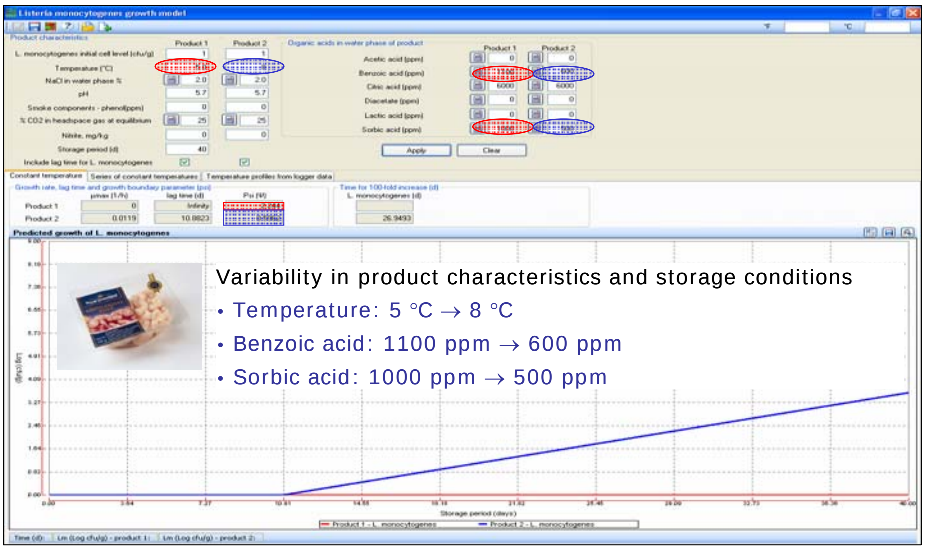


Application of models - examples

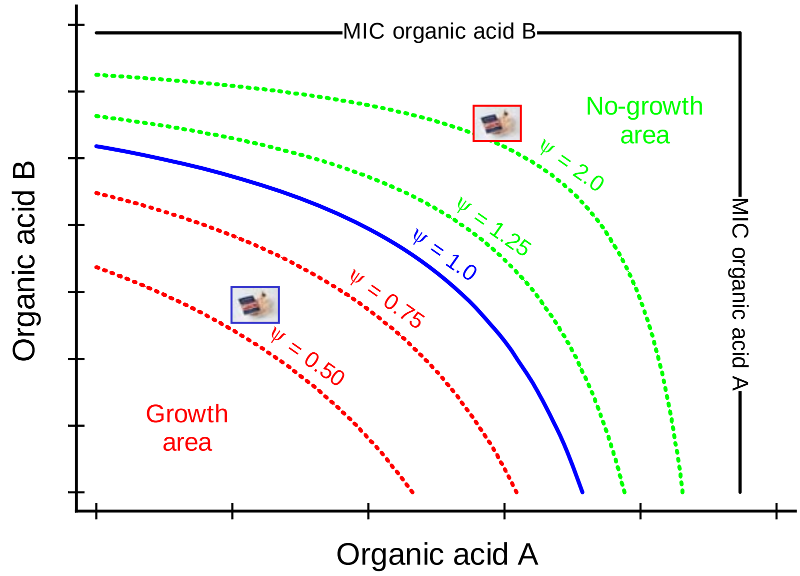


Application of models - examples

Distance to the growth boundary (psi-value)



Application of models - examples



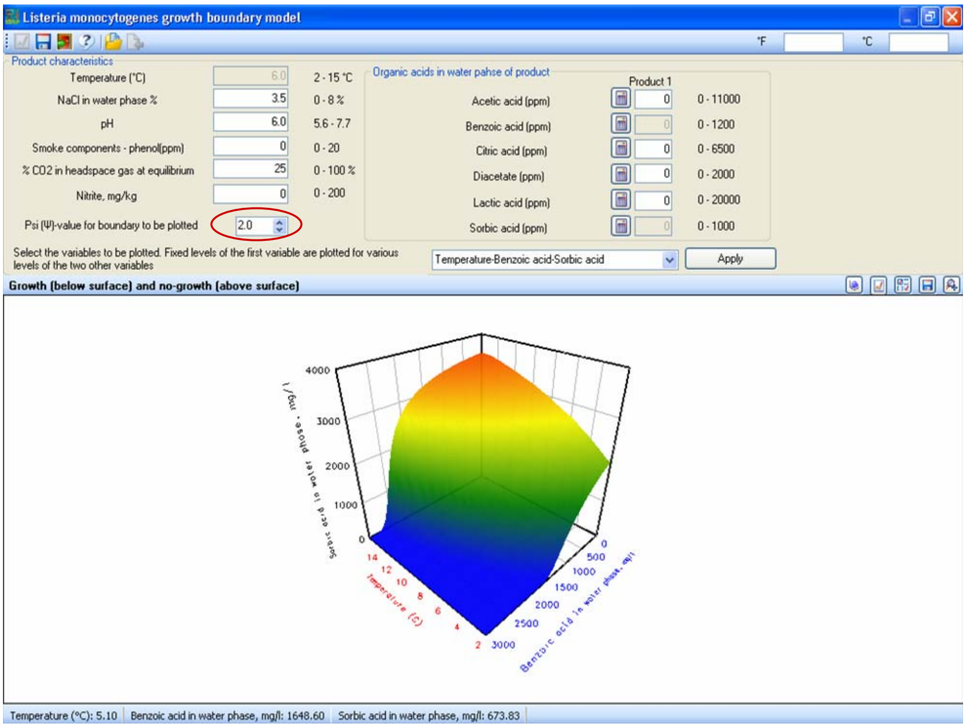
Application of models - examples

- International validation study

Predictive model	Fail-dangerous predictions	psi-value (mean ± SD)
Mejlholm & Dalgaard (2009)	47	1.22 ± 0.31

- Safety factor (psi-value) → mean + 2 SD = 1.84

Product	Temp. (° C)	NaCl (%)	pH	Phenol (ppm)	CO_2 (%)	Acetic acid (ppm)	Lactic acid (ppm)	psi-value
A	5	4.0	6.0	0	0	2000	9000	1.0
B	5	4.0	5.9	0	25	3450	13000	1.84
C	5	2.6	5.9	10	0	3450	13000	1.84



Outline

- Predictive models for *Listeria monocytogenes*
  - Why – predictive models
  - Available predictive models for *L. monocytogenes*
  - International validation study
- Application of models
  - Examples
  - Exercises

Application of models - exercises

Exercise 2: Distance to the growth boundary (psi-value)

Model: *Listeria monocytogenes* in chilled seafood → growth of *L. monocytogenes*

For a ready-to-eat food the following variability in product characteristics and storage conditions has been registered:

- Storage temperature: 5.0-7.0 °C
- 3.0-4.0% NaCl in the water phase
- pH 5.9-6.1
- Smoke components: 5-12 ppm phenol
- 20-30% CO_2 at equilibrium
- 2000-3000 ppm acetic acid in the water phase
- 7000-12000 ppm lactic acid in the water phase

- Initial concentration of *L. monocytogenes* = 1 CFU/g
- Storage period = 30 days

Application of models - exercises

Exercise 2: Distance to the growth boundary (psi-value) - continued

a) Predict the psi-value for the least and most preserving combination of product characteristics and storage conditions
Answer: Psi = and for the least and most preserving combination of product characteristics and storage conditions

b) How much should the concentration of acetic acid be increased to obtain a psi-value of 1.0 for the least preserving combination of product characteristics and storage conditions?
Answer: From 2000 ppm acetic acid to ppm acetic acid

c) By mistake the concentration of CO_2 is only 5% in the packages. How much is the psi-value reduced for the most preserving combination of product characteristics and storage conditions, and would it be necessary to repack the product? Yes/no
Answer: From 1.90 to

Application of models - exercises

Exercise 2: Distance to the growth boundary (psi-value) – continued

d) Type in the most preserving combination of product characteristics and storage conditions from exercise 2a). Rank the parameters (temperature, NaCl, pH, phenol, CO_2, acetic acid and lactic acid) in descending order with respect to their impact on the distance to the growth boundary (psi-value) (use changes as indicated in the table)

Parameters	Change	Psi-before	Psi-after	Psi-change	Rank
Temperature	5 °C → 7 °C	1.90	1.55	0.35	
NaCl	4% → 3%	1.90			
pH	5.9 → 6.1	1.90			
Phenol	12 ppm → 5 ppm	1.90			
CO_2	30% → 20%	1.90			
Acetic acid	3000 ppm → 2000 ppm	1.90			
Lactic acid	12000 ppm → 7000 ppm	1.90			

Exercises - solutions

Exercise 1 - solutions

Exercise 1: Growth of *L. monocytogenes* - continued

a) Is growth of *L. monocytogenes* prevented in this product? Yes/no. If no – what is the concentration of *L. monocytogenes* following storage for 21 days at 5 °C
Answer: 1.5 log (CFU/g)

b) How much should the concentration of acetic acid be increased to prevent growth of *L. monocytogenes* at 5 °C
Answer: From 500 ppm acetic acid to 2800 ppm acetic acid

c) How much should the concentration of acetic acid be increased to prevent growth of *L. monocytogenes* at 5 °C if the concentration of smoke components is 15 ppm phenol instead of 8 ppm phenol
Answer: From 500 ppm acetic acid to 1740 ppm acetic acid

Exercise 1 - solutions

Exercise 1: Growth of *L. monocytogenes* - continued

d) Use the initial characteristics from question a) and predict the concentration of *L. monocytogenes* at the end of the following storage period: 14 days (336 hours) at 5 °C (retail) + 2 hours at 15 °C (transport) + 7 days (168 hours) at 8 °C (home storage)
Answer: 2.5 log (CFU/g)

e) After how many days will the product reach the critical limit of 100 CFU/g (= 2 log CFU/g)
Answer: 18.6 days

Exercise 2 - solutions

Exercise 2: Distance to the growth boundary (psi-value)

a) Predict the psi-value for the least and most preserving combination of product characteristics and storage conditions
Answer: Psi = 0.68 and 1.90 for the least and most preserving combination of product characteristics and storage conditions

b) How much should the concentration of acetic acid be increased to obtain a psi-value of 1.0 for the least preserving combination of product characteristics and storage conditions?
Answer: From 2000 ppm acetic acid to 5010 ppm acetic acid

c) By mistake the concentration of CO_2 is only 5% in the packages. How much is the psi-value reduced for the most preserving combination of product characteristics and storage conditions, and would it be necessary to repack the product? Yes/no
Answer: From 1.90 to 1.80

Exercise 2 - solutions

Exercise 2: Distance to the growth boundary (psi-value)

d) Type in the most preserving combination of product characteristics and storage conditions from exercise 2a). Rank the parameters (temperature, NaCl, pH, phenol, CO_2, acetic acid and lactic acid) in descending order with respect to their impact on the distance to the growth boundary (psi-value) (use changes as indicated in the table)

Parameters	Change	Psi-before	Psi-after	Psi-change	Rank
Temperature	5 °C → 7 °C	1.90	1.55	0.35	2
NaCl	4% → 3%	1.90	1.84	0.06	6
pH	5.9 → 6.1	1.90	1.32	0.58	1
Phenol	12 → 5 ppm	1.90	1.72	0.18	5
CO_2	30% → 20%	1.90	1.84	0.06	6
Acetic acid	3000 ppm → 2000 ppm	1.90	1.62	0.28	4
Lactic acid	12000 ppm → 7000 ppm	1.90	1.56	0.34	3

Seafood safety and shelf-life prediction - a one-day workshop

14th January 2010, Reykjavik, Iceland

Evaluation	
Name (can be anonymous)	:
Has the workshop been useful in relation to the work you perform today and/or expect to carry out in the future?	:
Within which area do you expect primarily to use predictive models/computer software in relation to your work with seafood (shelf-life, safety, both or maybe not at all)?	:
Has the activities included in the workshop been sufficient for you to use the SSSP software within your future work?	:
Please suggest topic(s) that you feel should be included in future workshops of this type	:
Please suggest topic(s) that you feel should be excluded from future workshops of this type	:
Other suggestions?	: