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 Danielsdóttir, Dir. Food safety & Environment at Matis, Iceland
- Steinar B. Aðalbjörnsson, Marketing Director at Matis, Iceland
SSS PREDICTION Námskeið / SSS PREDICTION WORKSHOP

Paw Dalgaard, Anna Kristín Danielsdóttir, Steinar B. Ádalbjörnsson

12-10  29.04.2010

6025-1966


Spáforrit, sjávarútvegur, námskeið, geymsluþol ESB reglugerðir, fæðuöryggi, Listeria monocytogens

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.

Prediction software, seafood quality management, food safety, storage, EU regulations, Listeria monocytogens
TABLE OF CONTENT

1. INTRODUCTION ......................................................................................................... 1

   Icelandic ....................................................................................................................... 1

   English ....................................................................................................................... 1

2. MATERIAL & METHODS ......................................................................................... 2

   Software and documents ......................................................................................... 2

   Teacher and organizers ......................................................................................... 2

   Participants ............................................................................................................. 2

3. RESULTS ..................................................................................................................... 3

4. DISCUSSION & CONCLUSIONS ............................................................................. 3

5. ACKNOWLEDGEMENTS ........................................................................................ 3

6. REFERENCES ............................................................................................................. 3
1. INTRODUCTION

Icelandic


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 monocytogenes 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 Danielsdóttir and Steinar B. Aðalbjörnsson at Matís, Iceland.

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

Participants

1. Erlingur Brynjulfsson, 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 Jóhannesson, leo@opseafood.com – 38.000.- Greitt
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
<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.45 - 9.00</td>
<td>Registration</td>
</tr>
<tr>
<td>9.00 - 9.10</td>
<td>Welcome and opening</td>
</tr>
<tr>
<td>10.30 - 10.45</td>
<td>Presentation and PC exercises using the SSSP software</td>
</tr>
<tr>
<td>10.45 - 12.00</td>
<td>Predicting growth and inactivation of bacteria in seafood.</td>
</tr>
<tr>
<td>12.00 - 13.00</td>
<td>Presentation and PC exercises using SSSP and other freeware</td>
</tr>
<tr>
<td>13.00 - 14.00</td>
<td>Seafood safety prediction 1. Presentation and PC exercises concerning</td>
</tr>
<tr>
<td></td>
<td>histamine formation and histamine fish poisoning</td>
</tr>
<tr>
<td>14.00 - 14.15</td>
<td>Coffee break</td>
</tr>
<tr>
<td>14.15 - 15.45</td>
<td>Seafood safety prediction 2. Presentation and PC exercises concerning</td>
</tr>
<tr>
<td></td>
<td><em>Listeria monocytogenes</em> in ready-to-eat seafood</td>
</tr>
<tr>
<td>15.45 - 16.00</td>
<td>Evaluation and close of the workshop</td>
</tr>
</tbody>
</table>
Shelf-life prediction – effect of temperature

Paw Dalgaard

Seafood & Predictive Microbiology (Research group)
Section for Aquatic Microbiology & Seafood Hygiene
pad@aqua.dtu.dk

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

<table>
<thead>
<tr>
<th>Grade</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>No off-odour/flavour</td>
<td>I</td>
<td>Odour/flavour characteristic of species, very fresh, seaweedy</td>
</tr>
<tr>
<td>Acceptable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight off-odours/flavour</td>
<td>II</td>
<td>Slight off-odours/flavours such as mousy, garlic, bready, sour, fruity, rancid</td>
</tr>
<tr>
<td>Reject</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe off-odour/flavour</td>
<td>III</td>
<td>Strong off-odours/flavours such as stale cabbage, NH₃₂, H₂S or sulphides</td>
</tr>
</tbody>
</table>

Shewan et al. 1953
Sensory changes and shelf-life
an example with fresh fish

Quality index method (QIM) – simple scheme

<table>
<thead>
<tr>
<th>Quality parameter</th>
<th>Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface appearance</td>
<td>0 – 3</td>
</tr>
<tr>
<td>Skin</td>
<td>0 – 1</td>
</tr>
<tr>
<td>Slime</td>
<td>0 – 3</td>
</tr>
<tr>
<td>Stiffness</td>
<td>0 – 1</td>
</tr>
<tr>
<td>Clarity</td>
<td>0 – 2</td>
</tr>
<tr>
<td>Shape or pupil</td>
<td>0 – 2</td>
</tr>
<tr>
<td>Colour</td>
<td>0 – 2</td>
</tr>
<tr>
<td>Smell</td>
<td>0 – 3</td>
</tr>
<tr>
<td>Slime</td>
<td>0 – 2</td>
</tr>
<tr>
<td>Open surfaces</td>
<td>0 – 2</td>
</tr>
<tr>
<td>In throat cut</td>
<td>0 – 2</td>
</tr>
<tr>
<td>Sum of demerit points</td>
<td>0 – 23</td>
</tr>
</tbody>
</table>

Storage temperature – effect on shelf-life

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>% of samples or refrigerators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Denmark(^a)</td>
</tr>
<tr>
<td>&lt; 2</td>
<td>20</td>
</tr>
<tr>
<td>2 - 5</td>
<td>37</td>
</tr>
<tr>
<td>5 - 10</td>
<td>36</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>7</td>
</tr>
</tbody>
</table>

\(^a\) 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

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Shelf-life (days)</th>
<th>Relative rate of spoilage (RRS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.0</td>
<td>25</td>
<td>0.48</td>
</tr>
<tr>
<td>-1.5</td>
<td>17</td>
<td>0.72</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>2.3</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>6.3</td>
</tr>
</tbody>
</table>

- 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

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

**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:**

\[ RRS = \frac{\text{Shelf – life at } T_{\text{ref}}}{\text{Shelf – life at } T} = \exp[a \times (T - T_{\text{ref}})] \]

**Arrhenius RRS model:**

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

**Square-root RRS model:**

\[ RRS = \left(\frac{T - T_{\text{min}}}{T_{\text{ref}} - T_{\text{min}}}\right)^2; \quad T_{\text{min}} = -10^\circ\text{C} \Rightarrow RRS = 1 + 0.1 \times T^\circ\text{C} \]
Storage temperature – effect on shelf-life

Example with fresh fish

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Shelf-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2</td>
<td>19</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

Storage temperature – effect on shelf-life

\[ RRS(0°C) = (1 + 0.1 \times T°C)^2 = 4 \]

\[ \text{Shelf-life(10°C)} = \frac{\text{Shelf-life(T\text{ref °C})}}{\text{RRS(T°C)}} = \frac{12}{4} = 3 \text{ days} \]

Shelf-life at variable storage temperatures

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

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature profile and remaining shelf-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>0°C</td>
</tr>
<tr>
<td>3 days</td>
<td>+ 2°C</td>
</tr>
<tr>
<td>12 hours</td>
<td>+10°C</td>
</tr>
<tr>
<td>2 days</td>
<td>+ 3°C</td>
</tr>
</tbody>
</table>

Remaining shelf-life at 0°C

- Is it possible to store the products one more day at 2°C?
- Is it possible to store the products three more days at 2°C?
### Shelf-life at variable storage temperature

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

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature profile and remaining shelf-life</th>
<th>Example 1</th>
<th>Example 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>0°C</td>
<td></td>
<td>-2°C</td>
</tr>
<tr>
<td>3 days</td>
<td>+2°C</td>
<td></td>
<td>+2°C</td>
</tr>
<tr>
<td>12 hours</td>
<td>+10°C</td>
<td></td>
<td>+4°C</td>
</tr>
<tr>
<td>2 days</td>
<td>+3°C</td>
<td></td>
<td>+3°C</td>
</tr>
</tbody>
</table>

**Total 8.5 days**

**Remaining shelf-life at 0°C**

- None
- 1-2 days

---

**Seawood Spoilage and Safety Predictor**

- Relative rate of spoilage (RRS) models:
  - Fresh seafood from temperate waters
  - Square-root spoilage model
  - Fresh seafood from tropical waters
  - Exponential model for spoilage of fresh tropical seafood
  - Cold-smoked salmon
  - Sliced and vacuum-packed cold-smoked salmon
  - Cooked and brined shrimps
  - Cooked and brined MAP shrimps
  - RRS models with user-defined temperature characteristics
  - RRS models
  - Comparison of observed and predicted RRS data
  - Calculation of values for accuracy factors
  - Microbial spoilage models (MSM)
  - Histamine formation models
  - Listeria monocytogenes in chilled seafood
  - Listeria monocytogenes and lactic acid bacteria (LAB)

[DTU Food](http://sssp.dtuaqua.dk)
Seafood Spoilage and Safety Predictor (SSSP)
Shelf-life prediction for foods with known temperature sensitivity (RRS models)
Storage temperature – effect on RRS

![Graph showing the effect of storage temperature on RRS (Relative Rates of Spoilage)]

- **Cooked and brined MAP shrimps**: $E_A \approx 100$, $a \approx 0.15$
- **Fresh seafood - Tropical waters**: $E_A \approx 80$, $a \approx 0.12$
- **Fresh seafood - Cold waters**: $E_A \approx 20$, $a \approx 0.025$
- **Hot smoked and packed fish**: $T_{min} = -10^\circ C$
- **Packed cold-smoked salmon**: $E_A \approx 61$, $a \approx 0.09$

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

![Comparison of observed and predicted RRS data for cold smoked salmon](image)
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 allows data to be imported by copy and paste from spreadsheets (like MS Excel).

SSSP – Help menu

Table of content

Introduction to SSSP

Using SSSP

- Relative rate of spoilage (RRS) models and general information about SSSP
- Microbial spoilage models (MSM)
- Options and zoom functions available in SSSP to modify graphs

Relative rate of spoilage (RRS) models

- Introduction
- Fresh seafood from temperate waters
- Fresh seafood from tropical waters
- Cold-stored salmon
- Cooked and brined shrimps
- RRS models with user-defined temperature characteristics
- Comparison of observed and predicted RRS data
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

Shell-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.vitsab.com/

DTU Food 27/38

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

DTU Food 28/38
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
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
Exercise 1: RRS model with fixed temperature sensitivity

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)
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

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

- 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.

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

Primary growth models

\[
\begin{align*}
N & \quad \text{Cell concentration (cfu/g)} \\
\frac{dN}{dt} & \quad \text{Absolute growth rate (cfu/g/hour)} \\
\frac{(dN/dt)}{N} = \mu & \quad \text{Specific growth rate (1/hour)}
\end{align*}
\]
**Exponential growth model**

Differential form:
\[ \frac{dN}{dt} = N \times \mu_{\text{max}} \]

Integrated form:
\[ N_t = N_0 \times \exp(\mu_{\text{max}} \times t) \]

Integrated and transformed:
\[ \log(N_t) = \log(N_0) + (\mu_{\text{max}} \times t) \frac{\log(10)}{} \]

or
\[ \log(N_t) = \log(N_0) + \frac{\mu_{\text{max}} \times \log(10)}{\log(10)} \]

\[ \mu_{\text{max}} = \text{Slope} \times \log(10) = \frac{\log(N_{t2}) - \log(N_{t1})}{\text{time}_{2} - \text{time}_{1}} \times \log(10) \]
Logistic growth model

Differential form:

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

Integrated form:

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

Logistic growth model with delay

\[
N_t = \begin{cases} N_0, & t < t_{\text{lag}} \\ N_0 \times \frac{N_{\text{max}}}{N_0 + [N_{\text{max}} - N_0] \times \exp(-\mu_{\text{max}} \times \text{time})}, & t \geq t_{\text{lag}} \end{cases}
\]

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Log (cfu/g)</th>
<th>cfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>1.00</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>1.00</td>
<td>10</td>
</tr>
<tr>
<td>36</td>
<td>1.243</td>
<td>261</td>
</tr>
<tr>
<td>48</td>
<td>3.086</td>
<td>4034</td>
</tr>
<tr>
<td>60</td>
<td>4.508</td>
<td>89965</td>
</tr>
<tr>
<td>72</td>
<td>6.205</td>
<td>160181</td>
</tr>
<tr>
<td>84</td>
<td>7.392</td>
<td>2463647</td>
</tr>
<tr>
<td>96</td>
<td>7.978</td>
<td>86782927</td>
</tr>
<tr>
<td>108</td>
<td>7.957</td>
<td>99274580</td>
</tr>
<tr>
<td>120</td>
<td>8.000</td>
<td>99562263</td>
</tr>
</tbody>
</table>

DTU Food 12/48
The Baranyi and Roberts model is included in the DMFit and MicroFit software and this facilitates its use in practice.

**Baranyi and Roberts model**

Integrated form:

\[
\begin{align*}
\log(N_t) = \log(N_0) + \frac{1}{\mu_{\text{max}}} \left[ \exp(\mu_{\text{max}} \cdot \text{time}) \cdot q - \frac{1}{\mu_{\text{max}}} \right] \\
\end{align*}
\]

Differential form (simplified):

\[
\frac{dN}{dt} = N \times \mu_{\text{max}} \left( q \cdot \frac{N_t}{N_{\text{max}}} - 1 \right)
\]

DMFit/ComBase includes the Baranyi and Roberts model

Example:
- Data from Logistic model with delay
- Data input by copy and paste
- Estimated growth rate depends on the unit of the data
  - \(-\log(\text{cfu/g})\):
    - Maximum rate = \(\mu_{\text{max}}\) (1/h)
  - \(-\log(10)\text{(cfu/g)}\):
    - Maximum rate \(\times\log(10)\) = \(\mu_{\text{max}}\) (1/h)

DTU Food
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)**

  \[
  \frac{dLAB}{dt} = \mu_{LAB}^{max} \times \left(1 - \frac{LAB}{LAB_{max}}\right)
  \]

  \[
  \frac{dLm}{dt} = \mu_{Lm}^{max} \times \left(1 - \frac{Lm}{Lm_{max}}\right) \times \left(1 - \frac{LAB}{LAB_{max}}\right)
  \]

- **Logistic model for growth and interaction between LAB and *L. monocytogenes* (Lm)**
Primary inactivation models

Fig. 1. Commonly observed types of inactivation curves. Left plot: linear (γ, shape I), linear with tailing (κ, shape II), sigmoidal-like (Ω, shape III), linear with a preceding shoulder (ψ, shape IV). Right plot: biphasic (γ, shape V), concave (α, shape VI), biphasic with a shoulder (ξ, shape VII), and convex (Ω, shape VIII).

<table>
<thead>
<tr>
<th>Model</th>
<th>Differential form</th>
<th>Integrated form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log-linear</td>
<td>(\frac{dN}{dt} = N \times -k_{\text{max}})</td>
<td>(\log(N_i) = \log(N_0) \times \exp(-k_{\text{max}} \times \text{time}))</td>
</tr>
<tr>
<td>Log-linear with shoulder (S) and/or tailing: S, (time)</td>
<td>(\frac{dN}{dt} = N \times -k_{\text{max}} \times \left( \frac{1}{1+e^{-\delta t}} \right) \times \left[ 1 - \frac{N_i}{N_{\text{max}}} \right])</td>
<td>(\log(N_i) = \log\left( N_i - N_{\text{max}} \times e^{-\delta t} \times \left( \frac{e^{\delta t} - 1}{e^{\delta t} - 1} \times e^{-\delta t} \right) + N_{\text{max}} \right))</td>
</tr>
<tr>
<td>Weibull model: (concave, convex)</td>
<td></td>
<td>(\log(N_i) = \log\left( N_0 - N_{\text{max}} \times 10^{(\beta - \mu)} \times N_{\text{max}} \right))</td>
</tr>
<tr>
<td>Biphasic models:</td>
<td></td>
<td>(\log(N_i) = \log(N_q) + \log(f \times e^{\lambda_1 \cdot \mu} + (1-f) \times e^{\lambda_2 \cdot \mu}))</td>
</tr>
</tbody>
</table>

Geeraerd et al. (2005)
Primary inactivation model fitting - GInaFit

<table>
<thead>
<tr>
<th>Time</th>
<th>Measure</th>
<th>Parameter</th>
<th>R² (Coefficient of determination)</th>
<th>Standard Error</th>
<th>Mean Deviation of Squared Error</th>
<th>Relative Mean Deviation of Squared Error</th>
<th>Fit-Square</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.43</td>
<td>k0</td>
<td>0.95</td>
<td>0.93</td>
<td>0.09</td>
<td>0.073</td>
<td>0.956</td>
<td>0.93</td>
</tr>
<tr>
<td>0.19</td>
<td>0.42</td>
<td>k0</td>
<td>0.86</td>
<td>0.86</td>
<td>0.10</td>
<td>0.104</td>
<td>0.876</td>
<td>0.86</td>
</tr>
<tr>
<td>0.20</td>
<td>0.41</td>
<td>k0</td>
<td>0.87</td>
<td>0.87</td>
<td>0.11</td>
<td>0.114</td>
<td>0.896</td>
<td>0.87</td>
</tr>
<tr>
<td>0.49</td>
<td>0.30</td>
<td>k0</td>
<td>0.62</td>
<td>0.62</td>
<td>0.09</td>
<td>0.094</td>
<td>0.656</td>
<td>0.62</td>
</tr>
<tr>
<td>0.50</td>
<td>0.27</td>
<td>k0</td>
<td>0.63</td>
<td>0.63</td>
<td>0.10</td>
<td>0.104</td>
<td>0.656</td>
<td>0.63</td>
</tr>
<tr>
<td>0.60</td>
<td>0.50</td>
<td>k0</td>
<td>0.93</td>
<td>0.93</td>
<td>0.09</td>
<td>0.073</td>
<td>0.956</td>
<td>0.93</td>
</tr>
<tr>
<td>0.70</td>
<td>0.29</td>
<td>k0</td>
<td>0.62</td>
<td>0.62</td>
<td>0.10</td>
<td>0.104</td>
<td>0.656</td>
<td>0.62</td>
</tr>
<tr>
<td>0.80</td>
<td>0.41</td>
<td>k0</td>
<td>0.87</td>
<td>0.87</td>
<td>0.11</td>
<td>0.114</td>
<td>0.896</td>
<td>0.87</td>
</tr>
<tr>
<td>0.90</td>
<td>0.31</td>
<td>k0</td>
<td>0.61</td>
<td>0.61</td>
<td>0.09</td>
<td>0.094</td>
<td>0.656</td>
<td>0.61</td>
</tr>
<tr>
<td>1.00</td>
<td>0.13</td>
<td>k0</td>
<td>0.55</td>
<td>0.55</td>
<td>0.08</td>
<td>0.079</td>
<td>0.639</td>
<td>0.55</td>
</tr>
<tr>
<td>1.10</td>
<td>0.23</td>
<td>k0</td>
<td>0.63</td>
<td>0.63</td>
<td>0.10</td>
<td>0.104</td>
<td>0.656</td>
<td>0.63</td>
</tr>
<tr>
<td>1.20</td>
<td>0.42</td>
<td>k0</td>
<td>0.86</td>
<td>0.86</td>
<td>0.10</td>
<td>0.104</td>
<td>0.876</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Inactivation model identified:

For identification purposes, several models can be derived from numerical analysis of the data. The models can be selected based on goodness-of-fit criteria such as the root mean squared error (RMSE) or the coefficient of determination (R²).

Primary inactivation model fitting – Combase/DMFit

Choose a model:
1. Linear
2. Biphase
3. Trilinear
4. No log
5. No asymptote
6. Biphase (No log)
7. Biphasic (No asymptote)
8. Linear

Initial value for parameters:
- Log sigma: 1.5
- Shoulder: 0.1

Estimated parameters and standard errors:
- Linear
- Biphase
- Trilinear

Maximum number of iterations: 100

DTU Food cit.kuleuven.be/biotec/downloads/GInaFit/get_tool.php 19/48

DTU Food cit.kuleuven.be/biotec/downloads/Combase/DMFit 20/48
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

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 ($\lambda$)
- Growth rate ($\mu_{\text{max}}$)
- Maximum cell density ($N_{\text{max}}$)

**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

- Fitted data, growth rate ($\mu_{\text{max}}$) = 0.1 d\(^{-1}\)
- Predicted growth, $\mu_{\text{max}}$ = 0.2 d\(^{-1}\)

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

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

![Graph showing the relationship between storage time, concentration of microorganisms (TVC, SSO, Metabolites), and chemical spoilage index.]

**Application of predictive microbiology models**

1. Determine product characteristics and storage conditions of food
   - Temperature, $a_w$/NaCl, pH, organic acids, nitrit, smoke components, inhibiting microflora
2. Secondary model $\rightarrow$ lag time, growth rate, etc.
3. Primary model $\rightarrow$ 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

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th><em>P. phosphoreum</em> (cfu/g)</th>
<th>CO₂ (%)</th>
<th>Shelf-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>30</td>
<td>12,4</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>50</td>
<td>14,4</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>50</td>
<td>9,3</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>50</td>
<td>7,0</td>
</tr>
<tr>
<td>15</td>
<td>1000</td>
<td>50</td>
<td>1,4</td>
</tr>
<tr>
<td>15</td>
<td>1000</td>
<td>30</td>
<td>1,2</td>
</tr>
</tbody>
</table>
Effect of a simple temperature profile on growth of *P. phosphoreum* (SSO) and on shelf-life of fresh MAP fish

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**

<table>
<thead>
<tr>
<th>SSO Product</th>
<th>Freeware</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$S-producing <em>Shewanella</em></td>
<td>- Seafood Spoilage and Safety Predictor</td>
</tr>
</tbody>
</table>
| *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 successfully validated 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

Prevalence and conc. of hazard
Product characteristics
Storage conditions
Storage time (shelf-life)

Predictive microbiology model(s)

Output: Predicted concentration of hazard in food at the time of consumption

Predicted probability of illness per meal
Consumption patterns

Cases per 1 million meals
Cases per 100,000 (sub)-population

Model = deterministic + stochastic part

Predictive microbiology software (freeware)

- Predictive Microbiology Information Portal (PMIP; portal.arserrc.gov) and Pathogen Modeling Programme (PMP; pmp.arserrc.gov/PMPOne.aspx) (USA)
  - > 40 models of growth, survival and inactivation
  - Regularly 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 monocytogenes*

  - 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/biotech/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
Predicting growth of spoilage bacteria (Shewanella)

H_{2}S-producing *Shewanella* bacteria are well known spoilage microorganisms 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 contribute to spoilage of vacuum-pakked food. Use the SSSP model 'H_{2}S-producing Shewanella-Fresh seafood stored in air’ to predict the effect of growth of this spoilage bacterium on product shelf-life:

- With an 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 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).

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 exceed 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 temperature of 2.0°C – when the initial cell concentration is 10 cfu/g? Answer: ____ log(cfu/g)
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?)

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

Some fishmongers expose whole gutted fish in their shop window. These fish are not entirely 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 an 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)
### Growth of *Shewanella* and shelf-life – fishmonger example

Use the SSSP model ‘H₂S-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 kept the fish at 2°C during 48 hours.
   Answer: ____ days.

2. The fishmonger has kept 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: ______.

---

### Predicting growth of spoilage bacteria (*Photobacterium*)

*Photobacterium phosphoreum* is responsible for spoilage of fresh marine fish when stored in modified atmosphere 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% CO₂/75% N₂ at 0°C. Use the SSSP model ‘*Photobacterium phosphoreum*’ to predict the effect of storage temperature 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 increasing the concentration of CO₂ from 25% to 40%?
  Answer: ____ days.

- How much is shelf-life reduced by using vacuum-packing (corresponding to 0% CO₂) compared to MAP with 40% CO₂ and 60% N₂?
  Answer: ____ days.
Seafood Spoilage and Safety Predictor (SSSP)

Predicting growth of spoilage bacteria (*Photobacterium*)
**Seafood safety prediction 1.**
Presentation and PC exercises concerning histamine formation and histamine fish poisoning

**Paw Dalgaard**

Seafood & Predictive Microbiology (Research group)
Section for Aquatic Microbiology and Seafood Hygiene
pad@aqua.dtu.dk

---

**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 $\rightarrow$ Histidine decarboxylase $\rightarrow$ Histamine

- Significant growth is required $\rightarrow$ 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**: Fresh fish (4.4°C) with demands for rates of chilling (FDA/CFSAN 2001)
Histamine fish poisoning (HFP) - occurrence

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

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

<table>
<thead>
<tr>
<th>Seafood</th>
<th>Bacteria</th>
<th>Place and time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh tuna</td>
<td><em>Morganella morganii</em></td>
<td>Japan, 1955</td>
</tr>
<tr>
<td>Fresh tuna</td>
<td><em>Morganella morganii</em></td>
<td>Japan, 1965</td>
</tr>
<tr>
<td>Fresh tuna</td>
<td><em>Hafnia sp.</em></td>
<td>Prague, 1967</td>
</tr>
<tr>
<td>Fresh tuna</td>
<td><em>Raoultella planticola (Klebsiella pneumoniae)</em></td>
<td>California, 1977</td>
</tr>
<tr>
<td>Dried Sardine</td>
<td><em>Photobacterium phosphoreum</em></td>
<td>Japan, 2002</td>
</tr>
<tr>
<td>Tuna in chilisauce</td>
<td><em>Morganella psychrotolerans</em> or</td>
<td>Denmark, 2003</td>
</tr>
<tr>
<td></td>
<td><em>Photobacterium phosphoreum</em></td>
<td></td>
</tr>
<tr>
<td>Cold smoked tuna</td>
<td><em>Photobacterium phosphoreum</em></td>
<td>Denmark, 2004</td>
</tr>
<tr>
<td>Cold smoked tuna</td>
<td><em>Morganella psychrotolerans</em></td>
<td>Denmark, 2004</td>
</tr>
<tr>
<td>Tuna (packed in film)</td>
<td><em>Morganella morganii</em></td>
<td>Denmark, 2004</td>
</tr>
<tr>
<td>Fresh tuna</td>
<td><em>Photobacterium phosphoreum</em></td>
<td>Denmark, 2006</td>
</tr>
<tr>
<td>Dried milkfish</td>
<td><em>Raoultella ornithinolytica</em></td>
<td>Taiwan, 2006</td>
</tr>
</tbody>
</table>

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

**Growth**

**Histamine**

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**

![Graph showing spoilage progression](image)

- Log (cfu/g)
- Conc. of metabolites
- Storage time
- Minimal spoilage level
- Chemical spoilage index
- Shelf life

*Dalgaard, 1993*
**Prediction of histamine formation**

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

![Graph showing growth of bacteria over storage time](image)

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

![Graphs showing primary and secondary models](image)

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

DTU Food

Secondary square-root type model

Effect of storage temperature on growth rate

Ratkowsky et al. (1983)
Secondary square-root type model

Effect of temperature and NaCl/water activity

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

6.0 % NaCl in water phase \( a_w \approx 0.9641 \)

term for water activity

\[ \sqrt{(a_w - a_w_{\text{max}})/(a_w_{\text{opt}} - a_w_{\text{max}})} \]

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 \( \mu_{\text{max}} \)
of the histamine producing bacterium *Morganella psychrotolerans*

Emborg & Dalgaard (2008a)
Simplified cardinal parameter model for sub-optimum environmental conditions (*M. psychrotolerans*)

- Few parameters with (at least some) biological significance
- Include terms without dimension and with values between 0 and 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 $\mu_{max}$

\[
\text{Lag time} = RLT \cdot t_{gen} = RLT \cdot \ln(2) / \mu_{max}
\]

Ross and Dalgaard 2004
Modelling of growth and histamine formation

Growth model
\[
\frac{dN_i}{dt} = N_i \times \mu_{\text{max}} \times \left(1 - \left(\frac{N_i}{N_{\text{max}}}\right)^m\right)
\]

Histamine formation model
\[
\frac{d\text{Hist}}{dt} = \frac{\text{Hist}_{\text{ref}}}{\epsilon_{\text{ref}}} \times \frac{dN_i}{dt}
\]

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)

Growth model: Example for *M. psychrotolerans*

\[
\frac{dM_p}{dt} = M_p \times \mu_{max} \times \left(1 - \left(\frac{M_p}{M_{p, max}}\right)^m\right) \times \left(1 - \left(\frac{M_m}{M_{m, max}}\right)^m\right)
\]

Histamine formation model

\[
\frac{dHist}{dt} = Y_{Hist} \times \frac{dM_p}{dt} + Y_{Hist} \times \frac{dM_m}{dt}
\]
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.

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

Seafood safety prediction – histamine formation

**Exercise 1: Morganella – effect of storage temperature**

Histamine formation in fish can be due to both psychrotolerant 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:

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Time to 500 mg/kg</th>
<th>Most important bacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Seafood safety prediction – histamine formation

Exercise 2: *Morganella psychrotolerans* – effect of NaCl and CO₂

Histamine formation in chilled cold-smoked tuna can be due to *Morganella psychrotolerans*. Use the SSSP model ‘*Morganella psychrotolerans* – growth and histamine formation’ to predict the effect of salt (NaCl) and storage atmosphere (% CO₂ 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:

<table>
<thead>
<tr>
<th>% NaCl in water phase</th>
<th>% CO₂</th>
<th>Time to 500 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3%</td>
<td>0 %</td>
<td></td>
</tr>
<tr>
<td>3%</td>
<td>30 %</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>0 %</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>30 %</td>
<td></td>
</tr>
</tbody>
</table>

*(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)

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*.

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

- 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
  - CO₂
  - Acetic acid
  - Benzoic acid
  - Citric acid
  - Diacetat
  - Lactic acid
  - Sorbic acid
  - Interactions between all these parameters

12 parameters
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 CO₂ 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)

\[
\mu_{max} = \mu_{ref} \left( \frac{(T - T_{ref})}{T_{ref} - T_{min}} \right)^{a_{w} - a_{ref}} \left[ 1 - 10^{(pH_{opt} - pH)} \right] - \frac{[LAC]}{[MIC_{lactic acid}]} \left( \frac{P_{max} - P}{P_{max}} \right) \left( \frac{NIT_{max} - NIT}{NIT_{max}} \right) \left( \frac{CO_{2_{max}} - CO_{2_{opt}}}{CO_{2_{max}}} \right) \left( 1 - \frac{[DAC]}{[MIC_{diacetate}]} \right) \left( 1 - \frac{[AAC]}{[MIC_{acetic acid}]} \right) \left( \frac{1 - [BAC]}{[MIC_{benzoic acid}]} \right) \left( 1 - \frac{[CAC]}{[MIC_{citric acid}]} \right) \left( 1 - \frac{[SAC]}{[MIC_{sorbic acid}]} \right)
\]

\[
\psi = \sum_{i=1}^{2 \times \ell \times \ell} \frac{\phi_i}{2(1 - \phi_i)}
\]

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

[Link to paper](http://sssp.dtuaqua.dk/)
Other predictive models for *L. monocytogenes*

- **Pathogen Modeling Program** (http://pmp.arserrc.gov/)
  - Temperature
  - pH
  - NaCl
  - Nitrite

- **Combase predictor** (http://www.combase.cc/)
  - Temperature
  - pH
  - NaCl/aw
  - Acetic acid
Other predictive models for *L. monocytogenes*

- **PURAC**

**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

<table>
<thead>
<tr>
<th>Predictive Models</th>
<th>Temp.</th>
<th>NaCl/(a_w)</th>
<th>pH</th>
<th>Smoke comp.</th>
<th>CO₂</th>
<th>Nitrite</th>
<th>Acetic acid/diacetate</th>
<th>Lactic Acid</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delignette-Muller et al. (2006)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Augustin et al. (2005)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Zuliani et al. (2007)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PURAC (2007)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DMRI (2007)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mejlholm and Dalgaard (2009)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Danish Meat Research Institute
International validation study

Number of growth responses for *L. monocytogenes*

<table>
<thead>
<tr>
<th>Products</th>
<th>Growth</th>
<th>No-growth</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>442</td>
<td>260</td>
<td>702</td>
</tr>
<tr>
<td>Seafood</td>
<td>160</td>
<td>33</td>
<td>193</td>
</tr>
<tr>
<td>Poultry</td>
<td>50</td>
<td>14</td>
<td>64</td>
</tr>
<tr>
<td>Dairy</td>
<td>55</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>707</td>
<td>307</td>
<td>1014</td>
</tr>
</tbody>
</table>

- 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 ($\mu_{max}$)
  - 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

Bias/accuracy factors

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>702</td>
<td>2.3/2.4</td>
<td>2.1/2.5</td>
<td>1.3/2.1</td>
<td>1.4/1.8</td>
<td>1.1/1.5</td>
<td>1.0/1.5</td>
</tr>
<tr>
<td>Seafood</td>
<td>193</td>
<td>1.7/1.8</td>
<td>0.7/1.9</td>
<td>1.2/1.6</td>
<td>1.3/1.5</td>
<td>1.4/1.6</td>
<td>1.0/1.4</td>
</tr>
<tr>
<td>Poultry</td>
<td>64</td>
<td>1.5/1.9</td>
<td>2.0/2.1</td>
<td>1.0/1.5</td>
<td>1.0/1.5</td>
<td>1.2/1.5</td>
<td>0.9/1.5</td>
</tr>
<tr>
<td>Dairy</td>
<td>55</td>
<td>0.7/1.6</td>
<td>0.9/1.3</td>
<td>1.0/1.3</td>
<td>0.9/1.3</td>
<td>1.3/1.6</td>
<td>0.9/1.3</td>
</tr>
<tr>
<td>Total</td>
<td>1014</td>
<td>2.0/2.2</td>
<td>1.8/2.3</td>
<td>1.3/1.9</td>
<td>1.3/1.7</td>
<td>1.2/1.6</td>
<td>1.0/1.5</td>
</tr>
</tbody>
</table>

Delignette-Muller et al. (2006)
Augustin et al. (2005)
Zuliani et al. (2007)
PURAC (2007)
DMRI (2007)
Mejlholm & Dalgaard (2009)
### International validation study

![Graph showing growth rate predictions vs observed growth rates.](image)

**Bias/accuracy factors**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>702</td>
<td>2.3/2.4</td>
<td>2.1/2.5</td>
<td>1.3/2.1</td>
<td>1.4/1.8</td>
<td>1.1/1.5</td>
<td>1.0/1.5</td>
</tr>
<tr>
<td>Seafood</td>
<td>193</td>
<td>1.7/1.8</td>
<td>0.7/1.9</td>
<td>1.2/1.6</td>
<td>1.3/1.5</td>
<td>1.4/1.6</td>
<td>1.0/1.4</td>
</tr>
<tr>
<td>Poultry</td>
<td>64</td>
<td>1.5/1.9</td>
<td>2.0/2.1</td>
<td>1.0/1.5</td>
<td>1.0/1.5</td>
<td>1.2/1.5</td>
<td>0.9/1.5</td>
</tr>
<tr>
<td>Dairy</td>
<td>55</td>
<td>0.7/1.6</td>
<td>0.9/1.3</td>
<td>1.0/1.3</td>
<td>0.9/1.3</td>
<td>1.3/1.6</td>
<td>0.9/1.3</td>
</tr>
<tr>
<td>Total</td>
<td>1014</td>
<td>2.0/2.2</td>
<td>1.8/2.3</td>
<td>1.3/1.9</td>
<td>1.3/1.7</td>
<td>1.2/1.6</td>
<td>1.0/1.5</td>
</tr>
</tbody>
</table>

**Without** the effect of acetic and lactic acid
International validation study

![Graph showing growth rates observed vs predicted](image)

Augustin et al. (2005)

- Product with acetic acid/diacetate and lactic acid (n = 211)
  - Bias/accuracy factors = 3.1/3.3
- Product without acetic acid/diacetate and lactic acid (n = 392)
  - Bias/accuracy factors = 1.2/1.9

### Percentage of correct growth/no-growth predictions

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>702</td>
<td>63</td>
<td>76</td>
<td>82</td>
<td>65</td>
<td>81</td>
<td>86</td>
</tr>
<tr>
<td>Seafood</td>
<td>193</td>
<td>83</td>
<td>70</td>
<td>89</td>
<td>83</td>
<td>86</td>
<td>96</td>
</tr>
<tr>
<td>Poultry</td>
<td>64</td>
<td>78</td>
<td>78</td>
<td>84</td>
<td>78</td>
<td>95</td>
<td>97</td>
</tr>
<tr>
<td>Dairy</td>
<td>55</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1014</strong></td>
<td><strong>70</strong></td>
<td><strong>76</strong></td>
<td><strong>85</strong></td>
<td><strong>71</strong></td>
<td><strong>83</strong></td>
<td><strong>89</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fail-dangerous (%)</th>
<th>Fail-safe (%)</th>
<th>Interaction (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>15</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td>+</td>
</tr>
</tbody>
</table>
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

Product development/reformulation

Reduced content of salt: 3.0 → 2.0 % NaCl in the water phase

- Higher pH: 5.7 → 6.1
Benzoic and sorbic acids are not suitable for preservation of products with high pH → concentrations above the legal limit of 2000 ppm are needed to prevent growth of *L. monocytogenes*.

Substitution of benzoic, citric and sorbic acid with acetic and lactic acid.
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 \textit{L. monocytogenes}

Model: \textit{Listeria monocytogenes} in chilled seafood $\rightarrow$ growth of \textit{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 \textit{L. monocytogenes} = 1 CFU/g
- Storage period (shelf life) = 21 days
- No lag time for \textit{L. monocytogenes}

Application of models - exercises - continued

a) Is growth of \textit{L. monocytogenes} prevented in this product? Yes/no. If no - what is the concentration of \textit{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 \textit{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 \textit{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
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)

Psi ($\psi$) → measure of the distance between sets of environmental parameters (i.e., product characteristics and storage conditions) and the predicted growth boundary.

Application of models - examples

Organic acid A

Organic acid B

MIC organic acid B

No-growth area

Growth area

Predicted growth boundary ($\psi = 1.0$)
Application of models - examples

Distance to the growth boundary (psi-value)

Variability in product characteristics and storage conditions
- Temperature: 5 °C → 8 °C
Application of models - examples

Variability in product characteristics and storage conditions

- Temperature: 5 °C → 8 °C
- Benzoic acid: 1100 ppm → 900 ppm
- Sorbic acid: 1000 ppm → 800 ppm
Application of models - examples

Variability in product characteristics and storage conditions

• Temperature: 5 °C → 8 °C
• Benzoic acid: 1100 ppm → 600 ppm
• Sorbic acid: 1000 ppm → 500 ppm
Application of models - examples

- International validation study

<table>
<thead>
<tr>
<th>Predictive model</th>
<th>Fail-dangerous predictions</th>
<th>psi-value (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mejlholm &amp; Dalgaard (2009)</td>
<td>47</td>
<td>1.22 ± 0.31</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Temp. (°C)</th>
<th>NaCl (%)</th>
<th>pH</th>
<th>Phenol (ppm)</th>
<th>CO₂ (%)</th>
<th>Acetic acid (ppm)</th>
<th>Lactic acid (ppm)</th>
<th>psi-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>4.0</td>
<td>6.0</td>
<td>0</td>
<td>0</td>
<td>2000</td>
<td>9000</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>4.0</td>
<td>5.9</td>
<td>0</td>
<td>25</td>
<td>3450</td>
<td>13000</td>
<td>1.84</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>2.6</td>
<td>5.9</td>
<td>10</td>
<td>0</td>
<td>3450</td>
<td>13000</td>
<td>1.84</td>
</tr>
</tbody>
</table>
Outline

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

- Application of models
  - Examples
  - 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% CO₂ 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

---

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 CO₂ 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
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, CO₂, 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)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Change</th>
<th>Psi-before</th>
<th>Psi-after</th>
<th>Psi-change</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>5 ºC → 7 ºC</td>
<td>1.90</td>
<td>1.55</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>4% → 3%</td>
<td>1.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.9 → 6.1</td>
<td>1.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>12 ppm → 5 ppm</td>
<td>1.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>30% → 20%</td>
<td>1.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>3000 ppm → 2000 ppm</td>
<td>1.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>12000 ppm → 7000 ppm</td>
<td>1.90</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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

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: 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 CO₂ 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, CO₂, 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)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Change</th>
<th>Psi-before</th>
<th>Psi-after</th>
<th>Psi-change</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>5 °C → 7 °C</td>
<td>1.90</td>
<td>1.55</td>
<td>0.35</td>
<td>2</td>
</tr>
<tr>
<td>NaCl</td>
<td>4% → 3%</td>
<td>1.90</td>
<td>1.84</td>
<td>0.06</td>
<td>6</td>
</tr>
<tr>
<td>pH</td>
<td>5.9 → 6.1</td>
<td>1.90</td>
<td>1.32</td>
<td>0.58</td>
<td>1</td>
</tr>
<tr>
<td>Phenol</td>
<td>12 → 5 ppm</td>
<td>1.90</td>
<td>1.72</td>
<td>0.18</td>
<td>5</td>
</tr>
<tr>
<td>CO₂</td>
<td>30% → 20%</td>
<td>1.90</td>
<td>1.84</td>
<td>0.06</td>
<td>6</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>3000 ppm → 2000 ppm</td>
<td>1.90</td>
<td>1.62</td>
<td>0.28</td>
<td>4</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>12000 ppm → 7000 ppm</td>
<td>1.90</td>
<td>1.56</td>
<td>0.34</td>
<td>3</td>
</tr>
</tbody>
</table>
# Evaluation

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