Seafood Spoilage and Safety Predictor (SSSP) – Version 3 from 2008

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Outline:

- Key features of SSSP v. 2 from 2005
  - Flexible time-temperature integration tool for spoilage and shelf-life evaluation
- Histamine formation in seafood
  - *Morganella psychrotolerans* (0 - 20°C)
  - *M. psychrotolerans* and *M. morganii* (0 - 44°C)
- *Listeria monocytogenes* and lactic acid bacteria
  - New growth and growth boundary models
- Conclusions and perspectives
Key features of SSSP – relative rates of spoilage (RRS)

- 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

Http://www.difres.dk/micro/sssp/
Shelf-life can be predicted at different temperatures when:

1. Shelf-life at a constant temperature is known
2. RRS at different temperatures can be predicted

\[
\text{Shelf - life (T °C)} = \frac{\text{Shelf - life at } T_{\text{ref}} (°C)}{\text{RRS at } T (°C)}
\]

RRS-models can be used:
- Although spoilage microorganisms are not known
- Over wide ranges of storage temperatures

Spencer og Baines (1964), Olley og Ratkowsky (1973)
Key features of SSSP – relative rates of spoilage (RRS)

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

- Exponential model
- Square-root model
- Arrhenius model

\[ \text{Ln (Relative rates of spoilage)} \]

- Cooked and brined MAP shrimps
- Fresh seafood - Tropical waters
- Fresh seafood - Cold waters
- Packed cold-smoked salmon
- Hot smoked and packed fish

\[
\begin{align*}
\text{E}_A & \quad \text{a} \\
\sim 100 & \quad \sim 0.15 \\
\sim 80 & \quad \sim 0.12 \\
\sim 61 & \quad \sim 0.09 \\
\sim 20 & \quad \sim 0.025 \\
\end{align*}
\]

$T_{min} = -10°C$

Dalgaard (2000)
Key features of SSSP – microbial spoilage models

Time-Temperature Integration Software

- Seafood Spoilage and Safety Predictor (SSSP)
  - Relative rate of spoilage (RRS) models
  - Microbial spoilage models (MSM)
    - Photobacterium phosphoreum
      - Fresh MAP cod fillets
      - Fresh MAP plaice fillets
      - Fresh MAP salmon steaks
    - Shewanella putrefaciens
    - MS models with user-defined parameter values
    - Comparison of observed and predicted data
    - Listeria monocytogenes

Http://www.difres.dk/micro/sssp/
Key features of SSSP – microbial spoilage models

Product characteristics

- Initial cell density (cfu/g): 10
- Temperature (°C): 1.8
- Percentage CO₂: 28
- Shelf-life (days): 8.3
- Growth rate (μmax, 1/h): 0.093

Shelf-life prediction for:

- Series of constant temperatures
- Temperature profiles from logger data
- Calculation of % CO₂

Remaining shelf-life (hours)

- Temperature (°C): 1.8
- Storage time (hours): 240

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Time (h)</th>
<th>0 °C (h)</th>
<th>5 °C (h)</th>
<th>10 °C (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>291.8</td>
<td>116.25</td>
<td>62.05</td>
</tr>
<tr>
<td>1.8</td>
<td>240</td>
<td>-59.79</td>
<td>-23.82</td>
<td>-12.71</td>
</tr>
</tbody>
</table>

Predicted microbial growth

- Microbial growth
- RSL at 0°C
- RSL at 5°C
- RSL at 10°C
SSSP predicts the effect of product temperature profiles:

- Series of constant temperatures
- Data recorded by temperature loggers

Http://www.difres.dk/micro/sssp/
SSSP help-menu:

- How to use SSSP (e.g. for time-temperature integration)
- Information about individual predictive models:
  - Model development and equations
  - Model evaluation and validation
  - Range of applicability (products, storage conditions)
- Data formats (import, export)
- Other predictive microbiology software
SSSP users

- SSSP has been available since January 1999

- SSSP is used by more than 3000 people/institutions from more than 100 different countries:
  - Production and distribution of seafood: 33 %
  - Seafood inspection: 19 %
  - Research: 17 %
  - Teaching: 13 %

- SSSP is free and available in 12 different languages
  - www.difres.dk/micro/sssp/
Seafood Spoilage and Safety Predictor (SSSP)

Outline:

- **Key features of SSSP v. 2 from 2005**
  - Flexible time-temperature integration tool for spoilage and shelf-life evaluation

- **Histamine formation in seafood**
  - *Morganella psychrotolerans* (0 - 20°C)
  - *M. psychrotolerans* and *M. morganii* (0 - 44°C)

- **Listeria monocytogenes** and lactic acid bacteria
  - New growth and growth boundary models

- **Conclusions and perspectives**
Histamine formation in seafood

- Histamine fish poisoning (HFP) occurs worldwide and is the most common finfish-borne human disease
- HFP is due to marine finfish with >500-1000 mg histamine/kg
- Specific bacteria produce histamine in seafood
  - At above 0°C: *Morganella psychrotolerans, Photobacterium phosphoreum*
  - At above 7-10°C: *Morganella morganii, Raoultella planticola*
- EU regulation: 100-200 mg histamine/kg

*Dalgaard et al. 2008*
Histamine formation in seafood

• Models for histamine formation have been available for years:
  - 21.1 – 37.8°C: Frank et al. (1983)
  - -1.1 – 15.6°C: Frank & Yoshinaga (1987)

• Models for bacterial growth and histamine formation are new

Emborg & Dalgaard 2007
SSSP v. 3 includes models for histamine formation in seafood

http://www.difres.dk/micro/sssp/
Histamine formation in seafood

### Chilled tuna

<table>
<thead>
<tr>
<th>Product characteristics and storage conditions</th>
<th>Product 1</th>
<th>Product 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Morganella psychrotolerans, cfu/g</em></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>2.00</td>
<td>4.40</td>
</tr>
<tr>
<td>Water phase salt in product, %</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>pH</td>
<td>5.90</td>
<td>5.90</td>
</tr>
<tr>
<td>% CO₂ in headspace gas equilibrium</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Storage period (Days)</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Initial concentration of histamine (ppm)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Initial concentration of histidine (ppm), Max. 10750 ppm</td>
<td>10750</td>
<td>10750</td>
</tr>
</tbody>
</table>

### Chilled cold-smoked tuna

<table>
<thead>
<tr>
<th>Product characteristics and storage conditions</th>
<th>Product 1</th>
<th>Product 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Morganella psychrotolerans, cfu/g</em></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Water phase salt in product, %</td>
<td>3.00</td>
<td>5.00</td>
</tr>
<tr>
<td>pH</td>
<td>5.90</td>
<td>5.90</td>
</tr>
<tr>
<td>% CO₂ in headspace gas equilibrium</td>
<td>0.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Storage period (Days)</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Initial concentration of histamine (ppm)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Initial concentration of histidine (ppm), Max. 10750 ppm</td>
<td>10750</td>
<td>10750</td>
</tr>
</tbody>
</table>
A combined *M. psychrotolerans* and *M. morganii* model allows growth and histamine formation to be evaluated for a wide range of temperatures.
Outline:

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  - Flexible time-temperature integration tool for spoilage and shelf-life evaluation
- **Histamine formation in seafood**
  - *Morganella psychrotolerans* (0 - 20°C)
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- **Listeria monocytogenes** and lactic acid bacteria
  - New growth and growth boundary models
- **Conclusions and perspectives**
EU-regulation (EC 2073/2005) distinguish between ready-to-eat foods able or unable to support growth of *Listeria monocytogenes*

<table>
<thead>
<tr>
<th>Ready-to-eat foods</th>
<th>Critical limit</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support growth</td>
<td>None in 25 g</td>
<td>- When produced</td>
</tr>
<tr>
<td>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>
</tbody>
</table>
| Unable to support growth | 100 cfu/g | - Documentation  
- pH ≤ 4,4 or aw ≤ 0,92  
- pH ≤ 5,0 and aw ≤ 0,94  
- Shelf-life below 5 days |
SSSP v. 3 includes extensive growth and growth boundary models for *Listeria monocytogenes* and lactic acid bacteria.

Mejlholm and Dalgaard (2007a,b) - *J. food Prot*, **70**, 70-84 and 2485-2497
### Product characteristics and storage conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage period (d)</td>
<td>30</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>5.0</td>
</tr>
<tr>
<td>NaCl in water phase (%)</td>
<td>2.5</td>
</tr>
<tr>
<td>pH</td>
<td>6.1</td>
</tr>
<tr>
<td>Lactic acid in water phase (ppm)</td>
<td>&lt; 30000</td>
</tr>
<tr>
<td>Smoke components/phenol (ppm)</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>% CO2 in headspace gas equilibrium</td>
<td>0</td>
</tr>
<tr>
<td>Diacetate in water phase of product, mg/L</td>
<td>&lt; 2000</td>
</tr>
</tbody>
</table>

### Predicted microbial growth

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>L. monocytogenes</th>
<th>LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial cell density (cfu/g)</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Include lag time</td>
<td>✔</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$μ_{max}$ (1/h)</td>
<td>0.0123</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Lag time (d)</td>
<td>10.54</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Time for 100-fold increase (d)</td>
<td>26.85</td>
<td>7.37</td>
<td></td>
</tr>
</tbody>
</table>

### Predicted growth of LAB (blue curve) and Listeria monocytogenes (red curve)

- **Lactic acid bacteria**: Log(cfu/g): 7.43
- **L. monocytogenes**: Log(cfu/g): 1.22

**Time (d): 20.16**
L. monocytogenes - LAB interaction model

**Product characteristics and storage conditions**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage period (d)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>8.0</td>
<td>2 - 15 °C</td>
</tr>
<tr>
<td>NaCl in water phase (%)</td>
<td>4.5</td>
<td>2.5 - 8</td>
</tr>
<tr>
<td>pH</td>
<td>6.1</td>
<td>5.8 - 7.0</td>
</tr>
<tr>
<td>Lactic acid in water phase (ppm)</td>
<td>3000</td>
<td>&lt; 30000</td>
</tr>
<tr>
<td>Smoke components/phenol (ppm)</td>
<td>8</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>% CO2 in headspace gas equilibrium</td>
<td>0</td>
<td>0 - 80</td>
</tr>
<tr>
<td>Diacetate in water phase of product. mg/l</td>
<td>0</td>
<td>&lt; 2000</td>
</tr>
</tbody>
</table>

**Predicted microbial growth**

<table>
<thead>
<tr>
<th></th>
<th>L. monocytogenes</th>
<th>LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial cell density (cfu/g)</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Include lag time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmax (1/h)</td>
<td>0.0251</td>
<td>0.049</td>
</tr>
<tr>
<td>Lag time (d)</td>
<td>5.13</td>
<td>0</td>
</tr>
<tr>
<td>Time for 100-fold increase (d)</td>
<td>12.98</td>
<td>3.91</td>
</tr>
</tbody>
</table>

**Predicted growth of LAB (blue curve) and Listeria monocytogenes (red curve)**

- **Lactic acid bacteria**
- **Listeria monocytogenes**

**Graph Details**

- **Time (d):** 20.05
- **Lactic acid bacteria, Log(cfu/g):** 8.5
- **L. monocytogenes, Log(cfu/g):** 2.48
L. monocytogenes - LAB interaction model

Product characteristics and storage conditions:
- Storage period (d): 30
- Temperature (°C): 8
- NaCl in water phase (%): 4.5
- pH: 6.0
- Lactic acid in water phase (ppm): 8000
- Smoke components/phenol (ppm): 8
- % CO2 in headspace gas equilibrium: 25
- Diacetate in water phase of product, mg/l: 1500

Predicted microbial growth:
- Initial cell density (cfu/g): L. monocytogenes: 1; LAB: 10
- μmax (1/h): L. monocytogenes: 0.0054; LAB: 0.0375
- Log time (d): L. monocytogenes: 24.06; LAB: 0
- Time for 100-fold increase (d): L. monocytogenes: Not reached; LAB: 5.12

Predicted growth of LAB (blue curve) and Listeria monocytogenes (red curve)

Storage time (days):
- Time (d): 20.05
- Lactic acid bacteria, Log(cfu/g): 8.34
- L. monocytogenes, Log(cfu/g): 0
Listeria monocytogenes growth boundary model

Product characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>6.0</td>
<td>2 - 15 °C</td>
</tr>
<tr>
<td>NaCl in water phase (%)</td>
<td>4.5</td>
<td>2.5 - 8</td>
</tr>
<tr>
<td>pH</td>
<td>6.0</td>
<td>5.8 - 7.0</td>
</tr>
<tr>
<td>Lactic acid in water phase (ppm)</td>
<td>6000</td>
<td>&lt; 30000</td>
</tr>
<tr>
<td>Smoke components/phenol (ppm)</td>
<td>8</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>% CO2 in headspace gas equilibrium</td>
<td>0</td>
<td>0 - 80</td>
</tr>
<tr>
<td>Diacetate in water phase of product, mg/l</td>
<td>0</td>
<td>&lt; 2000</td>
</tr>
<tr>
<td>Nitrite, mg/kg</td>
<td>0</td>
<td>&lt; 350</td>
</tr>
</tbody>
</table>

Select the variables to be plotted. The first parameter is fixed, the second two are variable.

- Temperature-Lactic Acid-Diacetate
- Temperature-Lactic Acid-Diaceatate
- pH-Lactic Acid-Diacetate
- pH-NaCl-Phenol
- pH-CO2-Temperature

Temperature (°C): 4.98  Lactic acid in water phase, mg/l: 7963.71  Diacetate in water phase, mg/l: 1524.41
Conclusions and perspectives

- SSSP has stimulated the use of predictive models and time-temperature integration within the seafood sector.

- SSSP v. 3 includes new seafood safety models for:
  - Time-temperature integration
  - Product development and quality control
  - Documentation of seafood safety

- Future developments of SSSP may include:
  - Product specific e-learning modules
  - Improved links to other freeware

www.difres.dk/micro/sssp/ - pad@aqua.dtu.dk
Thanks

- Colleagues at DTU Aqua
- Financial support
- Industry collaboration

Last but not least

I thank you for your attention