



HoA workshop: Tools supporting food chain safety assessments,  
BfR, 8-9 February 2016



## Food Spoilage and Safety Predictor (FSSP) software – background and applications

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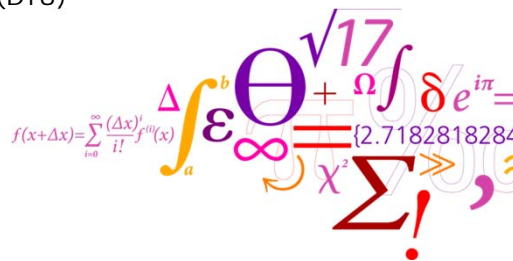
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National Food Institute

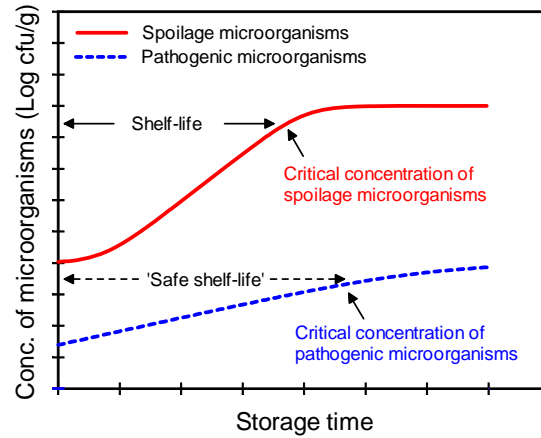
## Food Spoilage and Safety Predictor (FSSP) software – background and applications



### Outline

- Predictive microbiology - background
- Food Spoilage and Safety Predictor (FSSP) software
  - *Listeria monocytogenes* – an example
  - Help menu
  - Microbial interaction
  - Stochastic models
  - Documentation of food safety
- Time-temperature integration and food chain assessments
- Conclusions and perspectives

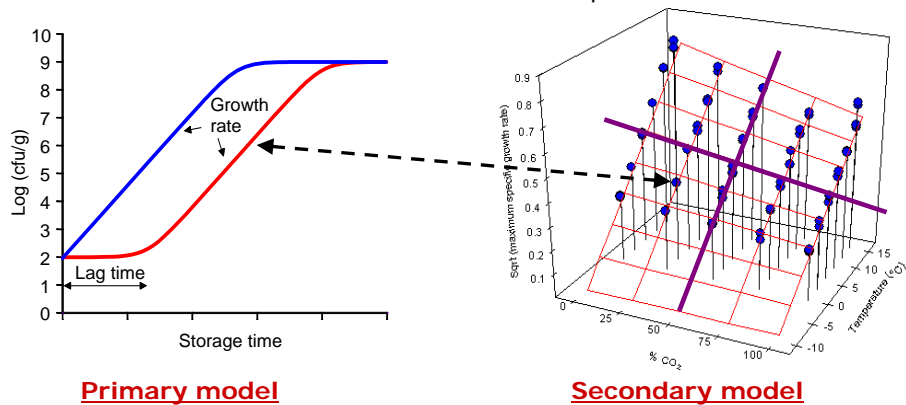
## Predictive mathematical modelling of growth



## Development of predictive microbiology models



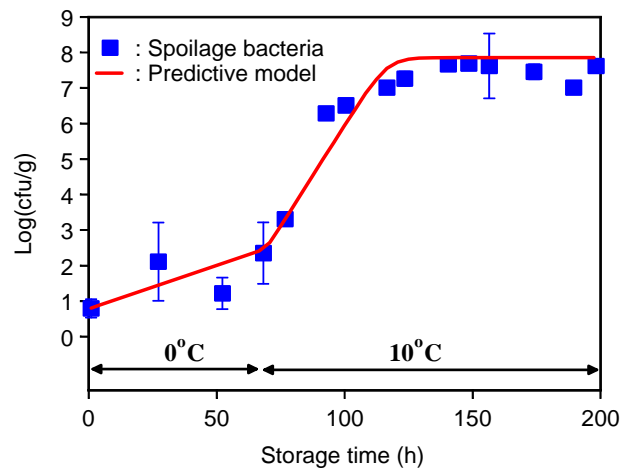
Models are usually developed in two steps from experiments including the effect of several environmental parameters



Models are included in software and can be used without detailed knowledge about equations and mathematics

## Evaluation/validation of growth models

- Predictive models must be evaluated by comparison of predictions with data from microorganism/food combinations of interest
- With good agreement between observed and predicted data the model is successfully validated



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## Application of successfully validated predictive microbiology models

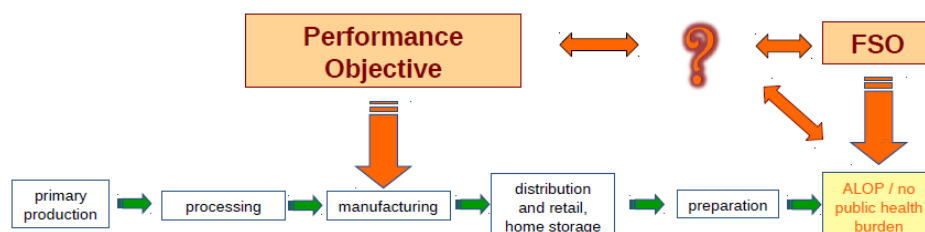
- Predict the effect of product characteristics and storage conditions on growth, survival or inactivation of microorganisms
  - Development or reformulation of products
- HACCP plans – establish limits for CCP
- Food safety objectives – equivalence of processes
- Education – easy access to information
- Quantitative microbiological risk assessment (QMRA)
  - The concentration of microbial hazards in foods may increase or decrease substantially (millions of folds) during processing and distribution

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McMeekin et al. (2006)

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## Application of successfully validated predictive microbiology models



$$PO \leq FSO - \Sigma I_{(distribution/consumer)} + \Sigma R_{(distribution/consumer)}$$

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# Food Spoilage and Safety Predictor (FSSP)



[HOME](#)

[FSSP FOR WINDOWS](#)  
Version 4.0 (July 2014)

[STOCHASTIC](#)  
FSSP models

[FSSP FOR OSX](#)  
Version 1.0 (December 2015)

[HELP](#)  
(English only)

[FSSP Installation guides](#)

The FSSP software is available free of charge:



[Download FSSP for Windows \(version 4.0\)](#)



[Download FSSP for OSX \(version 1.0\)](#)



[Download stochastic FSSP models](#)

The FSSP software has been developed to facilitate the practical use of mathematical models to predict growth of spoilage and pathogenic microorganisms in food. A major objective has been to develop a user-friendly software to predict the effect of constant or fluctuating temperature storage conditions on product shelf-life.

FSSP is a significantly expanded version of the Seafood Spoilage and Safety Predictor software which was first released in January 1999.

New features of FSSP from July 2014:

- Extensive models to predict the simultaneous growth of *Listeria monocytogenes* and lactic acid bacteria in chilled seafood, meat products and cottage cheese (FSSP for Windows).
- A generic model to predict growth of microorganisms in food depending on temperature, salt (NaCl/aw), pH, CO<sub>2</sub>, smoke intensity, nitrite and organic acids: Acetic/diacetate, benzoic, citric, lactic and sorbic acids (FSSP for Windows and OS X).

FSSP v. 4.0 includes:

- Four product-specific relative rate of spoilage (RRS) models
- Three generic RRS models
- Four product-specific microbial spoilage models

## FSSP news

[FSSP workshop with focus on \*Listeria monocytogenes\* - 28 October 2015 at DTU in Lyngby, Denmark \(in Danish\)](#)

[Predictive Food Microbiology course \(5 ECTS\) at DTU in January 2016](#)

## FSSP articles

[Article about the FSSP software in Eurofish magazine](#)

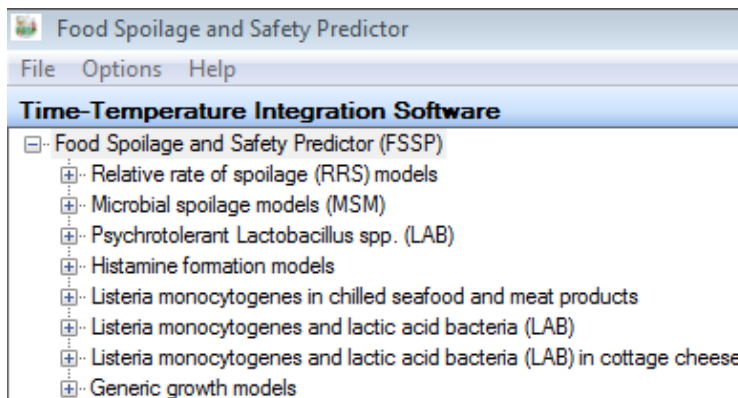
[Artikel om prædiktiv mikrobiologi og SSSP i "Fisk & Hav"](#)

## Links

[DTU Food](#)

[Home page of Paw Dalgaard](#)

<http://fssp.food.dtu.dk>

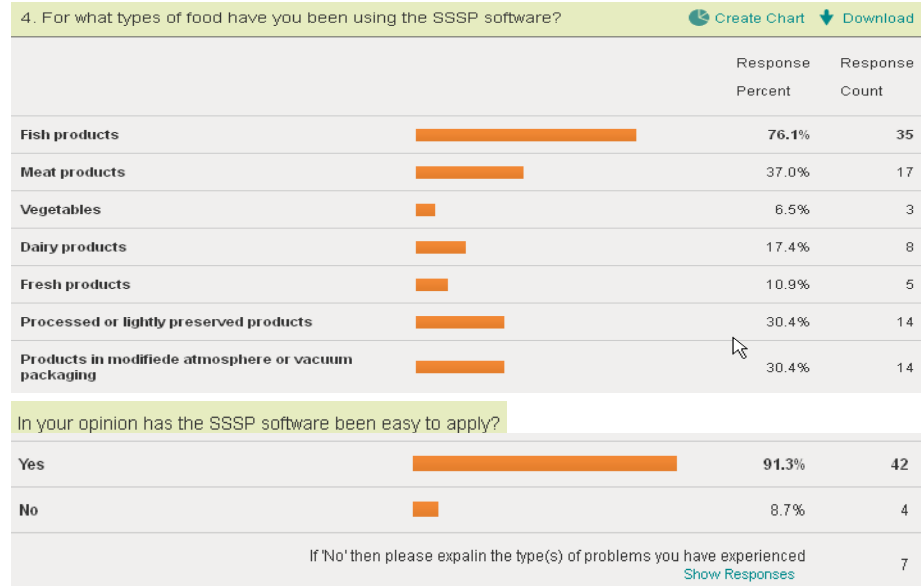


- Seafood, meat and dairy products
- Generic growth model
- Improved models for microbial interaction
- Stochastic models from FSSP homepage

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<http://fssp.food.dtu.dk>

## FSSP from 2014 is a new and expanded version of the Seafood Spoilage and Safety Predictor (SSSP) from 1999



## Software workshops worldwide (~ 1000 participants)

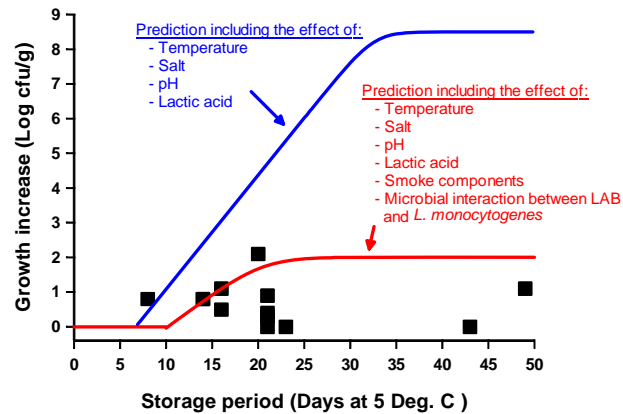


## Growth and growth boundary models



Complexity of food  $\leftrightarrow$  Complexity of predictive model

Example with *L. monocytogenes* in naturally contaminated vacuum packed cold-smoked salmon



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Dalgaard (2009) 13/40

## Growth and growth boundary models



Example for *L. monocytogenes*

- Temperature
- pH
- NaCl/water activity
- Smoke components (phenol)
- Nitrite
- CO<sub>2</sub>
- Acetic acid
- Benzoic acid
- Citric acid
- Diacetat
- Lactic acid
- Sorbic acid
- Interactions between all these factors

12 parameters

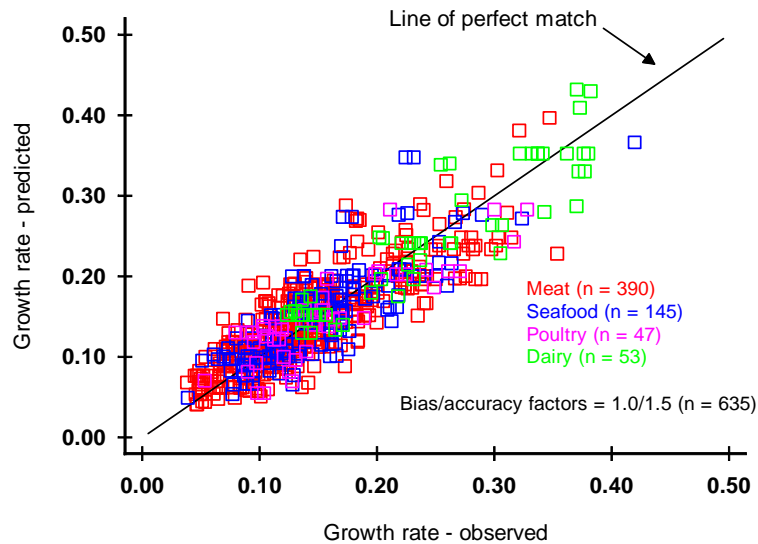


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Mejlholm & Dalgaard 2009, J. Food Prot. 72 (10), 2123-2143

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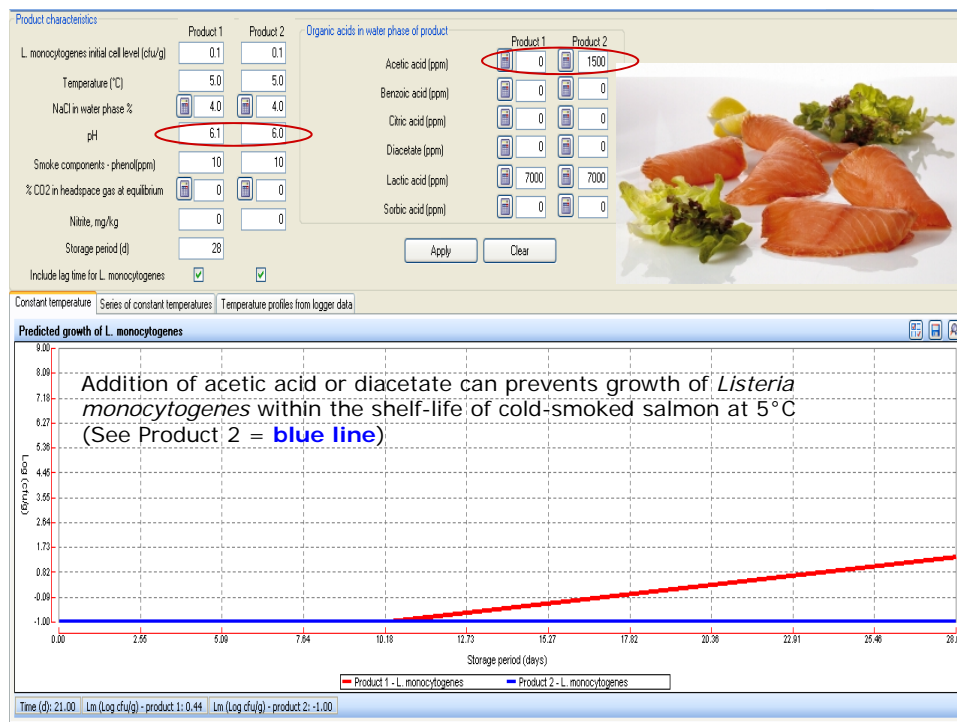
## Evaluation of predictive model in international validation study - Growth (n=707), No-growth (n=307)



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Mejlholm et al. 2010, Int. J. Food Microbiol. 141, 137-150

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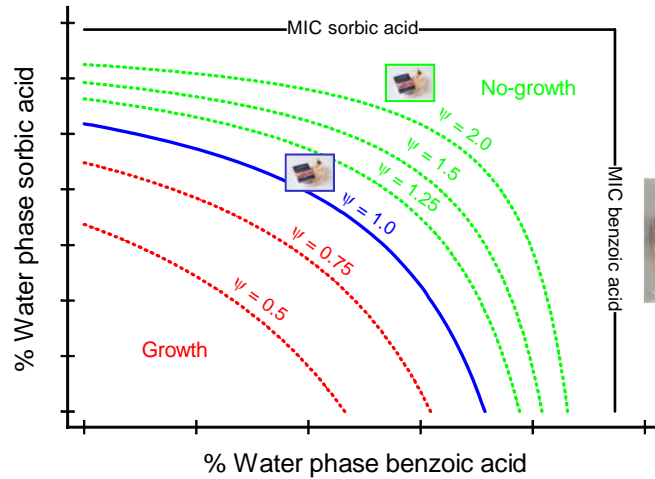




## Prediction of growth boundary

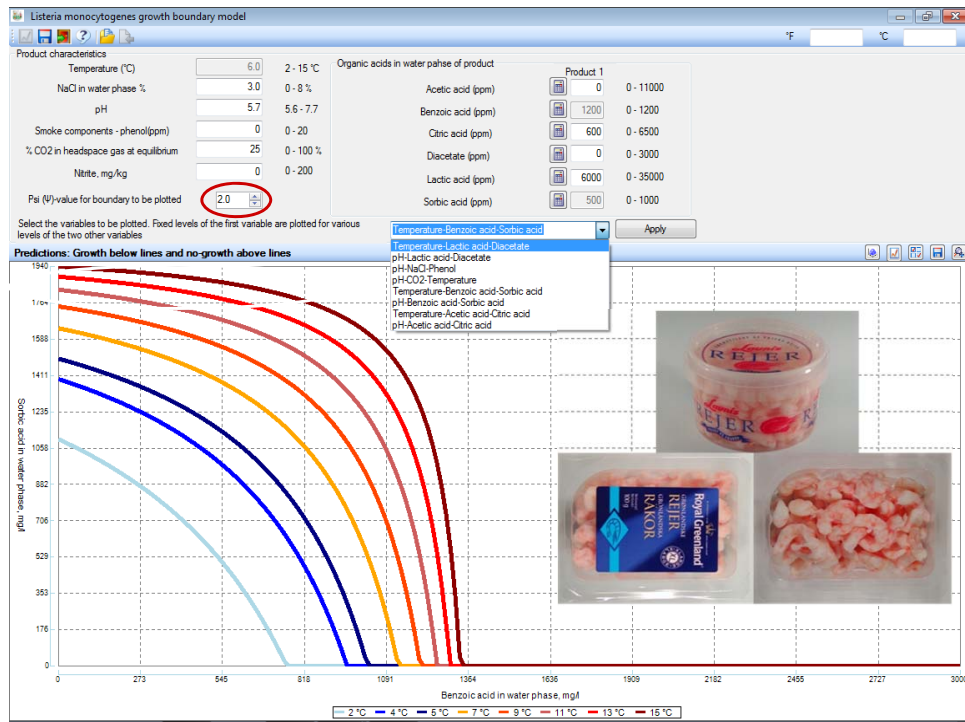


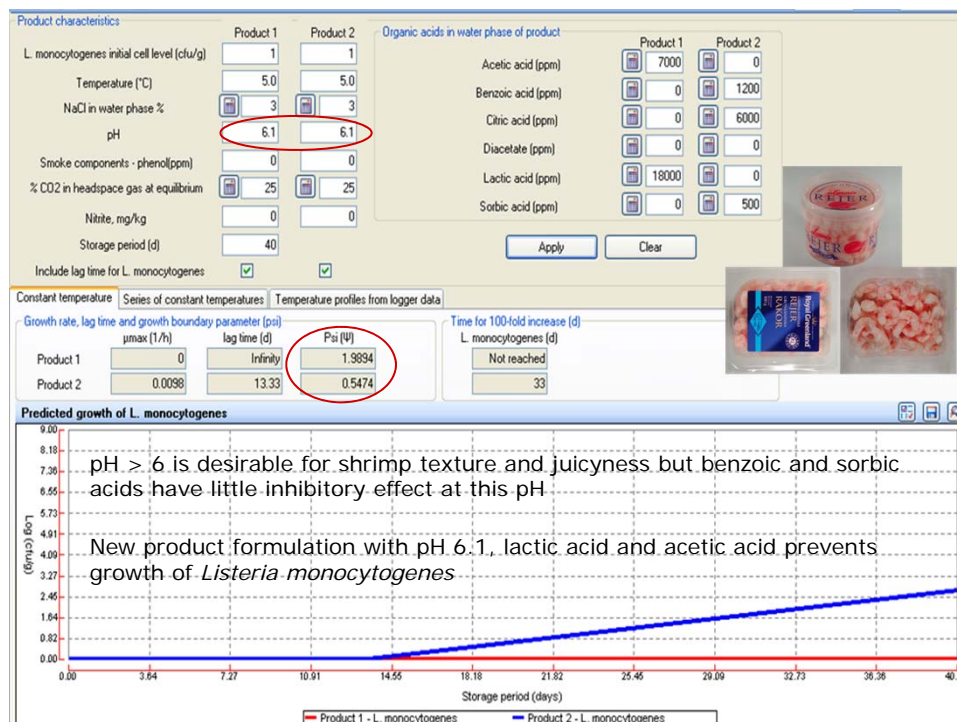
Software predicts combinations of product characteristics that prevent growth of *L. monocytogenes* in an appropriate distance from the growth boundary



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Mejlholm & Dalgaard (2009) 17/40





**FSSP includes and extensive help-menu with information on models and facilities in the software**



<b>Model</b>	<i>Listeria monocytogenes</i> and lactic acid bacteria (LAB) in lightly preserved seafood including ready-to-eat products
<b>References</b>	<p>Mejlholm, O. and Dalgaard, P. (2007b). Modeling and predicting the growth of lactic acid bacteria in lightly preserved seafood and their inhibiting effect on <i>Listeria monocytogenes</i>. <i>J. Food Prot.</i> 70 (11), 2485-2497.</p> <p>Mejlholm, O., Bøknæs, N., Dalgaard, P. (2014). Development and evaluation of a stochastic model for potential growth of <i>Listeria monocytogenes</i> in naturally contaminated lightly preserved seafood. <i>Food Microbiol.</i> <a href="http://dx.doi.org/10.1016/j.fm.2014.06.006">http://dx.doi.org/10.1016/j.fm.2014.06.006</a></p> <p>Mejlholm, O., Dalgaard, P. (2015). Modelling the simultaneous growth of <i>Listeria monocytogenes</i> and lactic acid bacteria in seafood and mayonnaise-based seafood salads. <i>Food Microbiol.</i> <a href="http://dx.doi.org/10.1016/j.fm.2014.07.005">http://dx.doi.org/10.1016/j.fm.2014.07.005</a></p>
<b>Primary growth model</b>	Logistic model with delay and including interaction between <i>Listeria monocytogenes</i> and LAB (Giménez and Dalgaard, 2004)
<b>Secondary growth model</b>	Simplified cardinal parameter type model
<b>Environmental parameters in model</b>	Temperature, atmosphere (CO <sub>2</sub> ), water phase salt/aw, pH, smoke components/phenol, nitrite and organic acids in water phase of product (acetic acids, benzoic acid, citric acid, diacetate, lactic acid and sorbic acids)
<b>Product validation studies</b>	Cold-smoked and marinated (including 'gravad') salmon, Greenland halibut and trout (Mejlholm & Dalgaard 2007b). Brined shrimp and mayonnaise-based seafood salads (Mejlholm and Dalgaard, 2015). Importantly, this model has been shown to accurately predict the simultaneous growth of <i>L. monocytogenes</i> and lactic acid bacteria in naturally contaminated cold-smoked salmon and naturally contaminated cold-smoked Greenland halibut (Mejlholm et al. 2014).
<b>Range of applicability</b>	<p>Temperature (2-25°C), atmosphere (0-100 % CO<sub>2</sub>), water phase salt (0.7-9.0 %), pH (5.6-7.7), smoke components/phenol (0-20 ppm), nitrite (0-150 ppm in product), acetic acid (0-11000 ppm in water phase), benzoic acid (0-1800 ppm in water phase), citric acid (0-6500 ppm in water phase), diacetate (0-3000 ppm in water phase), lactic acid (0-60000 ppm in water phase) and sorbic acid (0-1300 in water phase).</p> <p>For mayonnaise-based seafood salads the model included in FSSP should not be used for products with pH below 6.0 (Mejlholm and Dalgaard, 2015).</p>



## Food Spoilage and Safety Predictor (FSSP)



**Calculator**

Dry matter, %

NaCl in product, %

Water phase salt in product, %

**Calculator**

Temperature (°C)

Initial gas/product ratio

Initial %CO<sub>2</sub> in headspace gas

% CO<sub>2</sub> in headspace at equilibrium

**Calculator**

Dry matter, %

Acetic acid and acetate in product, %

OR

Sodiumacetate in product, %

Water phase acetic acid and acetate, %

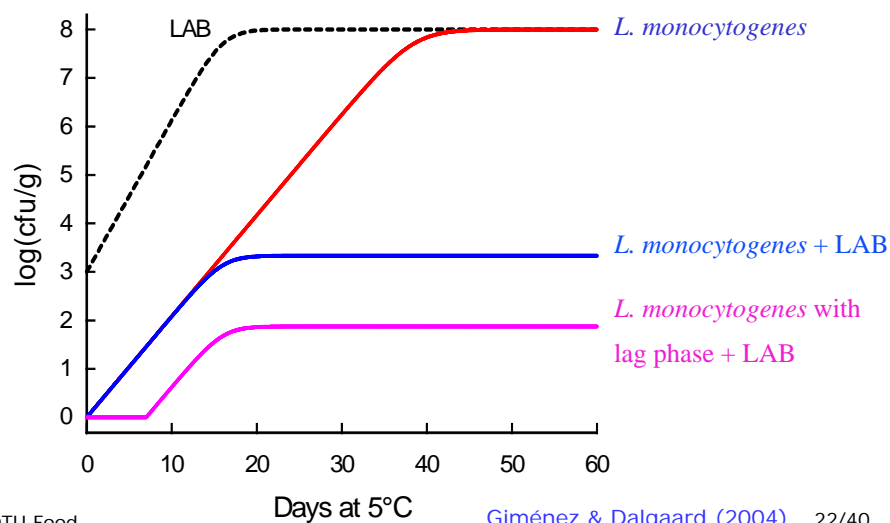
Water phase sodiumacetate, %

Acetic acid in water phase of product, mg/l

Build-in FSSP calculators facilitates determination of relevant water-phase concentrations of NaCl, CO<sub>2</sub> and organic acids

## Primary models for microbial interaction

Models are available to predict the inhibiting effect of high concentrations of lactic acid bacteria (LAB) on growth of *Listeria monocytogenes*





## *L. monocytogenes* - evaluation of growth model

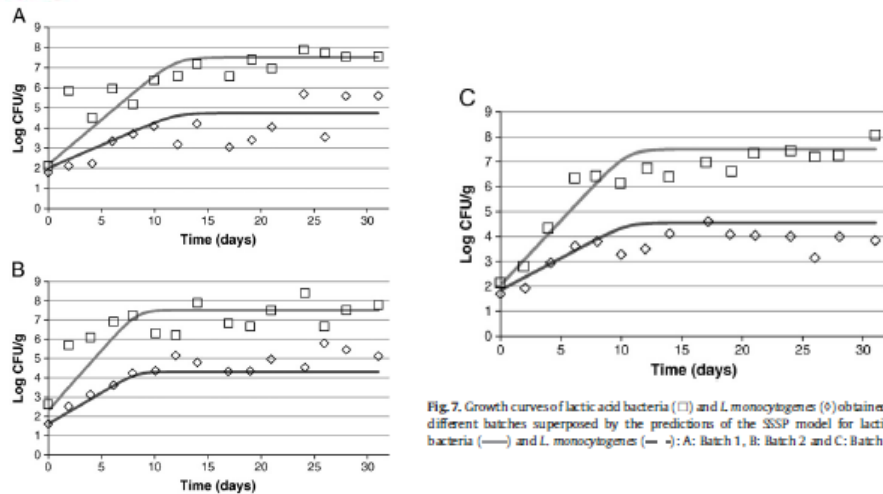
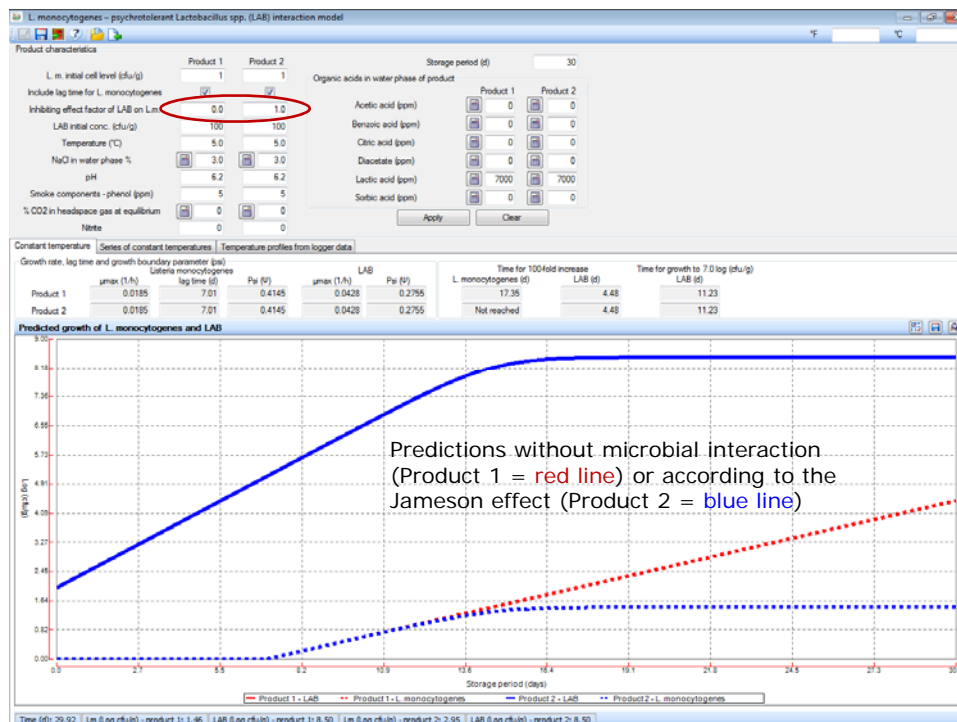


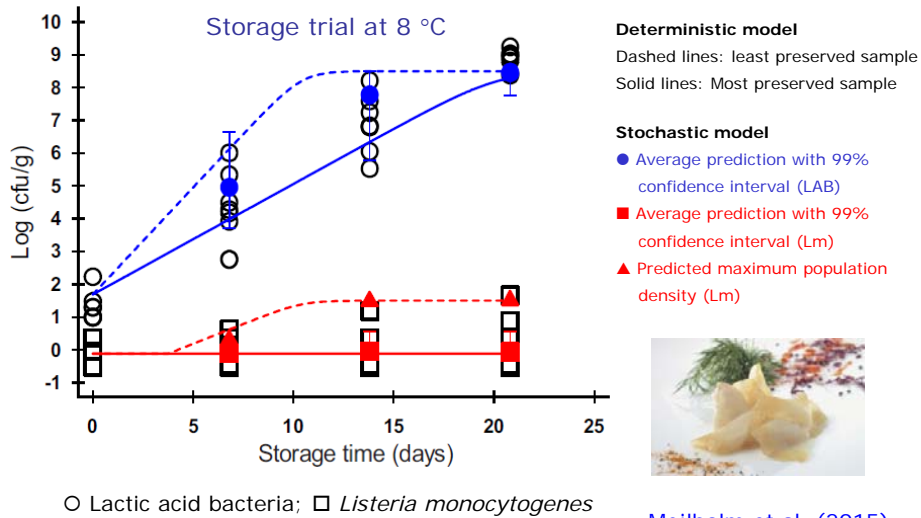
Fig. 7. Growth curves of lactic acid bacteria (□) and *L. monocytogenes* (○) obtained from different batches superposed by the predictions of the FSSP model for lactic acid bacteria (—) and *L. monocytogenes* (---): A: Batch 1, B: Batch 2 and C: Batch 3.

Challenge tests are not needed for cold-smoked salmon as the FSSP-software and the Mejlholm & Dalgaard model provided the same results



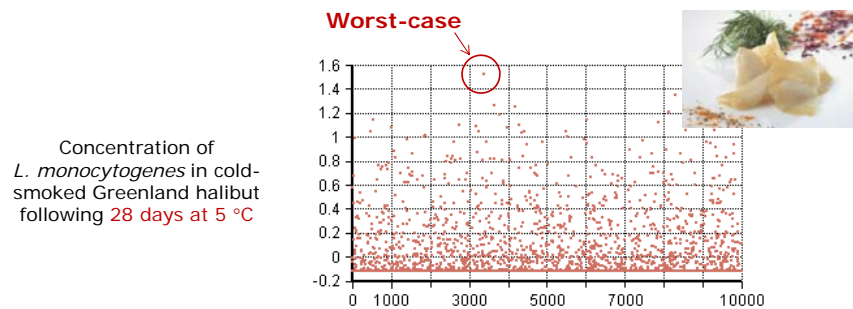
## Durability study and evaluation of stochastic lactic acid bacteria - *L. monocytogenes* interaction model

Naturally contaminated cold-smoked Greenland halibut  
with added acetic and lactic acids



## Effect of variability in product characteristics

- Cold-smoked Greenland halibut **added** acetic and lactic acids



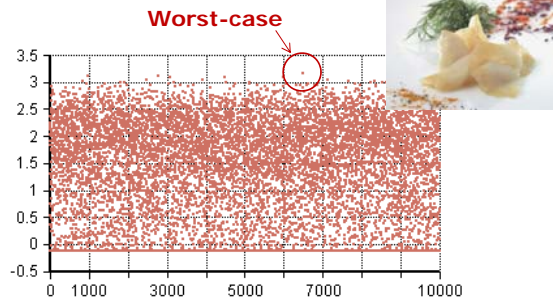
- Predicted maximum cell concentration of *L. monocytogenes*: 1.5 log (cfu/g)
  - In compliance with the EU-regulation
  - 99.9% of the repetitions were lower than 1.1 log (cfu/g)

## Effect of variability in product characteristics



- Cold-smoked Greenland halibut **without added** acetic and lactic acids

Concentration of  
*L. monocytogenes* in cold-smoked Greenland halibut following **28 days at 5 °C**



- Predicted maximum cell concentration of *L. monocytogenes*: **3.2 log (cfu/g)**
  - Not in compliance with the EU-regulation
  - 31% of the repetitions were higher than 2.0 log (cfu/g)

## Food Spoilage and Safety Predictor (FSSP) software – background and applications



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## ***Listeria monocytogenes* - microbiological criteria**



EU regulation (EC 2073/2005 and EC 1441/2007)

Ready-to-eat foods	Critical limits	Comments
Intended for infants or special medical purposes (Cat. 1.1)	Absence in 10 x 25 g	- Product placed on the market during their shelf-life
Support growth (Cat. 1.2)	100 cfu/g	- It must be <u>documented</u> that 100 cfu/g is not exceeded within the storage period
	Absence in 5 x 25 g	- When produced
Unable to support growth (Cat. 1.3)*	100 cfu/g	- <u>Documentation</u> - pH ≤ 4,4 or a <sub>w</sub> ≤ 0,92 - pH ≤ 5,0 and a <sub>w</sub> ≤ 0,94 - Shelf-life below 5 days

\* Growth should not exceed 0.5 log (cfu/g) ~ 3 cfu/g during the shelf-life

## **EU regulation (EC 2073/2005 – Annex II):**



**Documentation shall include specifications for:**

- Physico-chemical product characteristics
- Storage and processing conditions
- Contamination and foreseen shelf-life

**When necessary additional studies may include:**

- Predictive mathematical modelling for food in question
- Tests to investigate the ability to grow or survive

**Studies shall take into account variability for products, microorganisms, processing and storage conditions**

- 
- Predictive food microbiology models
  - Challenge testing - inoculated
  - Durability studies – naturally contaminated

# Listeria monocytogenes

Sådan vurderer du, om *L. monocytogenes* kan vokse i spiseklare og letkonserverede fiskeprodukter – brug edb-modeller



## I praksis

- Tag 1 filet fra 5 forskellige produktionsdage.
- 5 cm fra forreste ende af hver filet udtages 100 g fiskekød, som blendes.
- Brug det blandede fiskekød til at bestemme produktets egenskaber (pH, NaCl i vandfasen, røgkomponenter målt som phenol, tørstof og organiske syrer).
- Brug Seafood Spoilage and Safety Predictor (SSSP) programmet til at bestemme vækst af *L. monocytogenes* ud fra produktens egenskaber for hver af de 5 datasæt.
- Find det datasæt med mest vækst. Dette datasæt afgør, om produktet placeres i kategori 1.3 eller 1.2 i Mikrobiologiforordningen.
- Punkterne bør gentages mindst én gang om året og ved hver ændring i fremstillingsprocessen.

[http://www.foedevarestyrelsen.dk/SiteCollectionDocuments/25\\_PDF\\_word\\_filer%20til%20download/04kontor/Mikro%20zoonose/Listeria/Listeria%20fisk%20end.pdf](http://www.foedevarestyrelsen.dk/SiteCollectionDocuments/25_PDF_word_filer%20til%20download/04kontor/Mikro%20zoonose/Listeria/Listeria%20fisk%20end.pdf)

## Documentation of control for growth of *Listeria monocytogenes* in RTE seafood



- Use of FSSP software has been adapted by the Danish Veterinary and Food Administration
- Detailed characteristics are determined for samples from five independent production batches: pH, NaCl, dry matter, food preservatives (including naturally occurring lactate) and smoke components as relevant
- Predictions at relevant temperatures for each samples using the Mejlholm & Dalgaard (2009) model with lag phase for *L. monocytogenes*

[http://www.foedevarestyrelsen.dk/SiteCollectionDocuments/25\\_PDF\\_word\\_filer%20til%20download/04kontor/Mikro%20zoonose/Listeria/Listeria%20fisk%20end.pdf](http://www.foedevarestyrelsen.dk/SiteCollectionDocuments/25_PDF_word_filer%20til%20download/04kontor/Mikro%20zoonose/Listeria/Listeria%20fisk%20end.pdf)  
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## Documentation of control for growth of *Listeria monocytogenes* in RTE seafood



Batch	Product characteristics Values for five different fillets and average $\pm$ standard deviation					
	I	II	III	IV	V	Average $\pm$ Standard deviation
Salt in water phase of the product, %	4.7	3.4	4.2	4.7	4.0	4.19 $\pm$ 0.55
pH	6.16	6.22	6.17	6.16	6.22	6.19 $\pm$ 0.03
Acetic acid in water phase of the product, mg/L	2772	3617	2801	3359	3117	3133 $\pm$ 402
Lactic acid in water phase of the product, mg/L	7704	7349	7581	7430	6425	7298 $\pm$ 506
Phenol (Smoke intensity), mg/kg	11.8	11.5	10.4	10.8	8.8	10.7 $\pm$ 1.2
<b>Predicted growth responses at 5°C:</b>						
Lag time (days)	>90	>90	>90	>90	>50	>90
Growth rate (1/h)	<0.001	<0.001	<0.001	<0.001	<0.0025	<0.001
Increase during 28 days, cfu/g	0	0	0	0	0	0

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## SCIENTIFIC REPORT OF EFSA

### Scientific and technical assistance on the evaluation of the temperature to be applied to pre-packed fishery products at retail level<sup>1</sup>

European Food Safety Authority<sup>2,3</sup>

European Food Safety Authority (EFSA), Parma, Italy

- Pre-packed fresh fishery products can be stored at refrigeration temperatures above 0 °C (e.g. 3–5 °C) and be compliant with the current EU and international rules.
- Examples of combinations of product durability (maximum shelf-life) and packaging atmosphere that should enable compliance with the safety criteria for various storage temperatures at retail are provided.

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

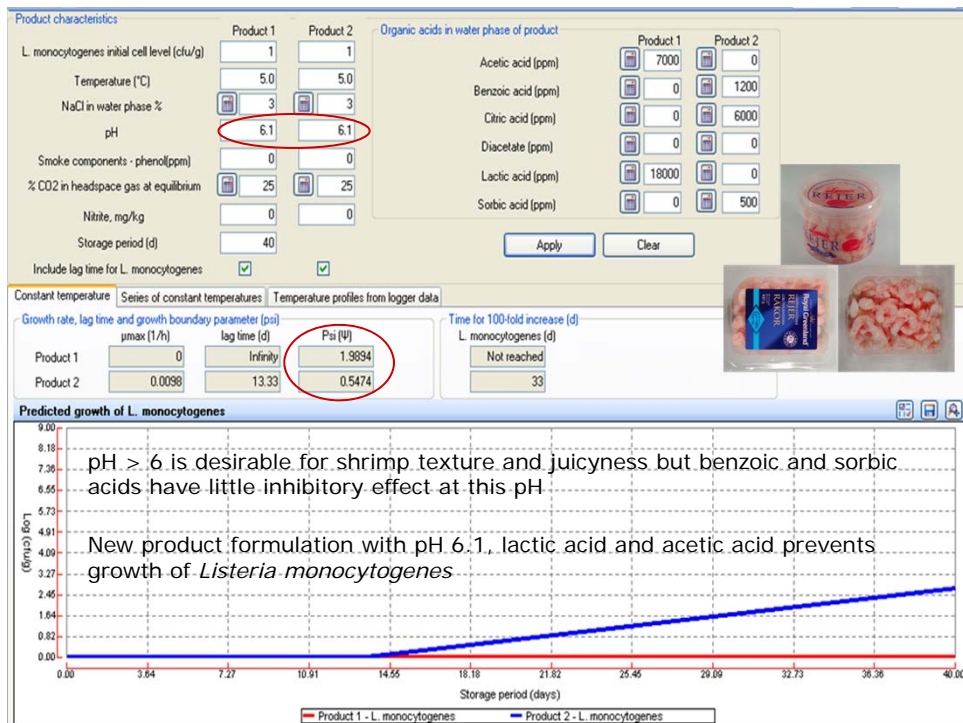


- A challenge for handling of temperature data



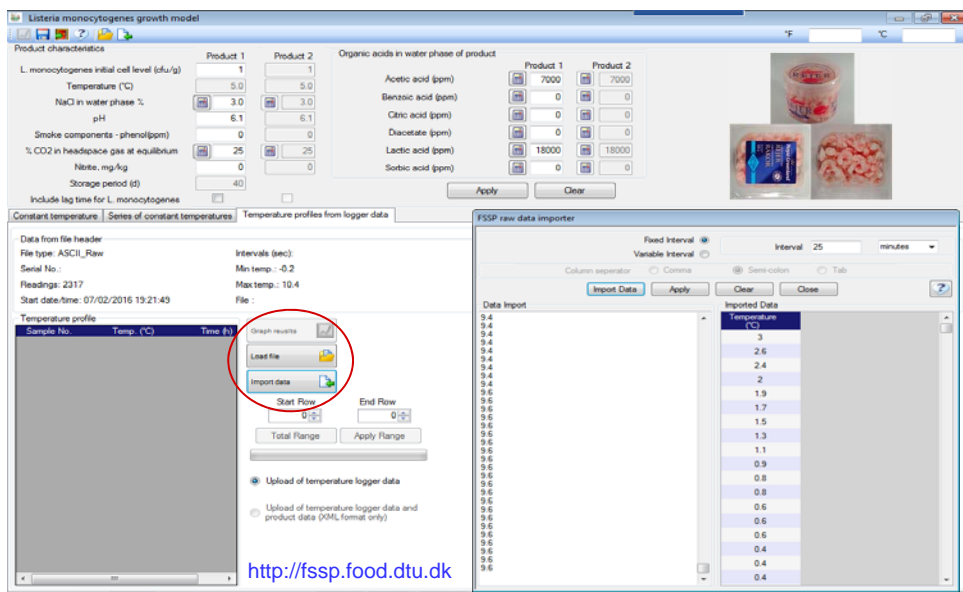
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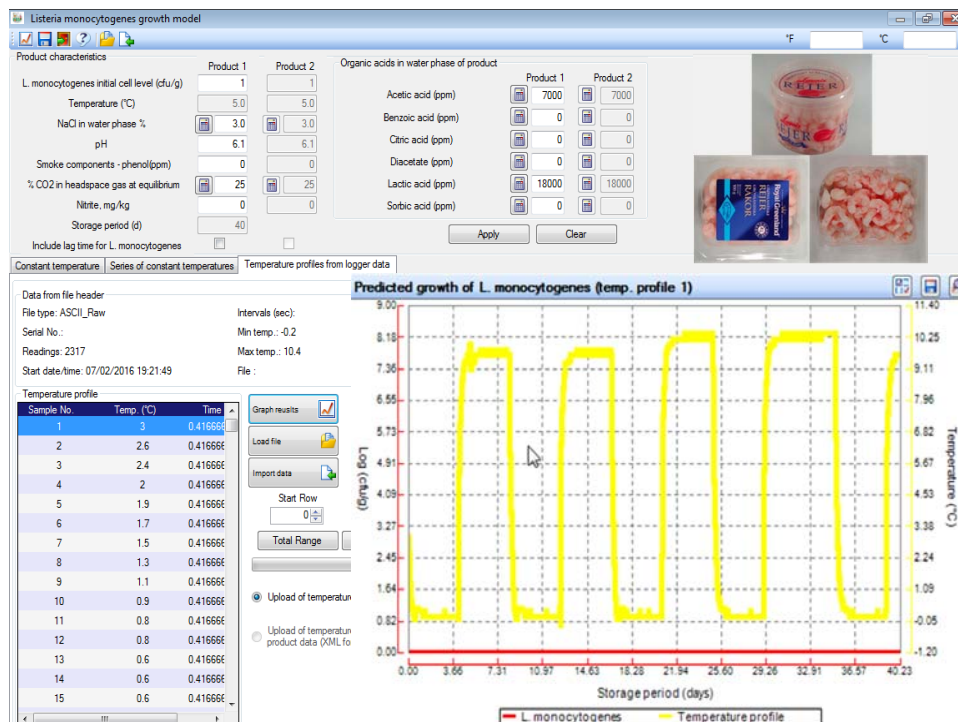
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## Time-temperature integration

To facilitate evaluation of product temperature profiles FSSP includes a module to import data (copy/paste) from spreadsheets





## Food Spoilage and Safety Predictor (FSSP) software – background and applications



### Conclusions and perspectives

- Complex predictive food microbiology models are often needed for assessment and documentation of food safety
- FSSP facilitate the practical application of validated predictive microbiology models with relevant complexity
- New research and further training is needed to help industry and authorities benefit from predictive microbiology models
- Increased collaboration between industry, authorities and academia is needed to accelerate progress