Predictive microbiology for the dairy industry

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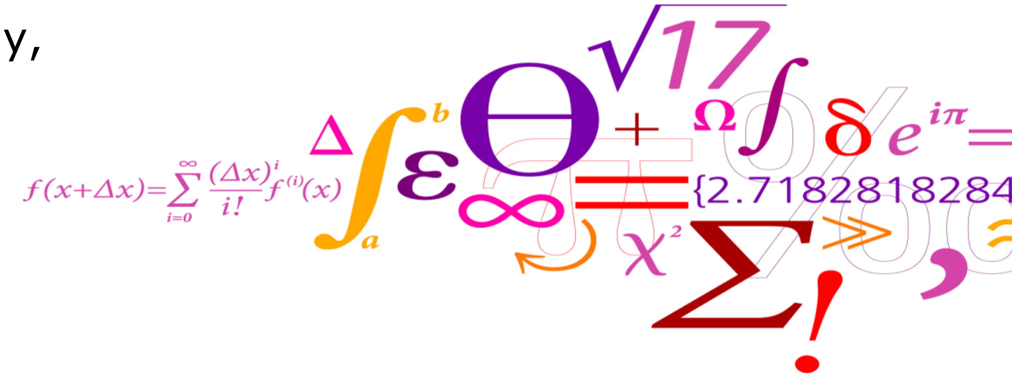

DTU Food
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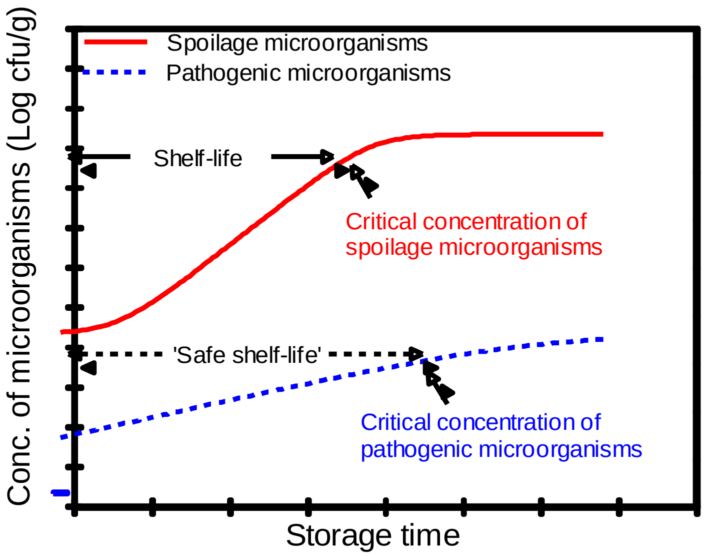
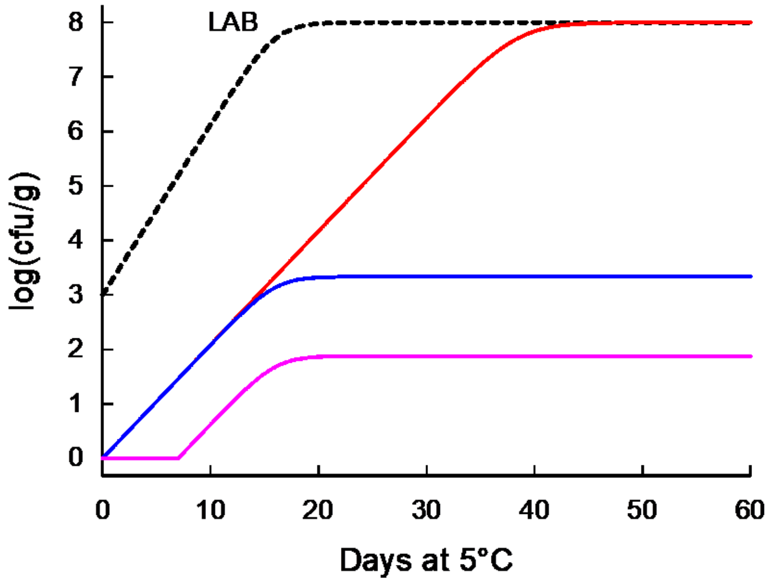
Outline

- Application of predictive food microbiology
- Evaluation of models and software
- New cardinal parameters and evaluation
- Examples of application for dairy products
- Conclusions and perspectives

Predictive microbiology - concept





Processing

Product characteristics

Storage conditions

Predictive models software

Safe shelf-life

Optimal product recipes

Spoilage and shelf-life

Growth of Listeria monocytogenes in cottage cheese in combination with LAB (starter or aroma culture)

°F °C

Product characteristics

	Product 1	Product 2
Listeria monocytogenes, cfu/g	1	1
Lactic acid bacteria, cfu/g	5000000	5000000
LAB aroma culture used	☑	☑
Worst case relative lag time	☐	☐
Storage period (d)	25	

	Product 1	Product 2
Temperature (°C)	7.0	7.0
Salt in water phase of product, %	1.0	1.0
Initial pH	5.47	5.20
Lactic acid in water phase of product, mg/l	450	1700
Sorbic acid in waterphase of product, mg/l	0	0

Apply Clear

Constant temperature | Series of constant temperatures | Temperature profiles from logger data

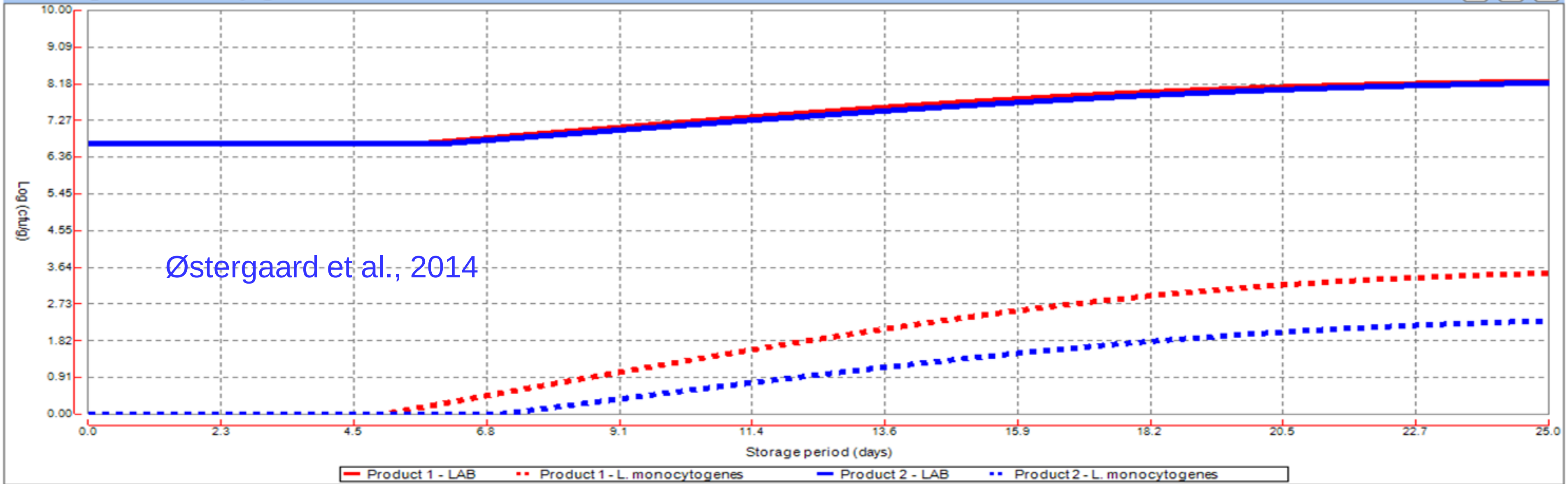
Listeria monocytogenes

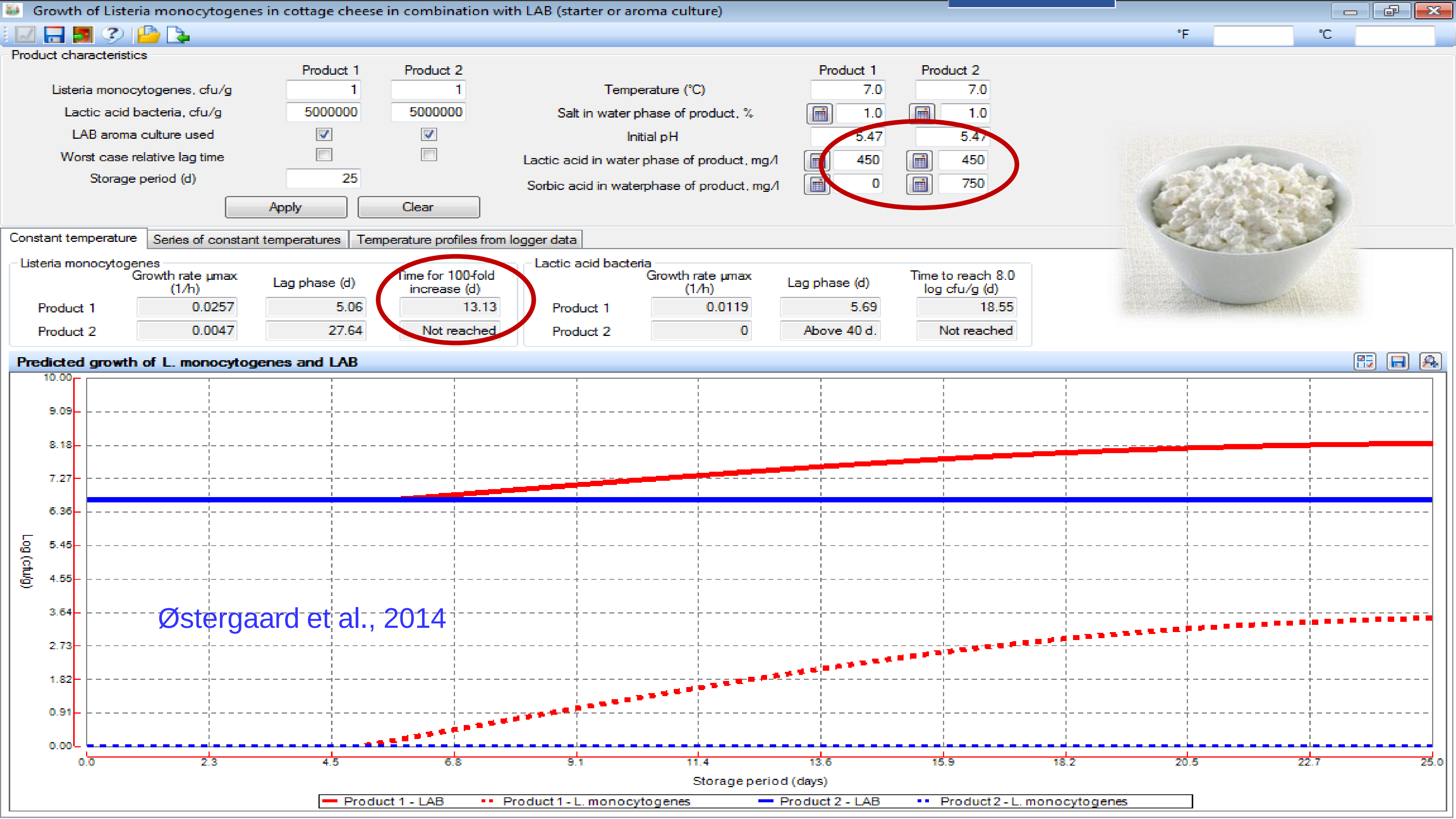
	Growth rate µmax (1/h)	Lag phase (d)	Time for 100-fold increase (d)
Product 1	0.0257	5.06	13.13
Product 2	0.0184	7.05	20.08

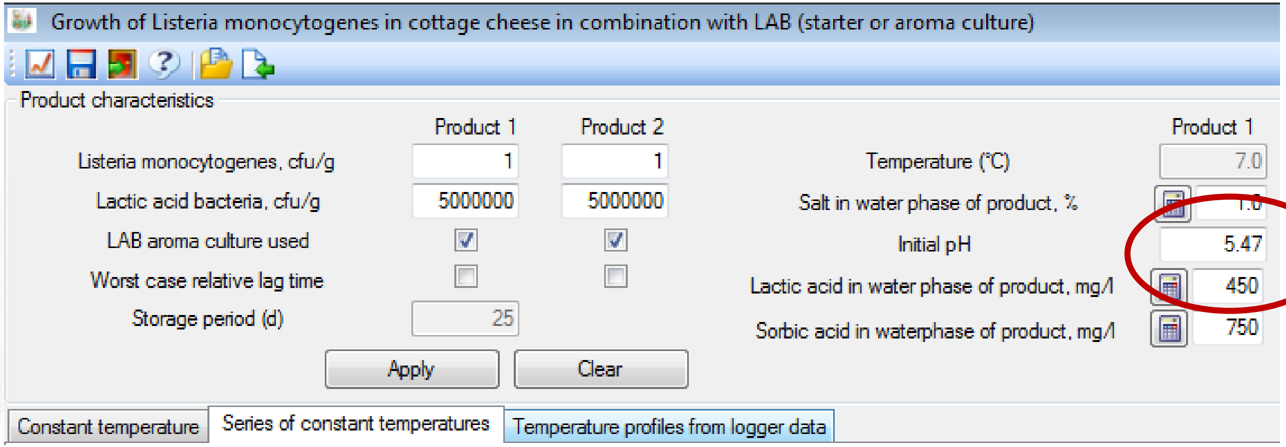
Lactic acid bacteria

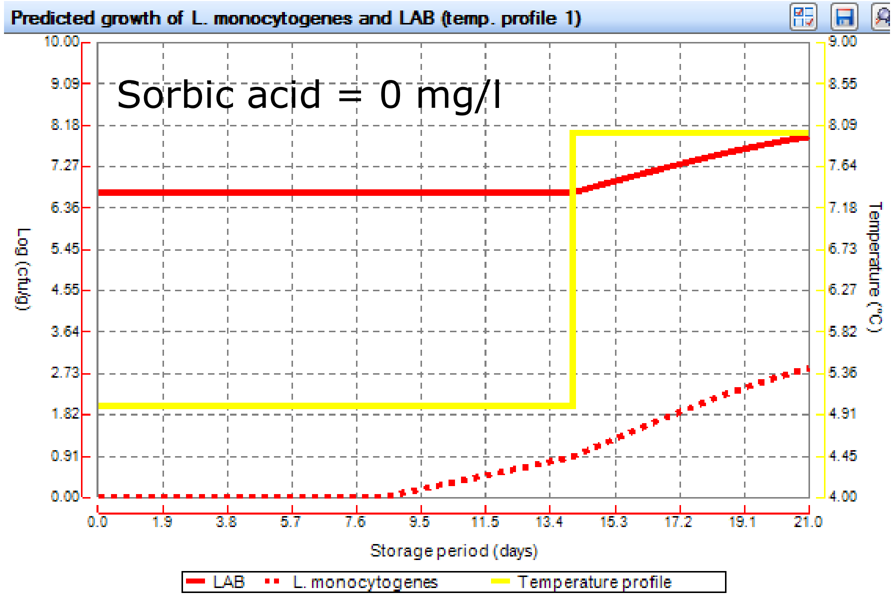
	Growth rate µmax (1/h)	Lag phase (d)	Time to reach 8.0 log cfu/g (d)
Product 1	0.0119	5.69	18.55
Product 2	0.0111	6.07	19.8

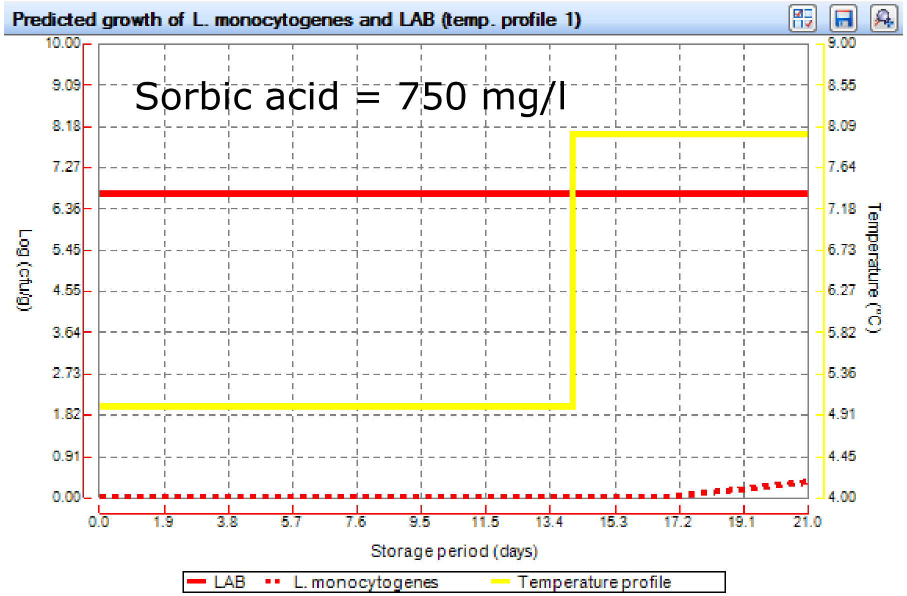
Predicted growth of L. monocytogenes and LAB




Growth of Listeria monocytogenes in cottage cheese in combination with LAB (starter or aroma culture)
°F
°C
Product characteristics
Product 1
Product 2
Listeria monocytogenes, cfu/g 1 1
Lactic acid bacteria, cfu/g 5000000 5000000
LAB aroma culture used
Worst case relative lag time
Storage period (d) 25
Apply
Clear
Temperature (°C) 7.0 7.0
Salt in water phase of product, % 1.0 1.0
Initial pH 5.47 5.47
Lactic acid in water phase of product, mg/l 450 450
Sorbic acid in waterphase of product, mg/l 0 750
Constant temperature
Series of constant temperatures
Temperature profiles from logger data
Listeria monocytogenes
Growth rate μmax (1/h)
Lag phase (d)
Time for 100-fold increase (d)
Product 1 0.0257 5.06 13.13
Product 2 0.0047 27.64 Not reached
Lactic acid bacteria
Growth rate μmax (1/h)
Lag phase (d)
Time to reach 8.0 log cfu/g (d)
Product 1 0.0119 5.69 18.55
Product 2 0 Above 40 d. Not reached
Predicted growth of L. monocytogenes and LAB
Log (cfu/g)
10.00
9.09
8.18
7.27
6.36
5.45
4.55
3.64
2.73
1.82
0.91
0.00
Østergaard et al., 2014
0.0
2.3
4.5
6.8
9.1
11.4
13.6
15.9
18.2
20.5
22.7
25.0
Storage period (days)
Product 1 - LAB
Product 1 - L. monocytogenes
Product 2 - LAB
Product 2 - L. monocytogenes


Growth of Listeria monocytogenes in cottage cheese in combination with LAB (starter or aroma culture)
Product characteristics
Product 1
Product 2
Listeria monocytogenes, cfu/g
1
1
Lactic acid bacteria, cfu/g
5000000
5000000
LAB aroma culture used
Worst case relative lag time
Storage period (d)
25
Apply
Clear
Product 1
Temperature (°C)
7.0
Salt in water phase of product, %
Initial pH
5.47
Lactic acid in water phase of product, mg/l
450
Sorbic acid in waterphase of product, mg/l
750
Constant temperature
Series of constant temperatures
Temperature profiles from logger data

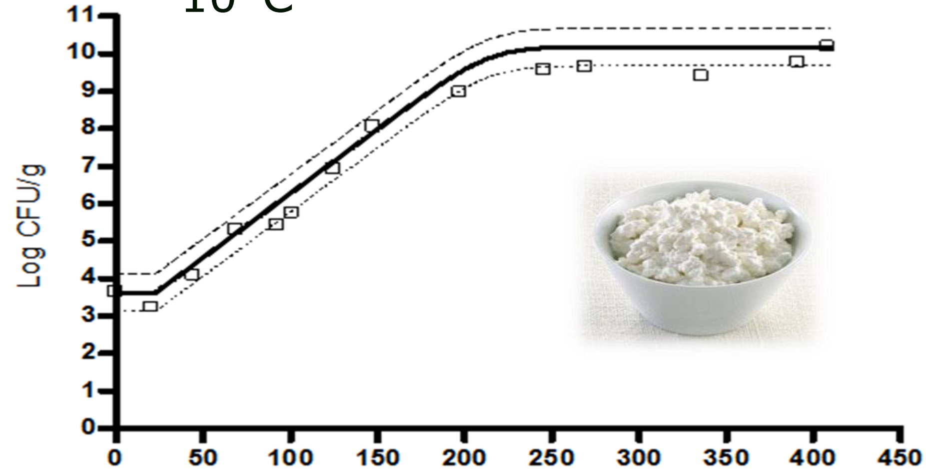

Predicted growth of L. monocytogenes and LAB (temp. profile 1)
Sorbic acid = 0 mg/l
Log (cfu/g)
Temperature (°C)
Storage period (days)
LAB
L. monocytogenes
Temperature profile

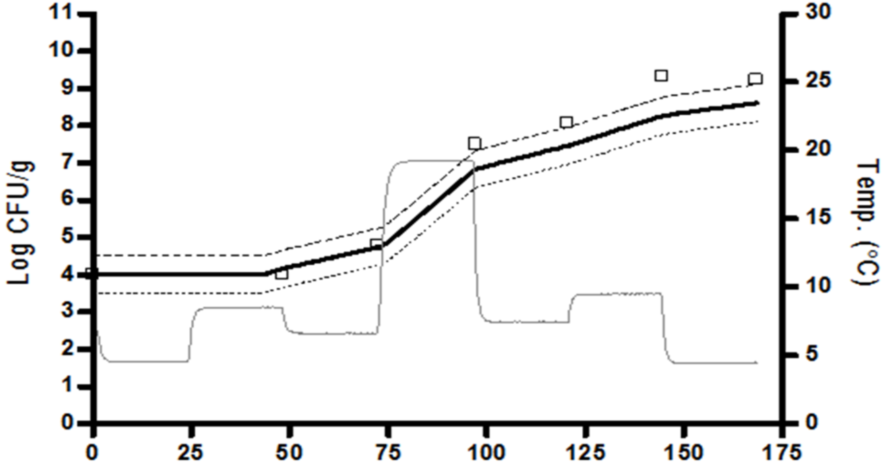

Predicted growth of L. monocytogenes and LAB (temp. profile 1)
Sorbic acid = 750 mg/l
Log (cfu/g)
Temperature (°C)
Storage period (days)
LAB
L. monocytogenes
Temperature profile

Pseudomonas – Validation of growth model

Predictions are compared with growth measured in products at constant and varying storage temperatures

10°C

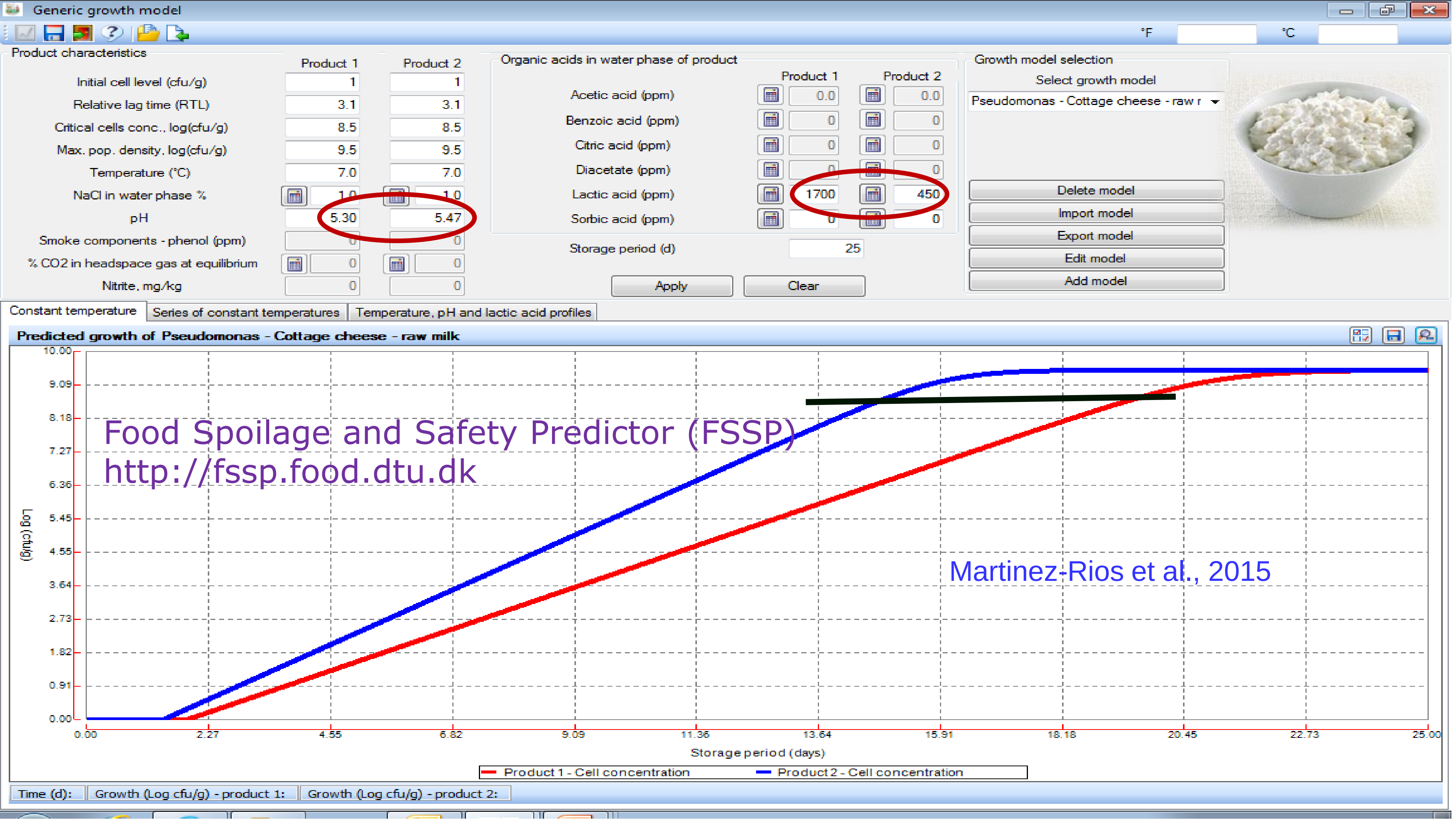




Storage time (hours)

Predicted and observed growth of psychrotolerant *Pseudomonas* in cottage cheese with dressing with aroma culture

Martinez-Rios et al., 2015


Generic growth model
°F
°C
Product characteristics
Product 1
Product 2
Initial cell level (cfu/g) 1 1
Relative lag time (RTL) 3.1 3.1
Critical cells conc., log(cfu/g) 8.5 8.5
Max. pop. density, log(cfu/g) 9.5 9.5
Temperature (°C) 7.0 7.0
NaCl in water phase % 1.0 1.0
pH 5.30 5.47
Smoke components - phenol (ppm) 0 0
% CO2 in headspace gas at equilibrium 0 0
Nitrite, mg/kg 0 0
Organic acids in water phase of product
Product 1
Product 2
Acetic acid (ppm) 0.0 0.0
Benzoic acid (ppm) 0 0
Citric acid (ppm) 0 0
Diacetate (ppm) 0 0
Lactic acid (ppm) 1700 450
Sorbic acid (ppm) 0 0
Storage period (d) 25
Apply
Clear
Growth model selection
Select growth model
Pseudomonas - Cottage cheese - raw r
Delete model
Import model
Export model
Edit model
Add model
Constant temperature
Series of constant temperatures
Temperature, pH and lactic acid profiles
Predicted growth of Pseudomonas - Cottage cheese - raw milk
Food Spoilage and Safety Predictor (FSSP)
http://fssp.food.dtu.dk
Martinez-Rios et al., 2015
Log (cfu/g)
10.00
9.09
8.18
7.27
6.36
5.45
4.55
3.64
2.73
1.82
0.91
0.00
0.00
2.27
4.55
6.82
9.09
11.36
13.64
15.91
18.18
20.45
22.73
25.00
Storage period (days)
Product 1 - Cell concentration
Product 2 - Cell concentration
Time (d):
Growth (Log cfu/g) - product 1:
Growth (Log cfu/g) - product 2:

EU regulation (EC 2073/2005) and documentation

- Predictive mathematical modelling can be used to support documentation of compliance with microbiological criteria when based on physico-chemical product characteristics; storage and processing conditions, contamination and foreseen shelf-life
- Models are increasingly used by the seafood and meat sectors
- Development and validation of predictive models with a wide range of applicability have been more successful for the seafood and meat sectors than for the dairy sector

Models and software for *L. monocytogenes*

Type of model	Type of products	Factors	References
Growth	Liquid dairy products	T, pH, a_w, nitrite, CO_2	Augustin et al. 2005
	Cheese	T, pH, a_w, nitrite, CO_2	Augustin et al. 2005
	Ready-to-eat meat and seafood	T, pH, NaCl, phenol, nitrite, CO2, AAC, DiAC, LAC	Mejlholm and Dalgaard, 2009
	Cottage cheese	T, pH, NaCl, LAC, SAC and **LAB***	Østergaard et al. 2014
	Smear soft cheese - (past. or un-past. milk)	T, pH, a_w	Schvartzman et al. 2011
	Food	T, pH, aw, LAC	Sym´Previus
	Cheese and dairy products	T, pH, aw	te Giffel and Zwietering, 1999
Inactivation	Cheese	T, pH, a_w, LAC, SAC	Coroller et al. 2012

*** Inhibiting effect of lactic acid bacteria (LAB)**

Evaluation of models and software

Evaluation of *L. monocytogenes* models and software by using data collected from the scientific literature for <u>different groups of cheeses</u>

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Prevalence of *Listeria monocytogenes* in European cheeses: A systematic review and meta-analysis

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Different groups of cheeses

		Number of *L. monocytogenes* positive (s) /total number of cheese samples (*n*)				
References	Survey year	Fresh	Ripened	Veined	Smear	Brined
Filiousis et al., 2009	2005-2006		4/20	[illegible]		0/10
Little et al., 2009	2006-2007		2/1240	[illegible]		
O'Brien et al., 2009	2007	0/29	1/104	[illegible]	14/79	
Di Pinto et al., 2010	2007-2009	[illegible]	[illegible]			
Pesavento et al., 2010	2008	[illegible]	[illegible]			
Prencipe et al., 2010	2005-2006	1/437	1/449	21/444	24/802	
Angelidis et al., 2012	2010	0/83		0/38	0/16	
Lambertz et al., 2012	2006-2[illegible]	[illegible]			0/62	
Dambrosio et al., 2013	2009-[illegible]	0/404				
Doménech et al., 2013	2005-2009	0/77				
Parisi et al., 2013	2008-2010	3/70				
Gyurova et al., 2014	[illegible]			0/7		0/34
Doménech et al., 2[illegible]	[illegible]-2012	[illegible]	[illegible]			
Schoder et al., 2015	NS[a]		1/15	0/50	1/22	
Spanu e[illegible]	2011-2013		3/50			7/33
Ianne[illegible]	[illegible]	[illegible]21	0/106	8/190	11/177	
Corone[illegible] 16	NS[a]					15/87
Total		17/2580	15/2101	32/1218	50/1158	24/164

Brined (11.8% CI: 3.5-33.3)

Smear (5.1% CI: 1.9-13.1)

Veined (2.4% CI: 0.9-6.3)

Ripened (2.0% CI: 0.8-4.9)

Fresh (0.8% CI: 0.3-1.9)

Martinez-Rios et al., 2018

Evaluation of growth models and software with literature data

Models are evaluated/validated by comparison of measured/observed and predicted values (kinetic parameters or shelf-life). The comparison can be graphical or mathematical

Bias factor (B_f) $$= 10^{\frac{\Sigma \log(\mu\text{-}predicted / \mu\text{-}observed)}{n}}$$

Accuracy factor (A_f) $$= 10^{\frac{\Sigma \left|\log(\mu\text{-}predicted / \mu\text{-}observed)\right|}{n}}$$

Conclusions

- Models for growth of *L. monocytogenes* in dairy products often include the effect of temperature, pH, salt/aw and some organic acids
  - ***Østergaard et al., 2014*** included the inhibiting effect of LAB and we **recommend** to use this model when **LAB are present**
- Cardinal parameter model including the effect of specific dairy components (**melting salts and gluconic acid**) have been developed for *Listeria monocytogenes* in an on going project DAIRY-PREDICT (2015-2019)
  - the model can be used for **re-formulation** of product, simulation of **storage conditions** and **documentation of safety**

Perspectives

- Anti-listerial compounds in fermented dairy products (**peptides and bacteriocins**) are interesting to include in extended model

- To benefit from the potential of predictive models further developments are needed within the dairy sector
    - Models for human pathogens other than *L. monocytogenes*
    - Collaboration between processors, culture-producers and scientists to include effects of dairy specific factors in validated models
- Help to establish safe shelf-life

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