Listeria monocytogenes: Predicting the growth boundary in seafood – a key to comply with new EU regulation

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Listeria monocytogenes

- Background and objectives
  - New EU-regulation (EC 2073/2005)
  - Predicting growth in cold-smoked salmon
- Preventing growth in lightly preserved seafood
- Predicting the growth boundary
- A key to comply with new EU regulation
- Conclusions and perspectives

New EU regulation (EC 2073/2005)

EU-regulation distinguish between ready-to-eat foods able or unable to support growth of *Listeria monocytogenes*

Ready-to-eat foods	Critical limit	Comment
Support growth	None in 25 g	- When produced
Support growth	100 cfu/g	- It must be documented that 100 cfu/g is not exceeded within the storage period
Unable to support growth	100 cfu/g	- Documentation - pH ≤ 4,4 or a_w ≤ 0,92 - pH ≤ 5,0 and a_w ≤ 0,94 - Shelf-life below 5 days

Predicting growth in cold-smoked salmon

Product characteristics:

- NaCl in the water phase 3 - 8 %
- pH 5.9 – 6.3
- Lactate in the water phase 4 - 15 g/liter
- Smoke components: 3 – 20 mg phenol/kg
- Microflora dominated by lactic acid bacteria (LAB)

Storage and distribution conditions:

- Vacuum-packed at about 5°C
- Modified atmosphere-packed at about 5°C

Variable product → Variable growth of *L. monocytogenes*

Jørgensen et al. 2000; Leroi et al. 2001; Espe et al. 2004

Predicting growth in cold-smoked salmon

Acceptable model includes the effects of temperatur, NaCl/a_w, pH, lactate, smoke components (phenol) and lactic acid bacteria (LAB)

Observed and predicted growth of *Listeria monocytogenes* in 13 batches of naturally contaminated products at 5 °C

	Observed growth	**Predicted growth**	
		With LAB	Without LAB
Avg., log cfu g^{-1}	**0.7**	**1.2**	2.7
Avg., cfu g^{-1}	**1 → 5 cfu / g**	**1 → 16 cfu / g**	1 → 500 cfu/g

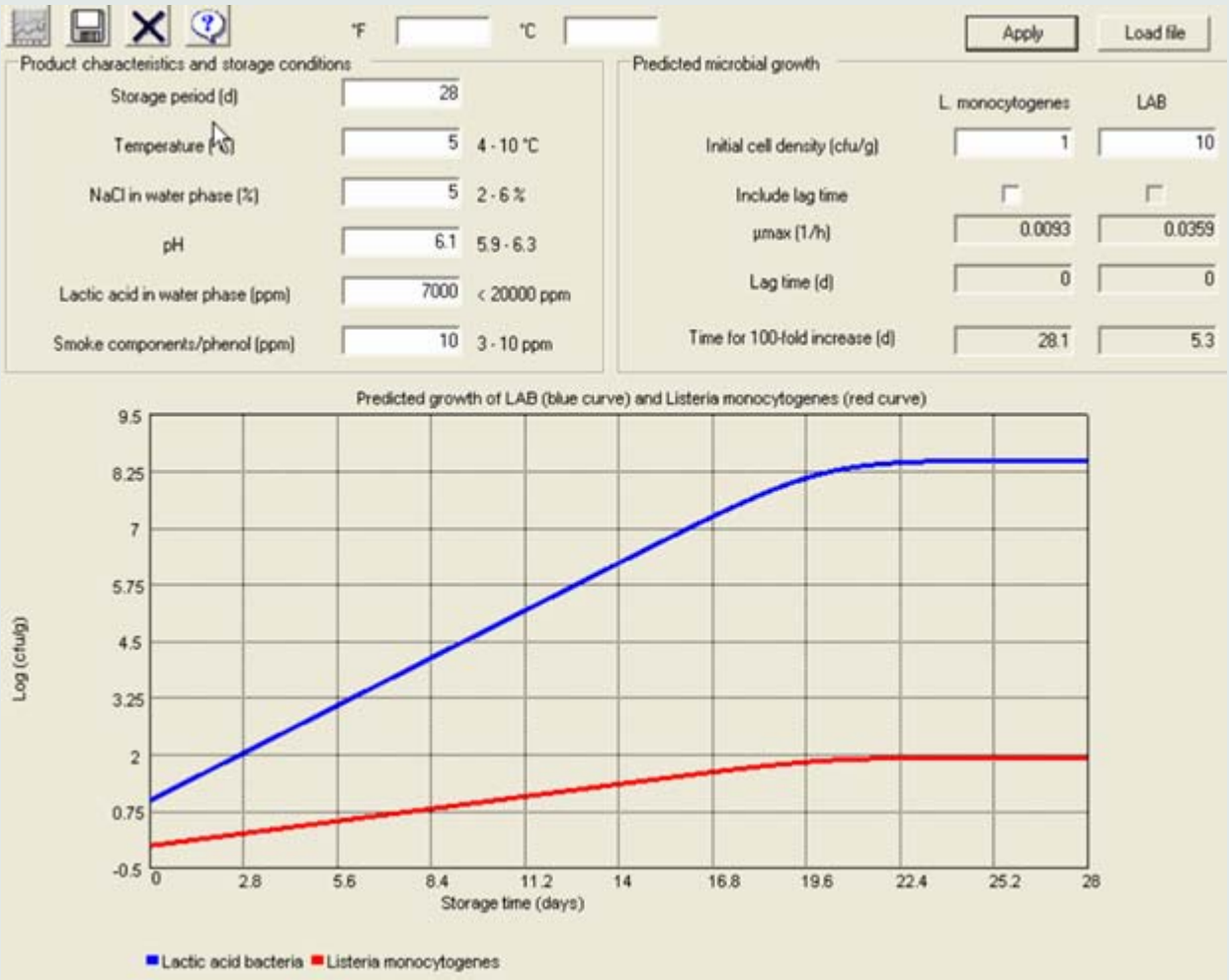
Giménez & Dalgaard (2004)

Predicting growth in cold-smoked salmon

SSSP software documents shelf-life depending on:

- Product characteristics
- Storage conditions

Seafood Spoilage and Safety Predictor (SSSP) is available free of charge at www.difres.dk/micro/sssp/



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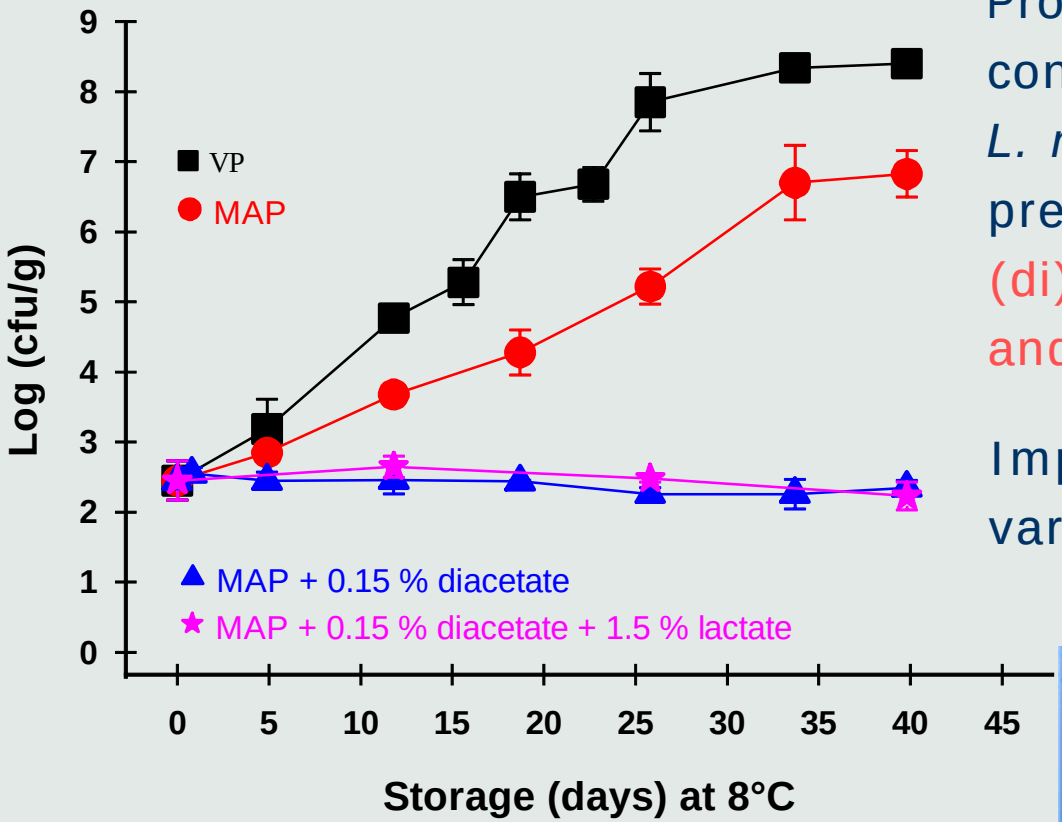
Objectives

- To prevent growth of *Listeria monocytogenes* in lightly preserved seafood
- To predict the growth boundary of *Listeria monocytogenes* depending on product characteristics and storage conditions

Preventing growth in lightly preserved seafood



Product characteristics and storage conditions determine if growth of *L. monocytogenes* can be prevented by addition of (di)acetate or if both (di)acetate and lactate are required

Important to prevent growth in various lightly preserved seafoods

Predicting the growth boundary

Existing growth model (Giménez and Dalgaard, 2004)

↓

Expand model with terms for effect of CO_2 and (di)acetate

↓

Add term for effect of interactions (Le Marc et al. 2002)

↓

Calibrate model to data for growth in well characterized lightly preserved seafoods (n = 39)

↓

Predict growth/no growth and the growth boundary

Predicting the growth boundary

$$\mu_{\max} = b \cdot ((T - T_{\min}) / (T_{ref} - T_{\min}))^2 \cdot (a_w - a_{w\,\min}) / (a_{w\,opt} - a_{w\,\min}) \cdot 1 - 10^{(pH_{\min} - pH)} \cdot 1 - \sqrt{\frac{[LAC_U]}{[MIC_{U\ LAC}]}} \cdot ((NIT_{\max} - NIT) / NIT_{\max})^2 \cdot (P_{\max} - P) / (P_{\max} - P_{opt}) \cdot (CO_{2\,\max} - CO_{2\,dissolved}) / (CO_{2\,\max} - CO_{2\,opt}) \cdot 1 - \sqrt{\frac{[AC_U]}{[MIC_{U\ AC}]}} \cdot \xi$$

Growth boundary model including the effect of temperature, NaCl/a_w, pH, lactate, nitrite, phenol (smoke), CO_2, diacetate and interactions between the parameters (ξ)

Each term results in a value between 0 and 1

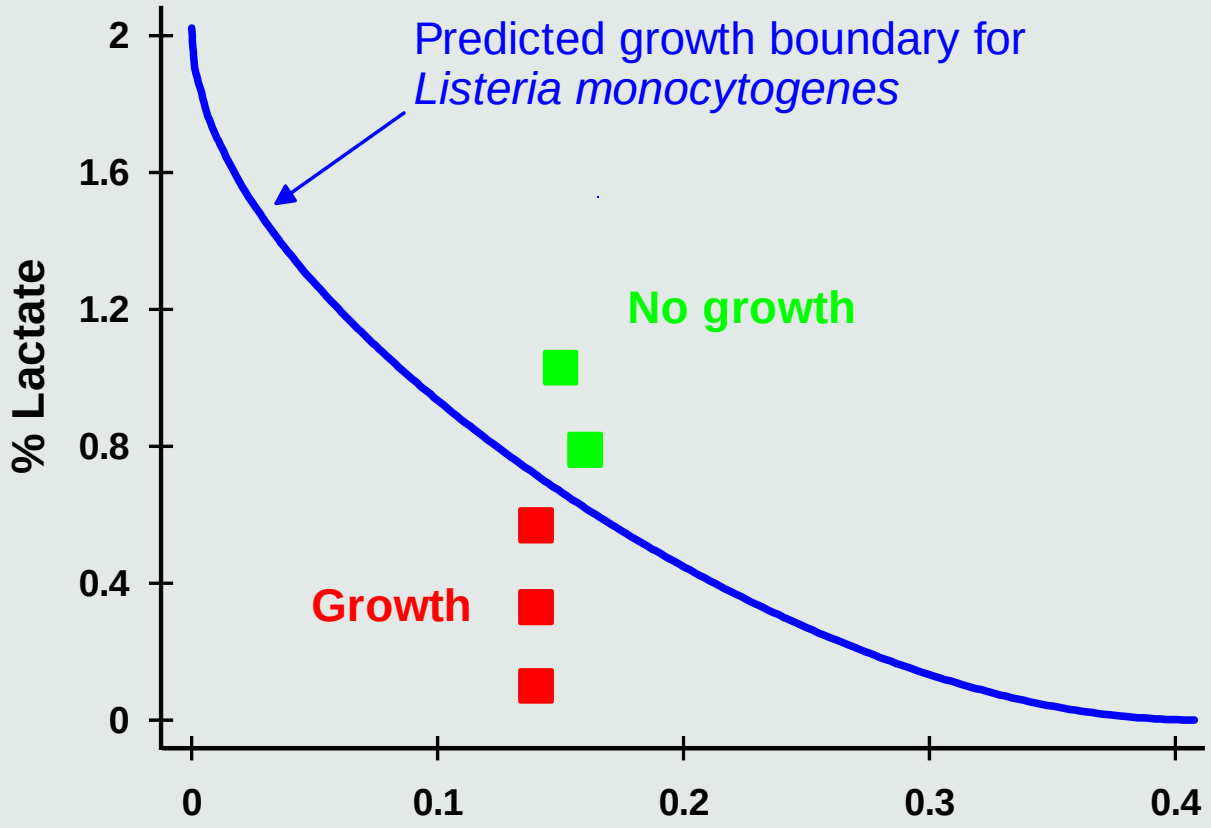
$$\xi(\varphi(T, a_w, pH, [LAC], NIT, P, CO_2, [AC])) = \begin{cases} 1 & ,\psi \le 0.5 \\ 2(1-\psi) & ,0.5 < \psi < 1 \\ 0 & ,\psi \ge 1 \end{cases}$$

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

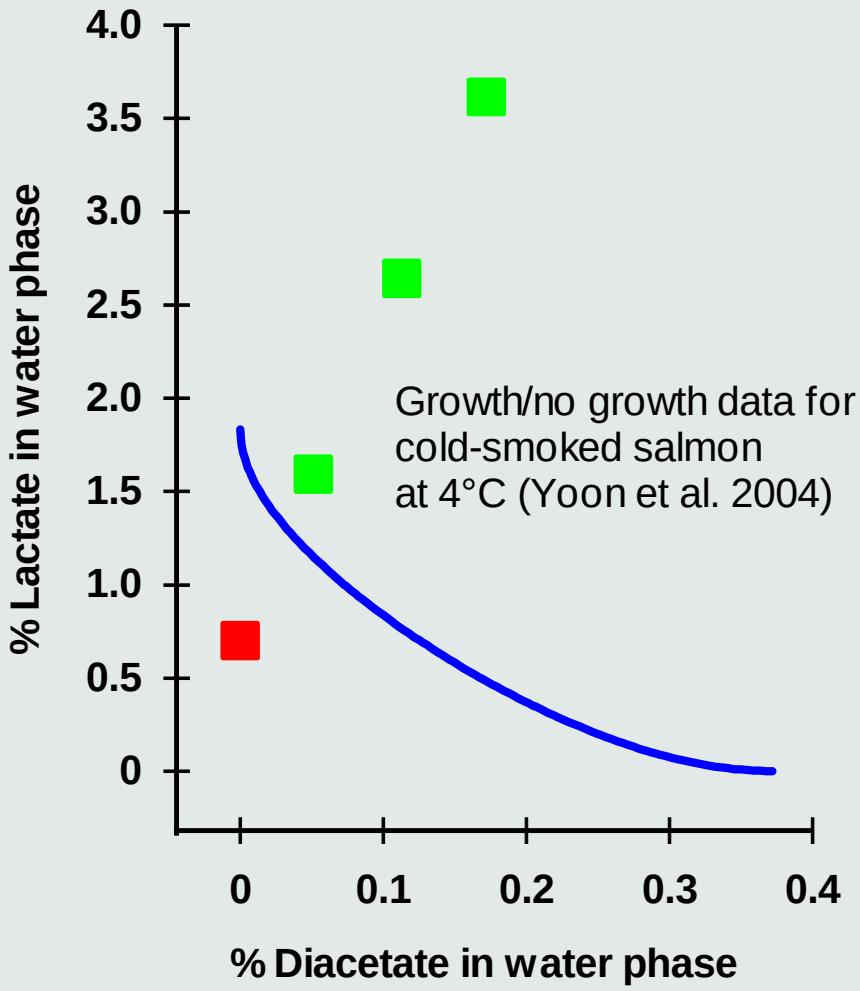
Interaction term (Le Marc et al. 2002)

Predicting the growth boundary

Interactions are essential when predicting the effect of product characteristics and storage conditions



Predicting the growth boundary



Correct prediction of growth/ no growth in 70 of 73 experiments

(96%)

Both inoculated and naturally contaminated lighlty preserved seafoods have been evaluated

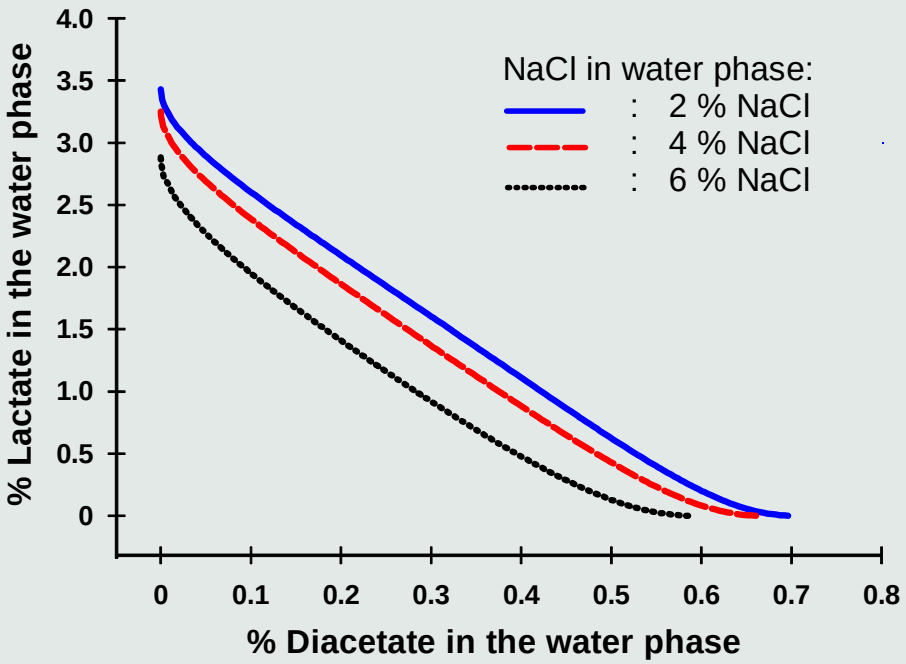
This study, n = 26

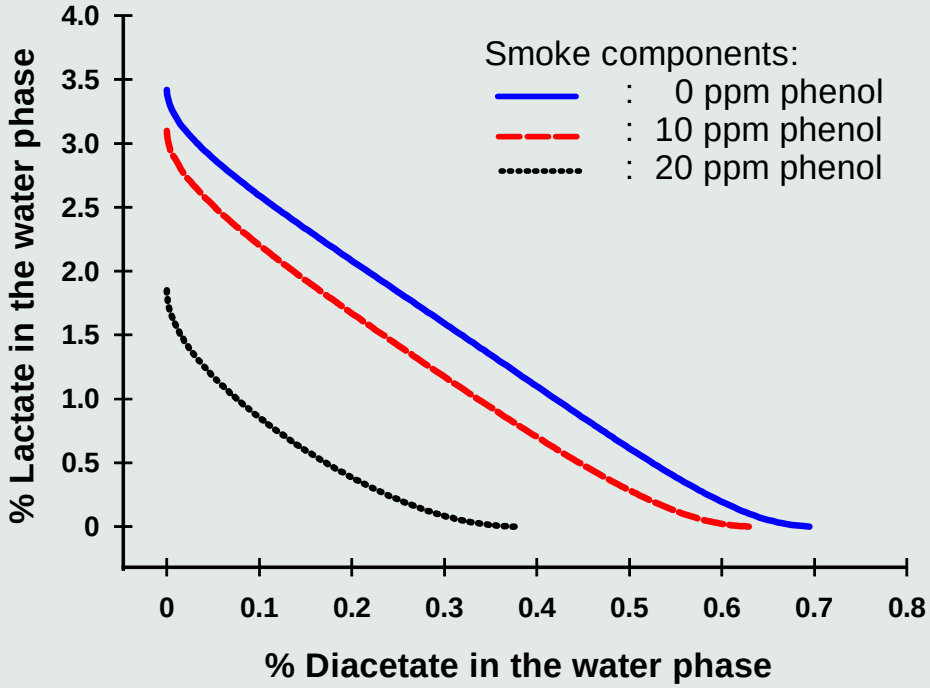
Literature, n = 47

The new model perform markedly better than existing growth boundary models (Augustin et al. 2005) (less than 70 %)

Predicting the growth boundary

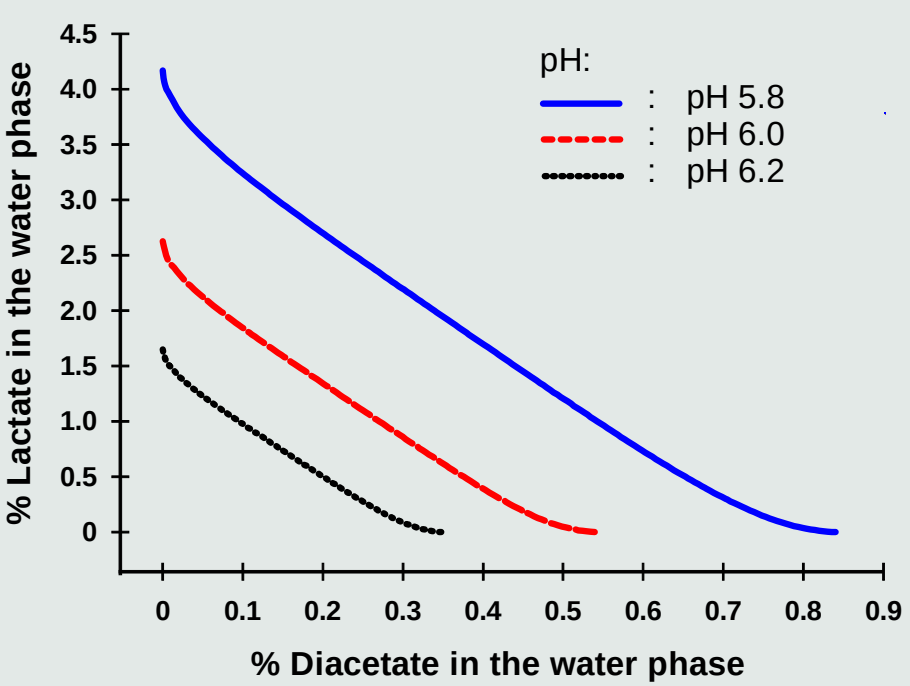
Smoke components are important to control growth of *Listeria monocytogenes* in lightly preserved seafood

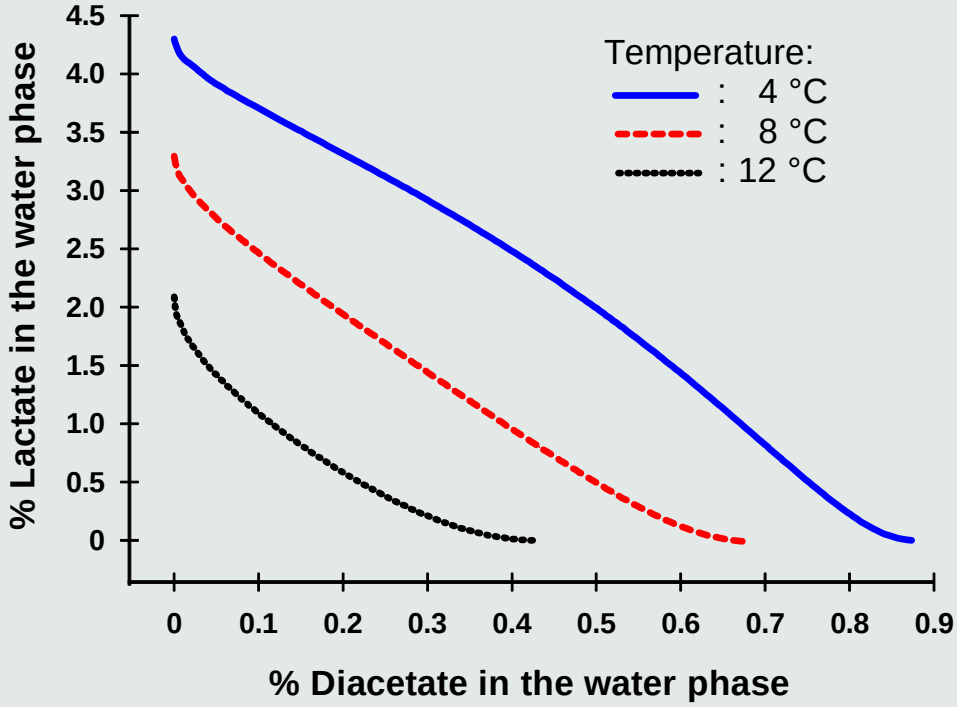




Predicting the growth boundary

Both pH and temperature (as expected) are important to control growth of *Listeria monocytogenes* in lightly preserved seafood



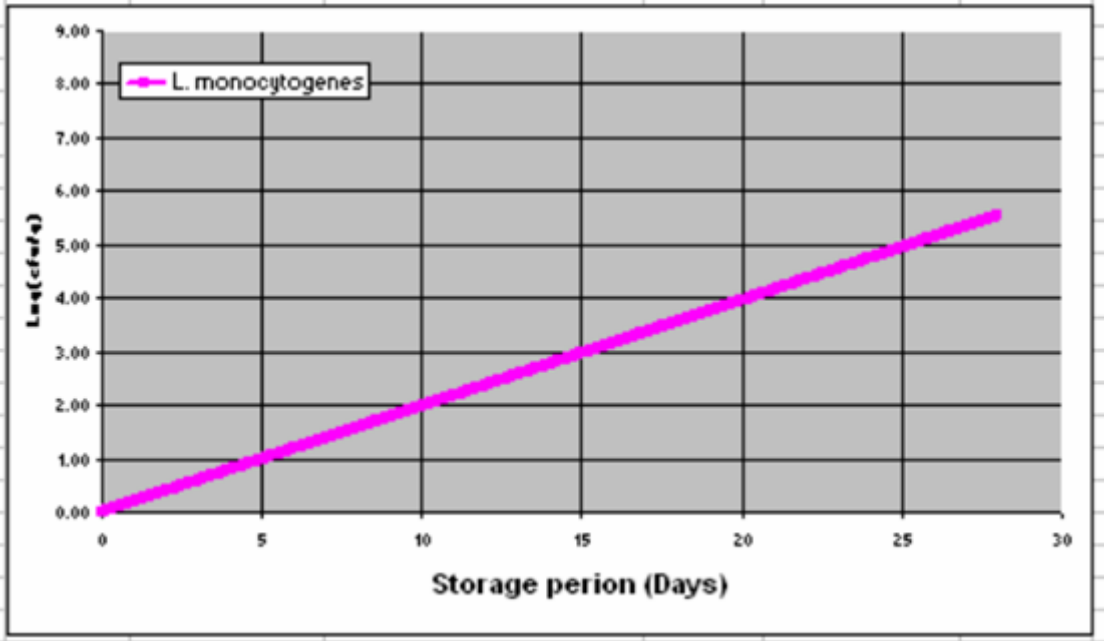


A key to comply with new EU regulation

Lightly salted seafood:

- Support growth of *L. monocytogenes* (more than 5 log cfu/g)
- Product only comply with new EU regulation if shelf-life is very short

Product characteristics and storage conditions		Range
Listeria monocytogenes, cfu/g	1	> 0
Storage periode	28	> 1
Temperature, °C	5.00	2 - 15 °C
NaCl in water phase, %	3.50	0 - 8%
pH	6.00	5.9 - 7.7
Lactate in water phase, mg/l	7000	0 - 20000
Smoke components (phenol, mg/kg)	0.0	0 - 20
% CO2 in equilibrium	0.0	0 - 100 %
Diacetate in water phase, mg/l	0	0 - 2000
Nitrite, mg/kg	0	0 - 200



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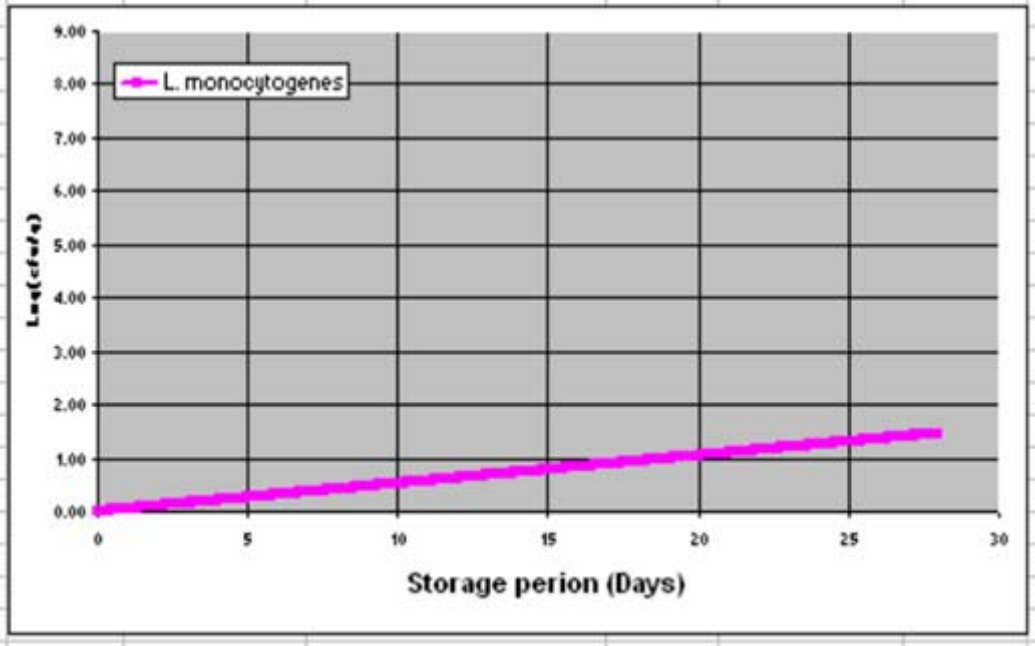
A key to comply with new EU regulation

Lightly salted product:

- Diacetate reduce but do not prevent growth of *L. monocytogenes* in this product (~ 1.5 log cfu/g in 28 days)
- Product with **3-4 weeks shelf-life** comply with new EU regulation

Product characteristics and storage conditions		Range
Listeria monocytogenes, cfu/g	1	> 0
Storage periode	28	> 1
Temperature, °C	5.00	2 - 15 °C
NaCl in water phase, %	3.50	0 - 8%
pH	6.00	5.9 - 7.7
Lactate in water phase, mg/l	7000	0 - 20000
Smoke components (phenol, mg/kg)	0.0	0 - 20
% CO2 in equilibrium	0.0	0 - 100 %
Diacetate in water phase, mg/l	1000	0 - 2000
Nitrite, mg/kg	0	0 - 200



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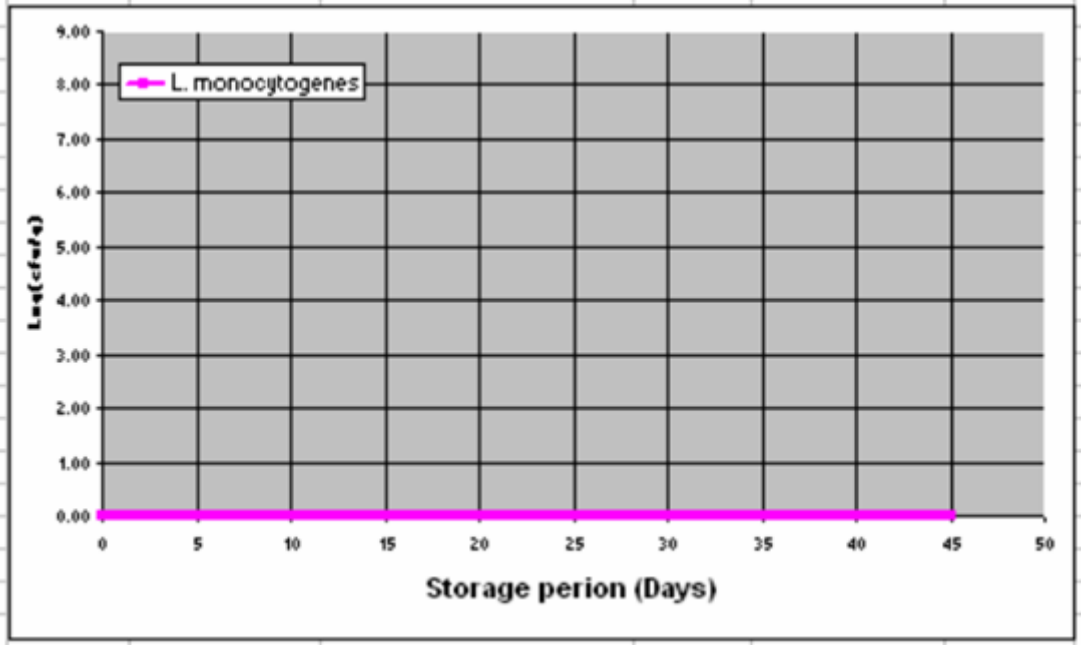
A key to comply with new EU regulation

Lightly salted product:

- Diacetate and smoke prevent growth of *L. monocytogenes* in this product

- Product comply with new EU regulation even with a **shelf-life of more than 4 weeks**

Product characteristics and storage conditions		Range
Listeria monocytogenes, cfu/g	1	> 0
Storage periode	45	> 1
Temperature, °C	5.00	2 - 15 °C
NaCl in water phase, %	3.50	0 - 8%
pH	6.00	5.9 - 7.7
Lactate in water phase, mg/l	7000	0 - 20000
Smoke components (phenol, mg/kg)	10.0	0 - 20
% CO2 in equilibrium	0.0	0 - 100 %
Diacetate in water phase, mg/l	1500	0 - 2000
Nitrite, mg/kg	0	0 - 200



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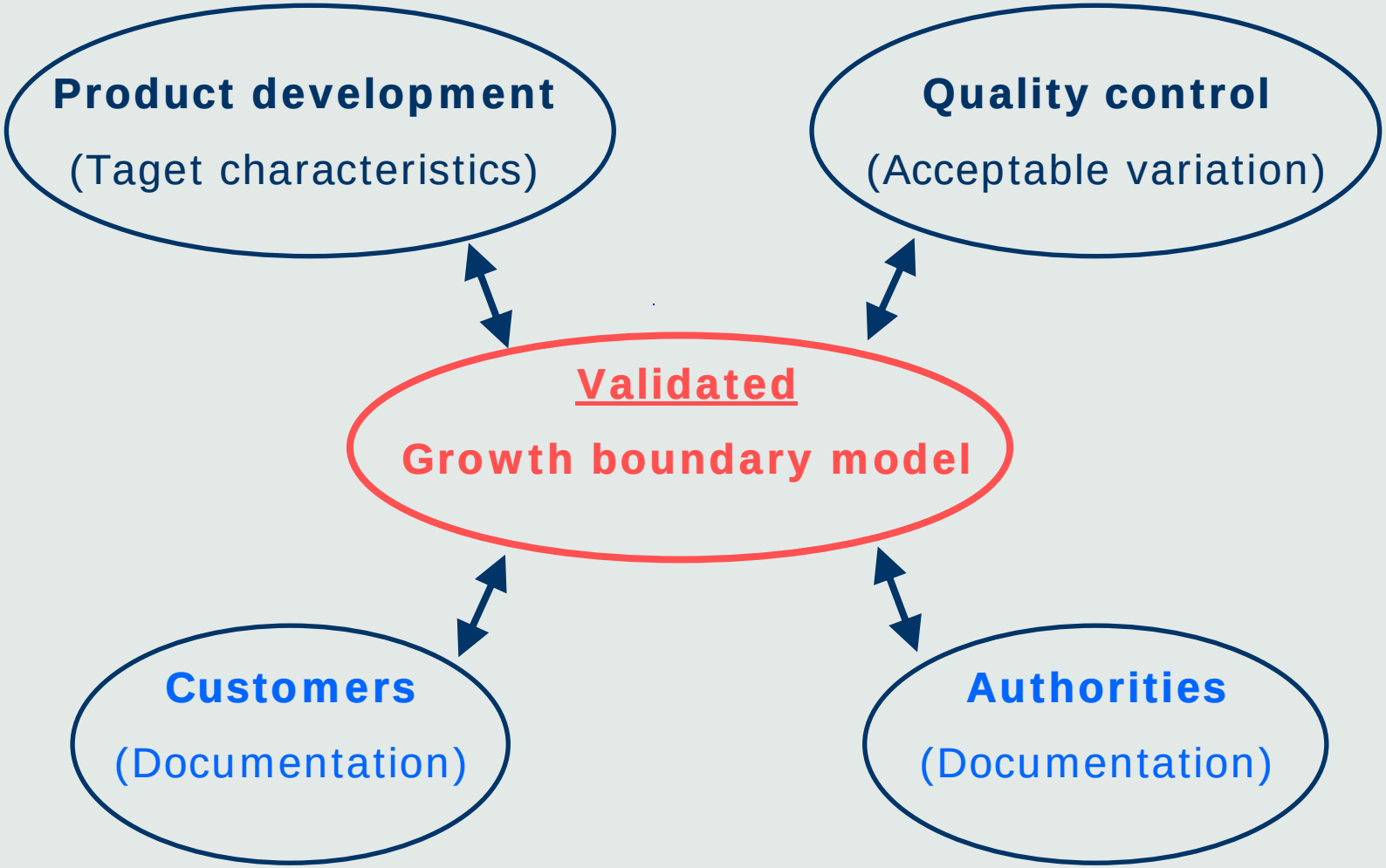
A key to comply with new EU regulation



A key to comply with new EU regulation

- Many different combinations of product characteristics and storage conditions can prevent growth of *L. monocytogenes*
- The new model facilitate identification of appropriate combinations for different products

Storage conditions				Product characteristics			
Temp. (°C)	CO_2 (%)	NaCl (% WPS)	pH	Phenol (ppm)	Nitrit (ppm)	Laktat (%)	Diacetat (%)
5.0	0	4.5	6.0	10.0	0	0.80	0.11
5.0	25	4.5	6.0	10.0	0	0.80	0.09
5.0	25	3.0	6.0	10.0	0	0.80	0.12
5.0	25	4.5	6.0	19.5	0	0.80	0
8.0	98	4.5	6.0	13.0	0	0.70	0
8.0	25	4.5	6.0	13.0	100	0.70	0.11

Conclusions and perspectives

- Diacetate (E 262) in combination with other product characteristics can prevent growth of *L. monocytogenes* in lightly preserved seafood
- The developed growth boundary model rapidly determines conditions that prevent growth of *L. monocytogenes* (A key to comply with new EU regulation)
- To improve its usefulness the growth boundary model will be included in application software

- The modelling approach seems useful for other antimicrobial agents and deserves further development

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