FOR BETTER FOOD & HEALTH

Listeria monocytogenes in cheese

a model for determining the level of undissociated lactic acid in cheese and predicting growth inhibition

Marjon.Wells-Bennik@nizo.com

Teagasc meeting – 25 May 2023

INNOVATING TOGETHER

NIZO

Ede, The Netherlands

- Confidential contract research for food industry
- Since 2009 independent company
- Founded in 1948 by the Dutch Dairy Companies (75 yr)
- Fields of work: dairy and non-dairy - plant based

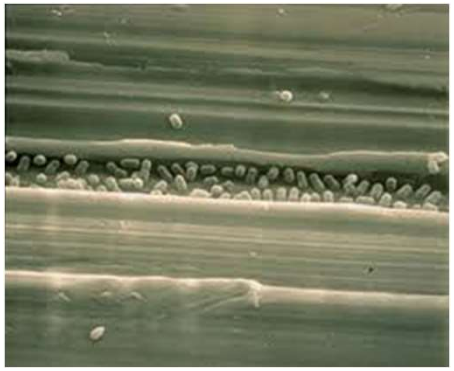
"Our passion is accelerating innovation together with our customers." - Gisella Frijlink (CEO NIZO)

NIZO

Collaborations Wageningen University

Inactivation and control - ensuring food safety and preventing outbreaks

- *L. monocytogenes:* Ubiquitously present
  - Soil, surface water, vegetation, animals, surfaces, moist/wet environments
- Inactivation by heating
  - Pasteurization e.g. 15s 72 °C; average reduction 10.4 log units
- Prevent contamination after heat treatment
  - GMP; sampling schemes processing areas, equipment, finished product
- Persistent!
  - Growth at low temperatures (refrigeration)
  - High acid resistance (no growth pH ≤ 4.4)
  - Growth in high salt concentrations and salt resistant
  - Biofilm former



Listeria on stainless steel

Listeria monocytogenes

control in the food chain -> ensuring food safety and preventing outbreaks

Dairy related outbreaks:

Most often:

- raw milk products
- contaminated soft cheeses

Other product categories

2015: ice cream, frozen yoghurt, Blue Bell: 3 deaths, 10 ill

2009: contaminated acid curd Prolactal: 7 deaths

1983: pasteurised milk 14 deaths, 49 ill (USA)

NEWS

FSA and UKHSA warn of listeria risk with Baronet soft cheeses

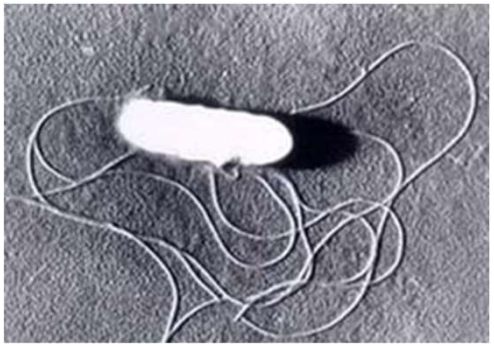
The Food Standards Agency (FSA) and UK Health Security Agency (UKHSA) are warning the public not to eat Baronet semi soft cheeses which have been recalled because they are contaminated with listeria

Last updated: 24 March 2023 View as PDF Print this page

One death, two people ill

Listeria monocytogenes in Ready-To-Eat (RTE) Foods

- Ready- to-eat products potentially highest risk
  - No heating before consumption



- EU regulation (EC) No 2073/2005:
  - criteria for *Listeria monocytogenes* in RTE foods

- Differentiation RTE Foods
  - Foods intended for infants and for special medical purposes (Category 1.1)
  - Foods that can support growth (Category 1.2)
  - Foods that cannot support growth (Category 1.3)

Products unable to support growth of *L. monocytogenes*?

- no support of growth, Category 1.3 products, e.g.:
  - High acid products, pH ≤ 4.4
  - Low water activity products, a_w ≤ 0.92,
  - pH ≤ 5.0 and a_w ≤ 0.94
  - shelf life < 5 days

- Other evidence for no growth potential:
  - predictive mathematical modelling
  - durability tests and/or challenge tests

- USA: zero tolerance (absent in 25 g)
  - products that can/cannot support growth

Quantitative assessment of relative risk to public health from *L. monocytogenes*

- Selected ready-to-eat foods including cheese (FDA, 2003)
  - Classification based on 'moisture on whole cheese content'

- Clusteranalysis of predicted per serving and per annum relative rankings

Risk	Cheese category	% moisture	Cheese type
High	soft unripened cheese	>50	cottage cheese, cream cheese, and ricotta
Moderate	soft ripened cheese	>50	brie, camembert, feta, and mozzarella
	semi-soft cheese	39-50	blue, brick, monterey, and muenster
	fresh soft cheese	>50	Queso Fresco, Queso de Creama, Queso de Puna
Very low	hard cheese	<39	cheddar, colby, emmental, and parmesan

- Dairy related outbreaks
  - Most often **raw** milk products / **soft** cheeses

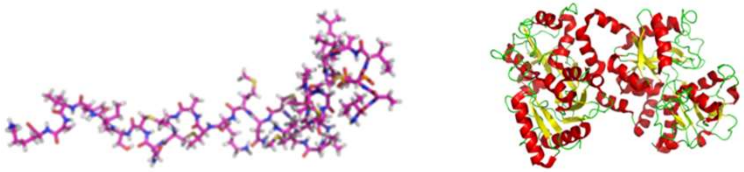
Undissociated lactate in Gouda cheese prevents growth of *L. monocytogenes*

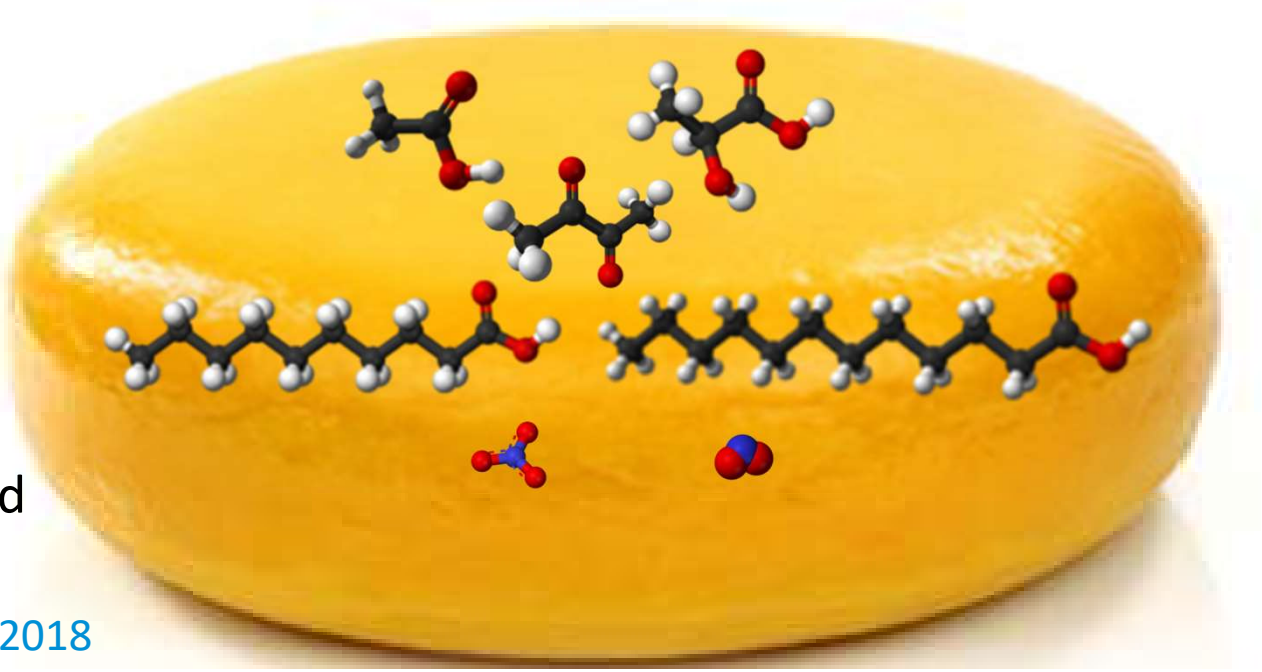
- Gouda cheese, semi hard-cheese
  - pH >5.0 (5.2-5.5), a_w > 0.94, typical moisture content 42w/w %
- No known listeriosis cases associated with Gouda made from pasteurised cheese
- 'no growth' *L. monocytogenes* established on Gouda cheese via challenge studies
  - no growth *L. monocytogenes* within 6 weeks, survival
    - Northolt et al., Neth. Milk Dairy J. 1988
  - No growth up to one year when inoculated during cheese making
    - Wemmenhove et al., Int Dairy J. 2013
  - No growth when added via brine
    - Wemmenhove et al., Int Dairy J. 2014

Potential bacterial growth inhibiting factors in Gouda cheese

Literature data and own data

- Temperature (T)
- pH
- water activity (Aw)
- **undissociated** acetic acid, lactic acid
- diacetyl
- free fatty acids
- nitrate (NO3-)
- nitrite (NO2-)
- lactoferrin
- nisin





Undissociated lactic acid established as major inhibitory factor

- Wemmenhove et al., Food Control, 2018

Undissociated lactic acid can fully inhibit growth of *L. monocytogenes*

- No growth >6.35 mM undissociated lactate (Hlac) in water phase
  - Aryani et al., 2015

- Calculation concentration undissociated lactate in water phase Gouda cheese

$$[HLac]_{water} = \frac{[HLac]_{Total}}{\varphi_{v\_water} + 10^{pH-pKa} \cdot \varphi_{v\_water} + P_{fw} \cdot \varphi_{V\_fat}}$$

- Information needed for calculation:
  - Lactate content cheese (total)
  - pH
  - Fat content
  - Moisture content

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Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Factors that inhibit growth of *Listeria monocytogenes* in nature-ripened Gouda cheese: A major role for undissociated lactic acid

E. Wemmenhove [a, b, c], H.J.F. van Valenberg [b], A.C.M. van Hooijdonk [b], M.H.J. Wells-Bennik [a, *], M.H. Zwietering [c]

Is HLac also critical for growth/no growth *L. monocytogenes* in other cheeses?

- Literature data for 10 different cheeses
- Calculation undissociated lactic acid
  - based on pH, total lactate, moisture, fat content
- Calculation growth rates of *L. monocytogenes*
  - using a predictive model (input pH, a_w undissociated lactic acid, temp)
  - Based on published growth (from challenge tests)
- Comparison predicted growth (model) and observed growth at T_{ref} 10°C
- Association with outbreaks?




Average HLac concentrations in water phase of cheeses

- HLac concentrations in water phase
  - calculated based on formula Wemmenhove et al. 2018.
- If > 6.35 mM
  - No growth of *L. monocytogenes*

Cheese type	Hlac in waterphase (mM)
Blue	1.7
Camembert	1.0
Cheddar	13.9
Cottage	0.3
Emmental	2.8
Feta	29.8
Gouda	11.8
Mozzarella (high-moisture)	3.1
Queso fresco	0.05
Ricotta	0.08

Predictions in cheeses - growth rates as function of different parameters

Specific growth rates (μ) calculated using gamma model (Zwietering et al., 1992)

$$\mu = \mu_{opt} * \gamma(T) * \gamma(pH) * \gamma(aw) * \gamma(HLac)$$

- μ_{opt} optimal growth rates in milk at 30-37°C (www.combase.com)
  1.69 h^{-1} (maximum value) and 0.73 h^{-1} (average value)

- γ gamma factors:
  - $\gamma(T)$ calculated by using T_{ref} = 10 °C, T_{min} = -1.5 °C, T_{opt} = 37 °C
  - $\gamma(pH)$ calculated by using pH_{min} = 4.4, pH_{opt} = 7.0
  - $\gamma(aw)$ calculated by using a_{wmin} =0.92
  - $\gamma(HLac)$ calculated by using MIC = 6.35 mM for full inhibition

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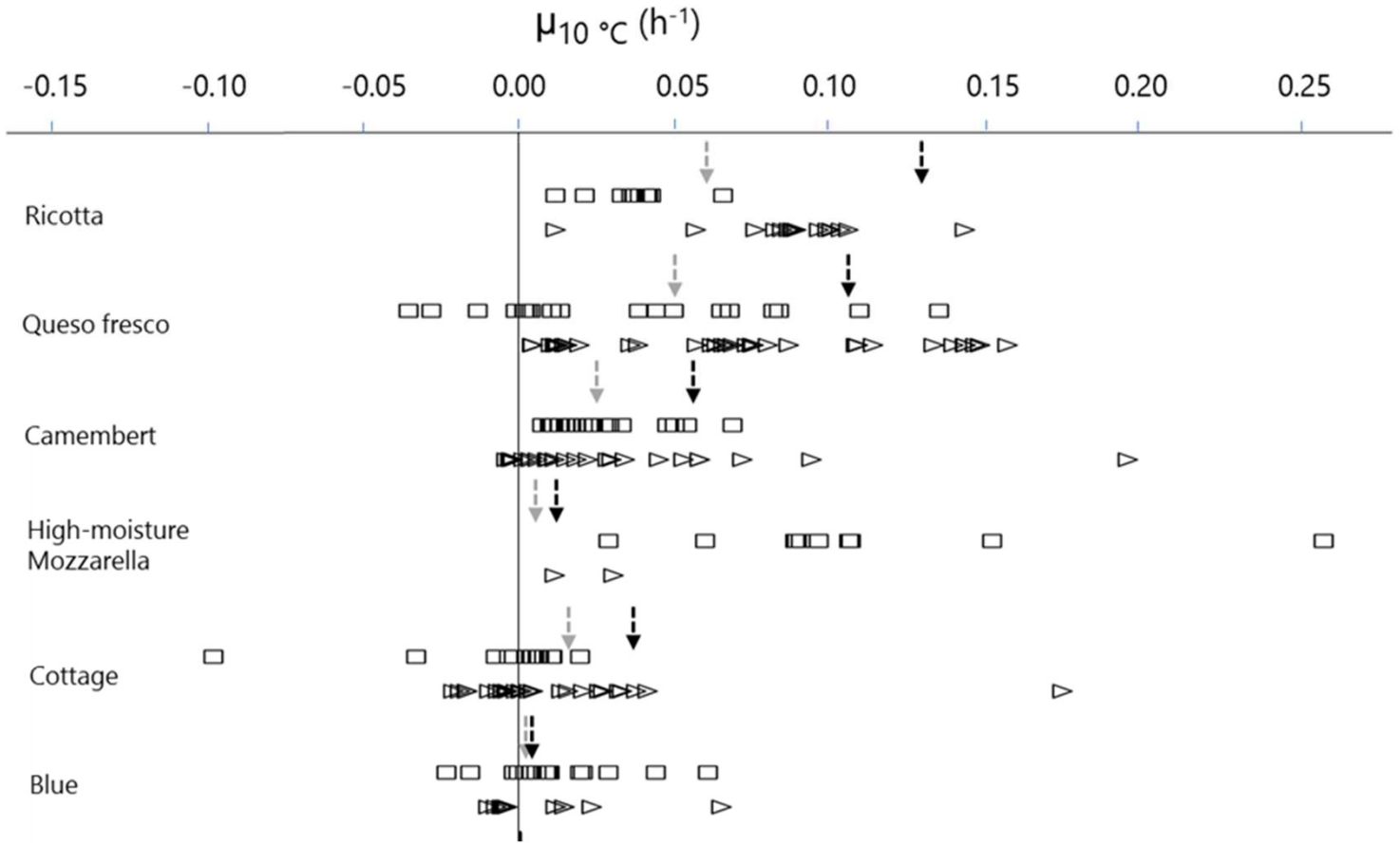
A model to predict the fate of *Listeria monocytogenes* in different cheese types – A major role for undissociated lactic acid in addition to pH, water activity, and temperature

E. Wemmenhove [a,b,1], M.H.J. Wells-Bennik [a,*], M.H. Zwietering [b,*]

[a] NIZO, Ede, the Netherlands
[b] Food Microbiology, Wageningen University, the Netherlands

Predicted and experimental growth rates at 10°C in different cheeses



Prediction
$\mu_{10\,°C}$ value calculation

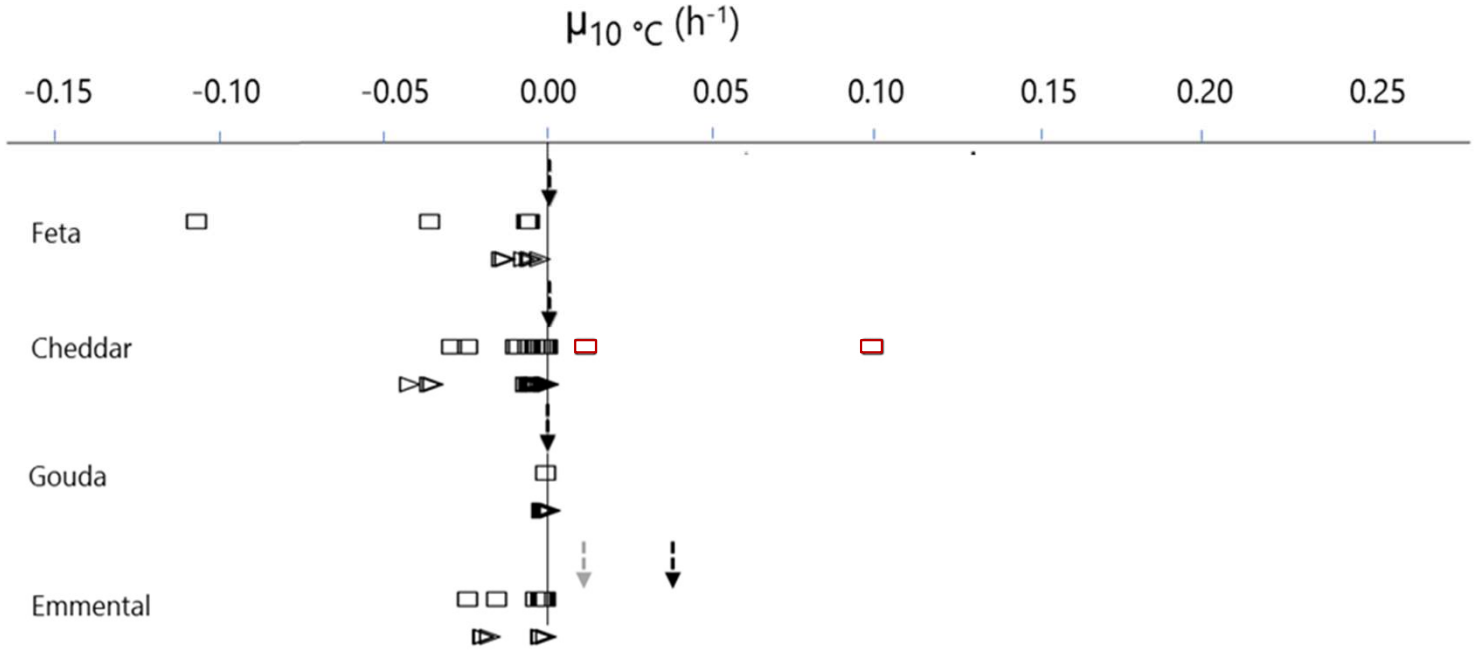
- μ_{opt} = 0.73 h^{-1} (average)
- μ_{opt} = 1.69 h^{-1} (maximum)

Calculated $\mu_{10\,°C}$ (challenge tests), contaminated at

- Surface
- Curd /milk

- Growth in Ricotta, Queso fresco, Camembert, high-moisture mozzarella, Cottage, blue

Predicted and experimental growth rates at 10°C in different cheeses



Prediction
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μ_{opt} = 1.69 h^{-1} (maximum)

Calculated $\mu_{10\,°C}$ (challenge tests), contaminated at

□ Surface

▷ Curd /milk

- Feta, Cheddar, Gouda: no growth predicted nor observed
- Emmental: growth predicted, no growth in practice
  - Other factors, e.g. acetic acid, proprionic acid, fatty acids important in Emmental
- Correct prediction of growth / no growth *L. monocytogenes:* 9 out of 10

Cheeses association with *L. monocytogenes* outbreaks

- Between 1983 and 2019 at least 41 outbreaks
  - Eurosurveillance, CDC reports, literature search
- Mainly soft cheeses
  - Examples ricotta, Queso Fresco, Camembert, blue
  - Growth predicted and seen
- Cheeses that support growth
  - Category 1.2 - regulation (EC) No 2073/2005
- Risk ranking moderate to high risk
  - FDA Risk assessment (2003) and zero tolerance




- Chain control: inactivation, prevent post-processing contamination and outgrowth

Plant based cheeses – *Listeria* risks?

FoodNavigator EUROPE

GUEST ARTICLE

Industry Insights from NIZO: Developing a plant-based product? Don't ignore Listeria risks!

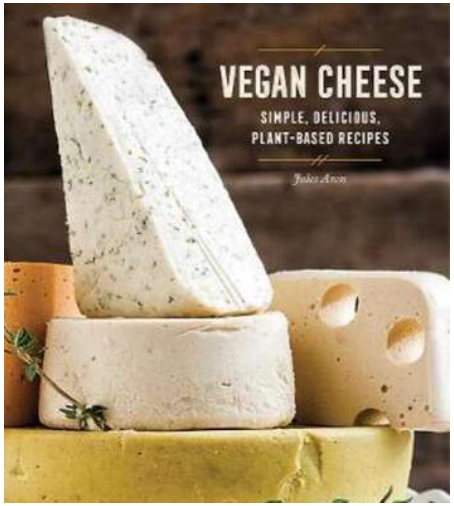
Vegan cheeses Jay & Joy

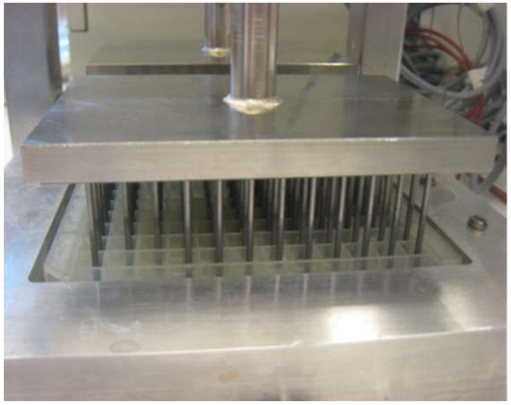
Recalls on vegan products (RASFF)

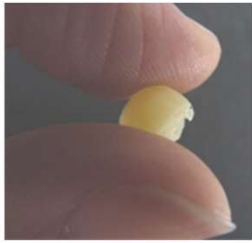
- Possible contamination with *Listeria monocytogenes* in vegan cheese alternatives from France - JAN 2023, France
- *Listeria monocytogenes* in vegan organic cheese and foie gras alternative - JAN 2023, Germany
- *Listeria monocytogenes* in hummus from the Netherlands - AUG 2022, DEC 2022, JAN 2023 The Netherlands
- *Listeria monocytogenes* in vegan cheese substitute from France - JUL 2022, Germany
- *Listeria monocytogenes* in vegan falafel - JUN 2022, The Netherlands
- *Listeria monocytogenes* in vegetal cheese-like speciality from France -JUN 2022, France
- *Listeria monocytogenes* in cheese substitute made from dehydrated cashew nuts - AUG 2021, France

Semi-hard and Cream cheese-like products

- Broad range of ingredients can be applied
  - based on starch
  - based on protein
- Target pH values typically 5.0 to 5.5
  - chemical acidification
  - fermentation using starter cultures
- Ingredients heated
  - yeast and moulds and vegetative bacteria inactivated
  - spores survive – *Clostridium* and *Bacillus* spp
  - post-processing contamination – slicing, extra additions?
- Potential risks:
  - Overgrowth starter during fermentation
  - Outgrowth contaminants during storage incl. post processing

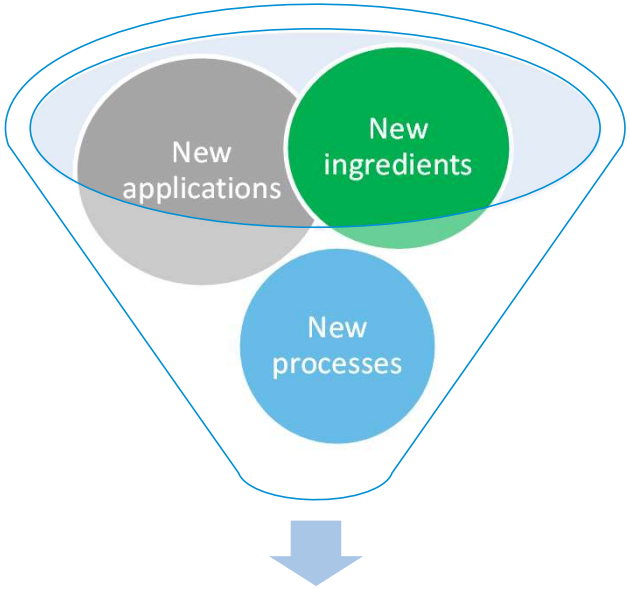






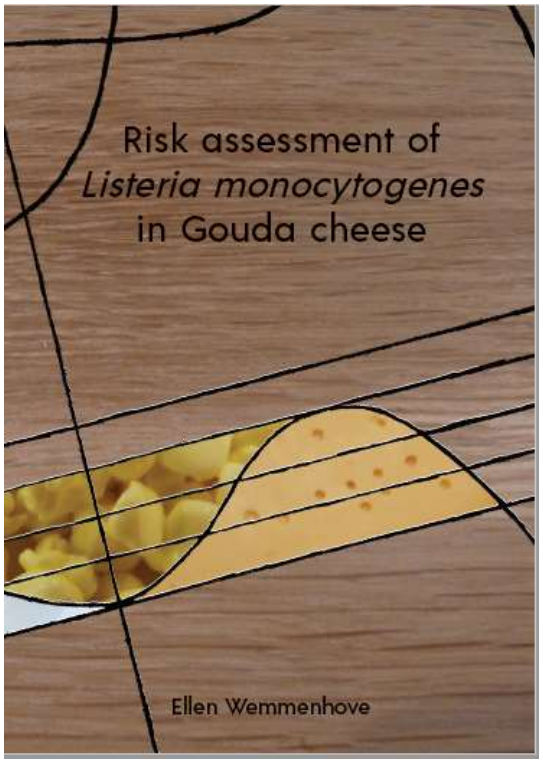
Concluding remarks

Growth (and inactivation models) help assess risks in products, taking entire food production chain into account



New issues during processing and in finished products, *e.g.* plant based ingredients

Acknowledgements



Thesis Ellen Wemmenhove
Request hard or digital copy
marjon.wells-bennik@nizo.com

Ellen Wemmenhove
current address Arla

Marcel Zwietering

Dutch Dairy Organization

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marjon.wells-bennik@nizo.com

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