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Listeria monocytogenes
Laboratorio Nazionale di Riferimento

Predicting the growth kinetics of *Listeria monocytogenes* and *Yersinia enterocolitica* in Italian-style fresh sausage: preliminary results

Luigi Iannetti **Romolo Salini**

Workshop dei LNR per *Listeria monocytogenes* e *Campylobacter*
25 e 26 novembre 2014 - Teramo

LEGAL FRAMEWORK

PREDICTIVE MODELS IN THE REGULATION 2073/2005

The use of predictive modelling techniques is clearly recommended by reg. 2073/2005 (annex II) to demonstrate the compliance with microbiological criterion 1.3 for *Listeria monocytogenes*

Categoria alimentare	Micro-organismo	Piano di campionamento		Limiti		Metodo di analisi di riferimento	Fase a cui si applica il criterio
		n	c	m	M		
Alimenti pronti che costituiscono terreno favorevole alla crescita di Listeria monocytogenes…	Listeria m.	5	0	100 UFC/g		EN/ISO 11290-2 (numerazione)	Durante la vita commerciale del prodotto

LEGAL FRAMEWORK

PREDICTIVE MODELS IN THE REGULATION 2073/2005

FBO can demonstrate that the level is less than 100 CFU/g through:

- Scientific literature
- Shelf life studies (durability studies and challenge tests)
- "predictive mathematical modelling established for the food in question, using critical growth or survival factors for the micro-organisms of concern in the product"

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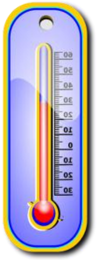
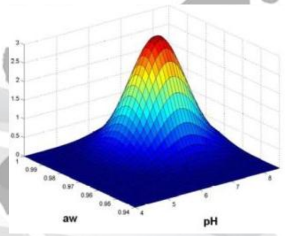
PREDICTIVE MICROBIOLOGY

- Interdisciplinary research area of food microbiology

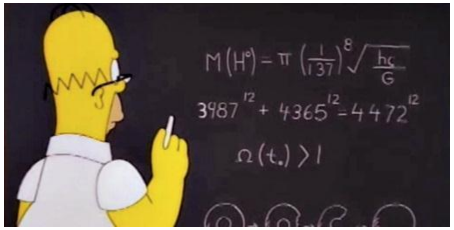
- Combines mathematics, statistics, microbiology, engineering and chemistry



- Develop and implement mathematical models in order to predict growth responses of microorganisms to conditons in question



PREDICTIVE MICROBIOLOGY



Mathematical models

Software development

→ Predictive microbiology

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PREDICTIVE MICROBIOLOGY

temp pH aw

Microbial responses to environmental conditions

$$\text{Change in log cell conc.} = f(temp, pH, aw)$$

PREDICTIVE MICROBIOLOGY

- **Primary models**:

Describe changes of the microbial number (growth, survival, death) as a function of time

- **Secondary models**:

describe parameters of the primary models as a function of environmental conditions (pH, temp, a_w)

- **Tertiary models**:

Computational implementation (software tools) of primary and secondary models.

PREDICTIVE MICROBIOLOGY

MODEL DEVELOPMENT:

- Experimental design – data collection
- Estimation of the parameters of the *primary model*
- Effect of the environmental variables of the *secondary model*
- Model validation
- Integration in a software tool (*tertiary model*)

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PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

Preliminary results on predictive model developed in collaboration with dr. József Baranyi of IFR (Institute of Food Research), Norwich, UK

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PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

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- Fresh Italian-style sausage: meat product/preparation from swine meat, traditional of Central Italy, frequently consumed raw

- Usually produced in small processing plant annexed to retail, for direct sale to the consumer

- The consumption of the raw product is usually after a variable storing period. Some changes happen in the product during this period

- Could we predict effects on pathogens' kinetics?

PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

- Risk of *Listeria monocytogenes* (Lm) and *Yersinia enterocolitica* (Ye) food poisoning, mostly when consumed raw or rare cooked

- Aim: designing and validate a secondary predictive model to predict Lm and Ye growth as a function of environmental conditions before consumption

PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

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Challenge tests

- Performed at the Laboratorio Trasformazioni Sperimentali of IZSAM, according to EURL Lm technical document (version 2008) with some modifications
- Traditional recipe (swine meat based)
- Contamination using mixture of 3 strains for each microorganism, one from international collection (ATCC for Lm, NCTC for Ye) and 2 wild strains from sausages

PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

- Observation period: 480 hours (20 days)
- Temperatures: 8°, 12°, 18°e 20°C
- Variables:

- Lm and Ye concentration as Log CFU/g (LogC)
- pH
- Water activity (a_w)
- Competitive microflora (lactic acid bacteria, micrococci, enterococci)

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- After initial growth and stationary phase, progressive decreasing of LogC was observed at all temperatures

- a_w (reduces as a function of time) seems to be the environmental variable with the biggest impact on Lm and Ye concentrations

PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

- PREDICTIVE MODEL UNDER DYNAMIC GROWTH-DEATH CONDITION in order to predict kinetics along the whole 20dd period

- It is an innovative model, as only growth or death models are commonly used for modelling in dynamic environment

PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

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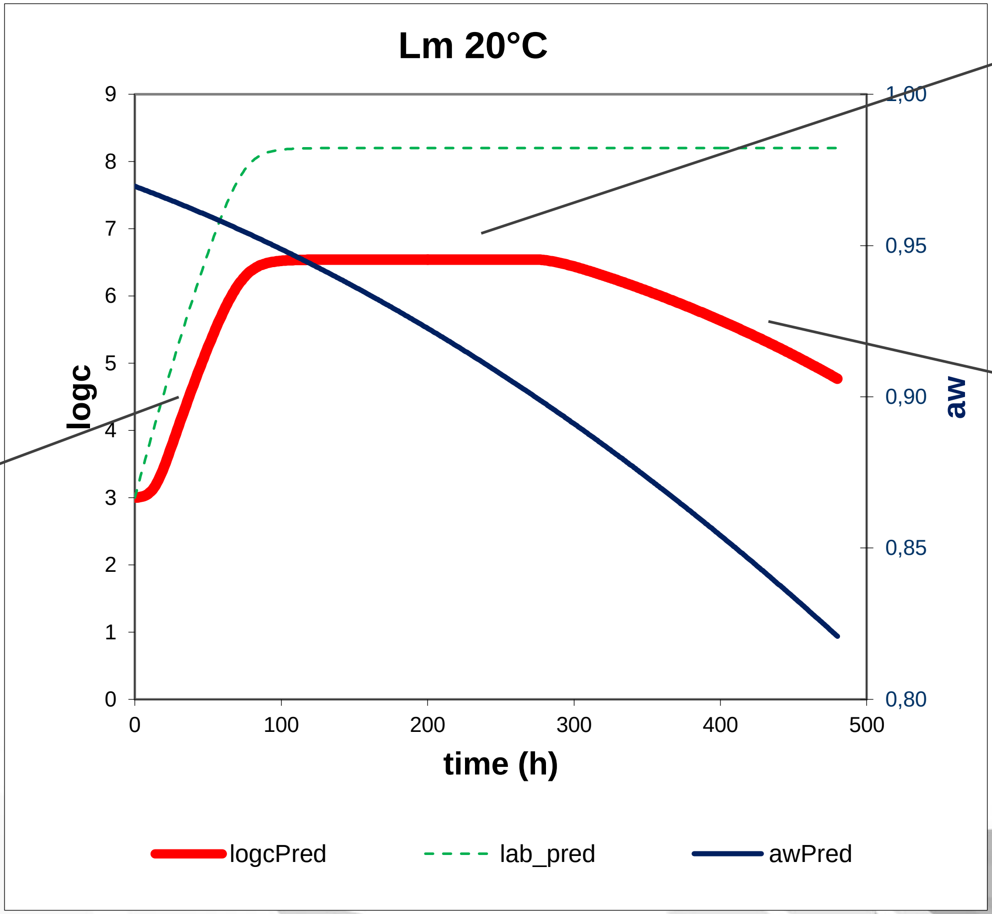
DYNAMIC GROWTH-DEATH CONDITIONS MODEL: «regions»



"growth-no growth region"

DEATH

GROWTH

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PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

- Environment quantification:
  - temperature (*temp*)
  - pH (*pH*)
  - water activity (a_w)

  where temperature and pH:
  - static, growth supporting

  Aw:
  - dynamic, decreasing from:
    *Growth region ($aw > aw_{Hi}$)
    *Growth-No growth region ($aw_{Lo}<aw<aw_{Hi}$)
    *Death region ($aw<aw_{Lo}$)

PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

Growth-No growth region

- It is the most “difficult” region in the model

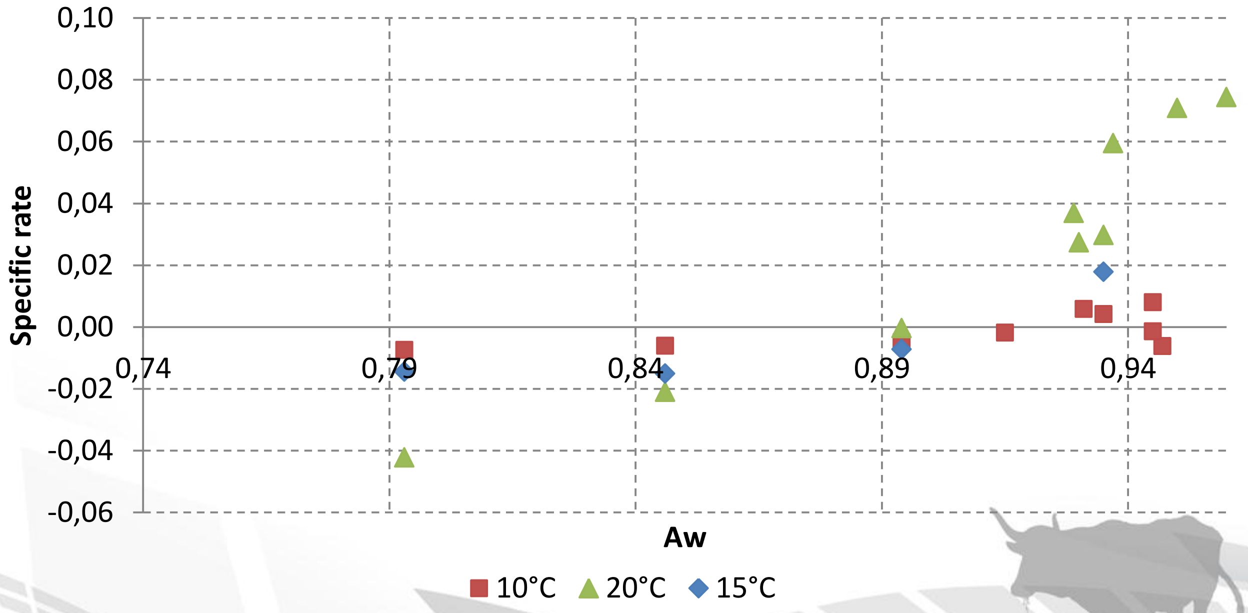
- When a_w is in the “Growth-No growth region” (unpredictable chaotic region), the model takes Logc (logCFU/g) would as a constant

- Width of this region?

PREDICTING KINETICS OF LISTERIA MONOCYTOGENES AND YERSINIA IN SAUSAGE

Growth-No growth region

- Width of this region for *Listeria monocytogenes*, according to data in literature, should be 0.03 (0.89-0.92), as confirmed by data collected from Combase e reported in this figure:



Growth-No growth region

- *Yersinia*: only a few data are in literature

- specific rate/a_w behaviour similar to *Listeria monocytogenes* was assumed

- same width (0.03) of growth-no growth boundary region, but "shifted" to right considering that its lower growth boundary is a_w 0.92-0.94 (FDA 2011)

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Competitive microflora inhibitory effect

- Lactic acid bacteria (lab) have the strongest impact on Lm and Ye growth
- The estimation of lab concentration over a certain level (labMax) was introduced as inhibitory factor of pathogens' growth rate (rateG)
- On the bases of observed values, labMax was taken as 8.2 log CFU/g

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CONCLUSION

- Preliminary results, need confirmation and validation
- Under the temporal change of the temperature variable (time ➔ temp) the model can predict growth/death kinetics at different storing conditions
- Innovative model under dynamic growth-death conditions
- Possible application of this type of model to other similar products with environmental changes during seasoning/storing