The use of predictive models for *Listeria monocytogenes* in the meat industry to support compliance with EU regulation 2073/2005 for Ready-to-eat products

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***Abstract*— To support the compliance with the EU regulation 2073/2005, particularly regarding *L. monocytogenes*, food business operators can make use of predictive microbiology. This study, in cooperation with 30 Flemish companies of the processed meat industry, aims to increase the implementation of these models in their production environment**

First, an inventory of the different processed meat products from the participating companies was made. Based on their intrinsic and extrinsic factors on the one hand and the process characteristics on the other hand, different categories were defined. Secondly, extended challenge tests (15 data points) were performed according to the EU technical guidance on two different batches of cooked ham and aspic products. Next to that, samples following a certain T-profile, were analyzed in threefold on day 0 and at the end of shelf-life to assess growth potential. Thirdly, available predictive models were evaluated regarding their performance towards these meat products. These models can be used to (*i*) support the companies in demonstrating their compliance with EU regulation 2073/2005 while reducing the amount of necessary challenge tests, (*ii*) stimulate their product innovation and (*iii*) determine the shelf-life of these products more precise.

Keywords— ***L. monocytogenes, challenge testing, growth potential, predictive models***

I. INTRODUCTION

As part of the control measures for *L. monocytogenes*, Food Business Operators (FBO) should conduct studies to identify growth potential of *L. monocytogenes* in products put on the market. Next to the specifications of physicochemical characteristics and available scientific literature, predictive microbiology can be used to prove compliance with the EU regulation 2073/2005. Therefore, it is important that existing predictive models are validated for a large category of products and that predictions are compared with results obtained from extensive challenge tests.

The objective of the study was the evaluation of the challenge test protocol described in the technical guidance document published by the EU Community Reference Laboratory (EU CRL, 2008) for *L. monocytogenes*. The concept of a simple challenge test to assess growth potential on actual data measurements at start and end of shelf life was compared to a modelling approach. Based on the intrinsic and extrinsic characteristics of different processed meat products, obtained from 30 Flemish meat companies, several categories were defined and model products were made on lab scale.

II. MATERIALS AND METHODS

A. Standardisation of the inoculum

All strains (LMG 23194, LMG 13305, LFMFP 392, LFMFP 491 and LFMFP 802) were taken from stock cultures stored at -80°C and were cultured in BHI at 37°C. In case of cold adaptation, a subculture was inoculated in fresh BHI broth and incubated at 7°C for

4 days (Vermeulen et al., 2011). To determine growth rates two monoculture strains were used, while for the growth potential tests a mixture of three *L. monocytogenes* strains was used.

B. Inoculation and packaging

Cooked ham was prepared on lab scale while the aspic products were purchased from a local producer. The products were immediately after production sliced in the lab and randomised before packing. After portioning in test units (150 ± 5 g), blanks were inoculated with 100 µl PPS (0.85% NaCl, 0.1% peptone) and the other samples with 100 µl of the diluted mixed culture (growth potential tests) or the monoculture (tests assessing growth rate). An inoculum of ca. 50 CFU/g was obtained. The aspic products were vacuum packed in high barrier packaging material (Vax090, Euralpack, Belgium) using a gas packaging chamber machine (Multivac A300/42, Sepp. Hagenmuller; Wolferschwenden, Germany). Cooked ham was MAP packed (30% CO_2 and 70% N_2) in a 1/1.8 G/P-ratio by using a tray sealer (Meca 900 VG, Mecapack, Pouzauges, France). The concentration of O_2 and CO_2 in the packages were determined using a O_2 CO_2 gas analyser (Checkmate, Gullimex, Belgium).

C. Storage conditions

In a first approach growth rates of two monocultures were determined for the meat products at constant temperature (7°C). In a second approach growth potential based on the actual measurements data of *L. monocytogenes* at the beginning en end of shelf-life was performed for different time-temperature profiles or were inoculated with different cultures (Table 1).

Table 1: overview of the different growth potential tests

N°	Pre-inoculation conditions	T-profile
1	Cold adapted	7d@8°C+15d@12°C
2	Not adapted	14d@4°C+8d@7°C
3	Cold adapted	14d@4°C+8d@7°C
4	Cold adapted	24d@4°C+12d@7°C

D. Microbial and physico-chemical analyses

For each growth curve at constant temperature total aerobic count (TAC), lactic acid bacteria (LAB) and *L. monocytogenes* count were analysed at 15 time points. This was performed for the blanks and two *L. monocytogenes* strains in monoculture. For growth potential tests the same parameters were analysed on day 0 en end of shelf-life in threefold. Enumeration of *L. monocytogenes* was performed according to ISO 11290-2 on ALOA (Biolife, 401605, Milan, Italy) using a reduced detection limit. The enumeration of TAC at 22°C was derived from ISO 6222 (4-5 days incubation of PCA (Oxoid, Hampshire, UK) at 22°C). LAB was determined according to ISO 15214 (4-5 days incubation of MRS (Oxoid) at 22°C).

On day 0 and the end of shelf life, the pH, a_w, % dry matter, % salt, % lactate and % acetate were determined according to the methods described in Vermeulen *et al*. (2011). Also the nitrite concentration was determined by an external laboratory.

E. Predictive modelling

The data of the extensive challenge tests (15 data points) were used to compare the growth rates predicted by SSSP (http://sssp.dtuaqua.dk) (one model with and one model without interaction with background flora) with the growth rates obtained by linear regression. Besides, these tertiary models and the modelling process as recommended by the EU technical guidance (EU CRL, 2008) were used to predict the growth potential for the different studied temperature profiles (Eq. 1 and Eq. 2).

$$\mu_{max} = \mu_{max_{ref}} \cdot \frac{(T - T_{min})^2}{(T_{ref} - T_{min})^2} \quad \text{(Eq. 1)}$$

with μ_{max} the maximum specific growth rate at temperature T, $\mu_{max,ref}$ the maximum specific growth rate at the reference temperature T_{ref} (i.e. 7°C), and T_{min} the minimum growth temperature of *L. monocytogenes* (-2 °C) (EU CRL, 2008).

$$\Delta \log CFU/g = \sum \mu_{max,i} \cdot d_i \quad \text{(Eq. 2)}$$

with Δ log CFU/g the logarithmic increase in cell count during the shelf-life, $\mu_{max,i}$ the maximum

specific growth rate at a certain temperature (T_i) and d_i the time of incubation at temperature T_i.

As input factors for the SSSP model the mean values of the experimentally determined intrinsic factors at day 0 were used. The lag phase was ignored as the *L. monocytogenes* originated from an adapted culture, except for condition 2.

III. RESULTS AND DISCUSSION

Extensive challenge or growth potential tests were performed on two batches for the two monocultures, cocktails and for blank samples. These tests showed a large variability on microbial growth within a batch and between different batches, as it was also seen for smoked salmon (Vermeulen et al., 2011). From commercial software packages, only the SSSP model was used as this model allowed to combine most of the intrinsic factors. For cooked ham the model showed good correspondence with the observed growth rates (Table 2). The model including nitrite and acetic acid underestimated the growth rate while the other model predicted much faster growth.

The growth potential was in a few cases underestimated (fail-dangerous) (Table 3). For the model incorporating nitrite and acetic acid, this is caused by the slower growth rate while for the other model this is due to the overestimation of the background flora which suppresses the growth of *L. monocytogenes* (Jameson effect). On the blank and inoculated samples, TAC and LAB count was initially very low (< 1 log CFU/g) and growth started only after six days (data not shown). It should be noted that this underestimation was still within the limits of microbial variability between the three replicates of the growth potential tests. Results also showed that growth potential was higher for the cold adapted cultures due to the absence of lag phase. This illustrates the importance of a standardized inoculum preparation. For the T-profile as suggested by the EU-protocol (condition 1), the prediction of growth potential was strongly deviating from the observed data. The linear model overestimated the growth potential far, even to unrealistic high levels, because it ignores the stationary phase of *L. monocytogenes*. The SSSP model, which takes into account the background flora underestimated the growth of *L. monocytogenes* due to the very low background flora in the samples. By consequence, the industrial trend towards food which is almost free from background flora (prolonged shelf-life) can compromise the food safety if *L. monocytogenes* is present in the food product.

The growth potential of *L. monocytogenes* in aspic products was in general relatively low (< 2.0 log CFU/g) (Table 4). This was mainly due to the low pH, the high acetic (0.1 %) and lactic acid (0.17 %) concentration and the fast growth of the background flora which reached the stationary phase after 10 days incubation at 7°C. For the aspic products the growth rate predicted by the SSSP model including nitrite and acetic acid underestimated far the growth of the two monocultures. Still the growth potential was not underestimated by this model as it does not consider the background flora.

Table 2: observed and predicted growth rates (log CFU/g . d)

			Observed	Growth rate SSSP[a]	SSSP[b]
Cooked ham	Batch 1	LMG 13305	0.2140	0.1782	0.2950
		LFMFP 802	0.2085		
	Batch 2	LMG 13305	0.2218	0.1303	0.2752
		LFMFP 802	0.1953		
Aspic	Batch 1	LMG 13305	0.1602	0.0323[c]	0.2085[c]
		LFMFP 802	0.1337		
	Batch 2	LMG 13305	0.1187	0.0219[c]	0.1917[c]
		LFMFP 802	0.1294		

[a] model without interaction with background flora

[b] model considering background flora, without nitrite and acetic acid

[c] pH was set on 5.6 (lowest value in the model), while the measured pH was 5.5

Table 3: Observed and predicted growth potential (log CFU/g) for *L. monocytogenes* in cooked ham

N°		Observed	Predicted		
			Linear	SSSP[a]	SSSP[b]
1	Batch 1	5.03	9.49	6.61	3.72
	Batch 2	5.86	11.00	6.70	3.50
2	Batch 1	2.12	3.40	1.18	1.89
	Batch 2	> 1.82	3.94	0.37	1.10
3	Batch 1	2.98	3.40	2.53	3.25
	Batch 2	2.34	3.94	1.73	2.76
4	Batch 1	2.46	5.38	3.92	2.95
	Batch 2	2.74	6.24	2.64	2.26

[a] model without interaction with background flora
[b] model considering background flora, without nitrite and acetic acid

Table 4: Observed and predicted growth potential (log CFU/g) for *L. monocytogenes* in aspic products

N°		Observed	Predicted		
			Linear	SSSP[a]	SSSP[b]
1	Batch 1	1.35	6.60	6.57	3.56
	Batch 2	1.72	9.37	6.26	4.03
2	Batch 1	-0.34	2.37	0.00	2.15
	Batch 2	1.99	3.35	0.00	2.62
3	Batch 1	0.53	2.37	1.33	3.50
	Batch 2	1.15	3.35	1.23	3.97
4	Batch 1	1.35	3.74	2.02	3.48
	Batch 2	1.18	6.20	2.18	3.93

[a] model without interaction with background flora
[b] model considering background flora, without nitrite and acetic acid

IV. CONCLUSIONS

The study proves that to fulfil the need from the industry to provide product specific, easy-to-use software models, more model validation is necessary. This is of utmost importance for the implementation of predictive models in assuring compliance with the EU-regulation and for the acceptance of this by the controlling agencies. Focus should be on (*i*) a better estimation of the background flora, (*ii*) the calculations of an adaptation factor to bridge the gap between challenge test and model prediction and (*iii*) the confidence interval on the predicted growth curves, to cover microbial variability. If the industry could use these models to prove compliance with the EU-regulation it can significantly reduce the costs of challenge tests and easily implement newly developed recipes or products.

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VI. REFERENCES

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