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Management of microbiological safety of ready-to-eat meat products by mathematical modelling: *Listeria monocytogenes* as an example

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Abstract

The recent Commission Regulation (EC) No 2073/2005 establishes microbiological criteria in foods. For the pathogen *Listeria monocytogenes* in the category *ready-to-eat foods able to support its growth, other than those intended for infants and for special medical purposes,* two different microbiological criteria are proposed: (i) *L. monocytogenes* levels should be <100 cfu/g throughout the shelf-life of the product, (ii) absence in 25 g of the product at the stage before the food has left the immediate control of the food business operator, who has produced it. The application of either the first or the second of these criteria depends on whether or not the manufacturer is able to demonstrate that the level of *L. monocytogenes* in the food product will not exceed 100 cfu/g throughout its shelf-life. This demonstration should be based on physico-chemical characteristics of the target product and consultation of scientific literature, and, when necessary, on quantitative models and/or challenge tests. Once the characteristics of the product as well as scientific literature show that the pathogen has potential to grow on a specific food commodity, it seems adequate to use quantitative models and/or perform challenge tests to study the extent to which *L. monocytogenes* could grow. In this study, we aim to illustrate with an example in cooked ham the application of quantitative models as a tool to manage the compliance with these criteria. Two approaches were considered: deterministic and probabilistic, in three different commercial brands (A, B, and C). The deterministic approach showed that the limit 100 cfu/g was exceeded largely at the end of the shelf-life of all three; however, when reducing the storage time, the level of *L. monocytogenes* remained below 100 cfu/g in B. The probabilistic approach demonstrated very low percentiles corresponding to 100 cfu/g; when reducing the storage time, percentiles for three products increased, especially in products B and C (from 4.92% to 75.90%, and from 0.90% to 73.90%, respectively). This study shows how different storage times influence the level of *L. monocytogenes* at the end of the shelf-life of cooked ham, and, depending on the level reached, the microbiological criterion applied should be different, as stated above. Beside this, the choice of either point-estimate or probabilistic approach should be determined by the competent sanitary authority, and, in case of selecting the second approach, a certain percentile for the level 100 cfu/g should be established.

Keywords: *Listeria monocytogenes*; Microbiological criteria; Ready-to-eat foods; Cooked ham; Shelf-life

1. Introduction

Research on food microbiology is continuously promoting revisions in Regulations, such as microbiological criteria in foodstuff, which are intended to be incorporated in national legislations.

In 1997, Codex Alimentarius (CAC, 1997) stated that «a microbiological criterion defines the acceptability of a product or a food lot, based on the absence or presence, or number of microorganisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot».

Listeria monocytogenes is a biological hazard for which a microbiological criterion is required. This microorganism has been recognized as a pathogen in animals for more than 70 years (Murray et al., 1926), but only in the last 25 years that it has been considered as a microorganism of concern especially for food industries, because of being a zoonotic agent which causes food-borne listeriosis.

In 1992, some measures were adopted in relation to *L. monocytogenes* in milk and dairy products through the Council Directive 92/46/CE (Council of the European Communities,

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1992), laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. This Directive established compulsory microbiological criteria for soft cheese and pasteurized milk (absence in 25 g), and other dairy products (absence in 1 g). Some years later, the Scientific Committee on Food (SCF) and the Scientific Committee on Veterinary relating to Public Health (SCVPH), from the European Commission, were asked to give scientific advice on *L. monocytogenes* as a food-borne pathogen, as no microbiological criteria had been established with the exception of those just mentioned above (Council Directive 92/46/CE) and due to the substantial controversy existing in the intra-Community trade as result of the absence of agreed reference values. Finally, two documents were presented, by both SCF (2000) and SCVPH (1999). Specifically, SCVPH discusses *L. monocytogenes* levels which could be relevant in a possible further effort to lower the incidence of food-borne listeriosis, and distinguishes between 2 categories of foods; foods supporting the growth of *L. monocytogenes*, and foods which do not. Regarding the former, *L. monocytogenes* should not be detected in 25 g at the time of production, while in the latter, *L. monocytogenes* levels should be <100 cfu/g at the time of consumption, and therefore throughout the shelf-life of the commodity. SCF supported the recommendation of the SCVPH, and stated that manufacturers' efforts should be focused on the food commodity types where *L. monocytogenes* can multiply in order to ensure that products are in compliance with this recommendation.

The recent Commission Regulation No. 2073/2005 *on microbiological criteria for foodstuffs* included the above microbiological considerations given by SCVPH, and added other aspects, such as the type of population (high risk population), sampling plan or actions in case of unsatisfactory results. For *L. monocytogenes*, in the food category *ready-to-eat (RTE) foods able to support the growth of L. monocytogenes other than those intended for infants and for special medical purposes*, two different microbiological criteria are proposed: (i) *L. monocytogenes* levels should be <100 cfu/g throughout the shelf-life of the product, (ii) absence in 25 g of the product before the food has left the immediate control of the food business operator, who has produced it. The application of either the first or the second of these criteria depends on whether or not the manufacturer is able to demonstrate that the level of *L. monocytogenes* in the food product will not exceed 100 cfu/g throughout its shelf-life. The Annex II of the Regulation states that this demonstration should be based on physico-chemical characteristics of the product studied and consultation of scientific literature, and, when necessary, on quantitative models and/or challenge tests. Once the characteristics of the product as well as scientific literature show that the pathogen has potential to grow on a specific food commodity, it seems adequate to use quantitative models and/or perform challenge tests to study the extent to which *L. monocytogenes* could grow. If the manufacturer is unable to demonstrate this, the food product should therefore comply with the criterion of absence in 25 g.

This demonstration is a matter of high interest for manufacturers of food industries which produce RTE foods, as *L. monocytogenes* can potentially be present on them and grow as long as the food product and storage characteristics support it. *L. monocytogenes* may be present on processing equipment and facilities (walls, floors, drains, etc.) (Flores et al., 2004), and contaminate food via water droplets, splashing, dust particles from the ceiling, and contact surfaces, including transfer by workers hands (Grau, 1993).

Beside this, several studies have reported the presence of *L. monocytogenes*, i.e. prevalence, in various food commodities (De Simon et al., 1992; Macgowan et al., 1994; Gaya et al., 1998; SCVPH, 1999; Nørrung et al., 1999; Gombas et al., 2003; Vitas et al., 2004). For instance, SCVPH reported the prevalence in various food products: 7–36% in minced meat, 0–52% in meat products, 4–60% in fish products and 1–12% in salads. Consequently, RTE foods seem to be a potential vehicle for this pathogen which, if present in the RTE product, must be assessed in terms of numbers at the time of consumption.

Predictive microbiology is an area of food microbiology in which microbial responses to environmental factors are measured under defined and controlled conditions. The responses are quantified and summarized in the form of mathematical equations which, by interpolation, can predict responses to novel sets of conditions, i.e. those which were not actually tested (Ross and McMeekin, 1995). A mathematical model must be validated in order to ensure that predictions of the model are applicable to real situations (Brocklehurst, 2004). Once a model has been validated in a product, it can be used at all stages of the food chain from harvesting, through production and processing, to distribution and retail sale. Modelling is already being incorporated into Hazard Analysis Critical Control Point (HACCP) systems, product development, and quantitative risk assessment (Baranyi and Roberts, 2000).

This study shows another application of quantitative models, which is its use as a tool to help manufacturers to evaluate the compliance or not of RTE foods with microbiological criteria. In this paper, a food commodity is given as an example: cooked ham. The objective of this work was (i) to assess the level of *L. monocytogenes* at the end of the shelf-life of three commercial brands of cooked ham by means of predictive models from two approaches: point-estimate and probabilistic; and (ii) management of the safety of cooked ham.

2. Materials and methods

2.1. Scope

The product subjected to the present study is prepackaged cooked ham. The starting point of this study was set at the stage when the product is just manufactured and ready to be placed in the market, being the final point the end of its shelf-life. During this time, cooked ham was supposed to be kept at refrigeration temperatures due to its perishable nature. The growth of *L. monocytogenes* was then modelled during distribution and storage at refrigeration.

It was assumed a contamination of cooked ham at the end of its manufacture, when subjected to slicing (cross-contamination), as cross-contamination is the usual source of contamination for

cooked meat products (Bell and Kyriakides, 2003). For this reason, the lag phase of *L. monocytogenes* was considered.

2.2. Predictive models

Two secondary models were selected on the basis of food product specificity and validation of the model: lag time (λ) (Eq. (1)) and maximum specific growth rate (μ_{max}) (Eq. (2)), both of them being response surface models reported by Devlieghere et al. (2001):

$$\begin{aligned}\mathrm{Ln}\lambda = {} & 51.1874 - 0.4967 \cdot T \\ & + 6.0668 \cdot 10^{-04} \cdot CO_2 - 44.8051 \cdot a_w + 0.5566 \cdot \mathrm{NaL} \\ & + 1.4918 \cdot 10^{-02} \cdot T^2 - 4.8613 \cdot 10^{-02} \cdot \mathrm{NaL}^2 \\ & + 3.1252 \cdot 10^{-04} \cdot \mathrm{NaL} \cdot CO_2 \end{aligned} \quad (1)$$

where λ is the lag phase; T is temperature; CO_2 is the concentration of dissolved CO_2 (mg l^{-1}); a_w is water activity, and NaL is the concentration of Na-lactate (% w/w).

$$\begin{aligned}\sqrt{\mu_{max}} = {} & -1.0636 - 0.1469 \cdot T + 5.8168 \cdot 10^{-04} \cdot CO_2 \\ & + 1.1532 \cdot a_w - 2.8161 \cdot 10^{-04} \cdot \mathrm{NaL} \\ & + 0.1733 \cdot T \cdot a_w - 2.5119 \cdot 10^{-03} \cdot T \cdot \mathrm{NaL} \\ & - 4.4792 \cdot 10^{-06} \cdot T \cdot CO_2 - 6.0063 \cdot 10^{-04} \cdot a_w \cdot CO_2 \end{aligned} \quad (2)$$

where μ_{max} is the maximum specific growth rate; T, CO_2, a_w and NaL are as described in Eq. (1).

In the equations above, the concentration of dissolved CO_2 (mg l^{-1}) was calculated by means of combining the equation suggested by Devlieghere et al. (1998), which predicts the concentration of CO_2 in the water phase as a function of the percentage of CO_2 in the initial headspace gas, the initial gas/product volume ratio and Henry's constant, and the equation which predicts the Henry's constant as a function of temperature (between 0 and 160 °C), giving realistic predictions for concentrations of dissolved CO_2, as reported by Ross and Dalgaard (2004).

Finally, the reparameterized version of the Gompertz equation (Eq. (3)) (Zwietering et al., 1990) was used to calculate the final number of cells of *L. monocytogenes* at the end of the shelf-life of the products.

$$\mathrm{Ln}(n) = \mathrm{Ln}(n_0) + A\exp\left(-\exp\left(\frac{\mu_{max} \cdot \exp(1)}{A}(\lambda - t) + 1\right)\right) \quad (3)$$

where μ_{max} is the maximum specific growth rate (h^{-1}); n_0 is the initial number of cells (cfu/g), i.e. when the product is ready to be placed on the market; A = is the maximum level of increase Ln (n_∞/n_o); t is the shelf-life of the product (h), and λ is the lag phase (h).

In order to limit the exponential growth at a certain level, i.e. maximum population density (MPD), raw growth data for ham generated by Leatherhead Food Research Association (LFRA) was taken from ComBase v.3 (Baranyi and Tamplin, 2004); two groups of records were used on the basis of water activity values near to those found in the samples collected: 0.988 in one hand and 0.981 in the other hand. The maximum levels of cells reported in ComBase for both groups of data were organized by temperatures, as can be observed in Table 1. A linear regression was performed to link temperature (between 2 and 8 °C) to MPD within each a_w group. The equations calculated at a_w=0.988 and a_w=0.981 were, respectively, Eqs. (4) and (5):

$$\mathrm{MPD} = 0.3323 \cdot T + 5.255 \quad (4)$$

$$\mathrm{MPD} = 0.2857 \cdot T + 3.695 \quad (5)$$

where MPD is the maximum population density ($\log_{10}$ cfu/g), and T is the temperature (°C). Eq. (4) was used in the case of brand A, while Eq. (5) was used for brands B and C (see a_w values for the three brands in Table 2).

For temperatures above 8 °C, a MPD constant value of 8 $\log_{10}$ cfu/g was assumed.

2.3. Samples

Three commercial brands of cooked ham were sampled from local markets. Ideally, they should have been collected at the stage when they were just manufactured and ready to be placed in the market, but it was not possible due to confidentiality. Samples were analyzed in order to get information as required in Eqs. (1) and (2), with the exception of temperature and storage time, which will be discussed below. This information is shown in Table 2.

2.4. Point-estimate approach versus probabilistic approach

In the point-estimate approach, the temperature value entered in the model is that recommended by the producer and stated on the label of the product; the shelf-life was not revealed by the producer because of being confidential, so it was assumed to be the time elapsed between the date of collection of the product and the "use-by" date, stated on the label of the product. It will be called "actual shelf-life" throughout the paper.

Table 2 shows the temperature and shelf-life values for the products tested.

Also, an estimate of the initial level of *L. monocytogenes* (at the stage when the product is ready to be placed on the market)

Table 1
Maximum population density (MPD) observed in experiments performed in ham and reported by Leatherhead Food Research Association (LFRA) in ComBase v.3

T[a]	a_w[b]	MPD[c]
2	0.981	4.52
	0.988	6.35
4	0.981	4.95
	0.988	5.79
6	0.981	4.41
	0.988	7.52
8	0.981	6.60
	0.988	7.99

[a] T: temperature (°C).
[b] a_w: water activity.
[c] MPD: maximum population density ($\log_{10}$ cfu/g). Each MPD value is the average of 6 records.

Table 2
Physical and chemical characteristics, actual shelf-life (days) and storage temperature (°C) for three commercial brands of cooked ham

Commercial brand	Initial % CO_2 [a]	G/P [b]	NaL [c]	a_w [d]	Actual shelf-life	Storage temperature
A	13.8333	1.9787	0	0.988	41	5
B	13.5667	1.2581	2	0.979	55	5
C	30.1333	0.359	0	0.980	34	5

[a] Initial % CO_2: percentage of CO_2 in the initial headspace gas.
[b] G/P: initial gas/product volume ratio.
[c] NaL: concentration of Na-lactate (% w/w).
[d] a_w: water activity.

was selected as follows: Garrido et al. (2004) reported the level of *L. monocytogenes* found in 252 samples of sliced cooked meat products; the mode range (10–100 cfu/g) was considered as the initial level, and, for a point-estimate approach, the geometric mean of this range was calculated, resulting in 32 cfu/g.

For the probabilistic approach, Monte Carlo simulations (with 10,000 iterations) were carried out with Palisade @Risk Professional© software (Newfield, NY USA). A normal distribution was fitted to the frequency distribution of temperatures reported by Azevedo et al. (2005), with a mean of 7.3 °C, and a standard deviation of 2.31; it was truncated at 2 °C as minimum temperature and 13 °C as the maximum. The shelf-life was the same as above (actual shelf-life). The initial distribution of cells was a cumulative distribution built from data reported by Garrido et al. (2004); The minimum and maximum values were 0 and 4 $\log_{10}$ cfu/g, respectively; the median was 1.22 log cfu/g and the percentile associated to 100 cfu/g was 84.03%.

2.5. Management of the safety of the products

After implementation of the model, another period of time for shelf-life (it will be denoted as "novel shelf-life") was explored for both approaches. A novel shelf-life of 10 days was selected as an example.

3. Results and discussion

In this paper, a quantitative demonstration of the achievement or not of 100 cfu/g of *L. monocytogenes* at the end of the shelf-life of RTE products was illustrated with an example: prepackaged cooked ham.

In the point-estimate approach, the levels reached for the three brands of cooked ham at the end of the actual and novel shelf-life are shown in Table 3. The level of *L. monocytogenes* exceeded largely the microbiological criterion of 100 cfu/g at the end of the actual shelf-life in the products initially contaminated (9.7% prevalence). In such case, the producer should comply with the microbiological criterion of absence in 25 g, with the sampling plan applied ($n=5$, $c=0$). However, if the producer still observes a certain prevalence of *L. monocytogenes* in the product, he may make the decision of shorting the shelf-life; for a novel shelf-life of 10 days, the level of *L. monocytogenes* in all products were reduced, but only the product B resulted in acceptable levels (32 cfu/g); in this case, only the manufacturer of product B should comply with the criterion <100 cfu/g at the stage when products are placed on the market and during their shelf-life, and, subsequently, the presence of the microorganism at the assumed initial level or below, is allowed.

Table 3
Level N (cfu/g) of *L. monocytogenes* at the end of the actual shelf-life (see Table 2) and at the end of the novel shelf-life (10 days) of three brands of cooked ham

Commercial brand	Actual shelf-life	Novel shelf-life
A	7.3E+06	2.6E+02
B	9.2E+05	3.2E+01
C	1.3E+05	1.1E+02

Table 4
Percentiles (%) corresponding to ≤100 cfu/g of *L. monocytogenes* at the end of the actual shelf-life (see Table 2) and at the end of the novel shelf-life (10 days) for three brands of cooked ham

Commercial brand	Actual shelf-life	Novel shelf-life
A	0	14.51
B	4.92	75.90
C	0.90	73.90

In the probabilistic approach, the percentiles corresponding to 100 cfu/g at the end of the actual and novel shelf-life for the three brands of prepacked cooked ham, can be seen in Table 4. When calculating the level of *L. monocytogenes* at the end of the actual shelf-life, it was found that all products presented very low percentile values corresponding to 100 cfu/g, which can be translated into very high probability (100% for product A) of exceeding the limit 100 cfu/g at the end of the actual shelf-life. When applying the novel shelf-life of 10 days, it was observed that the most noticeable changes in percentiles corresponding to 100 cfu/g were those of products B and C

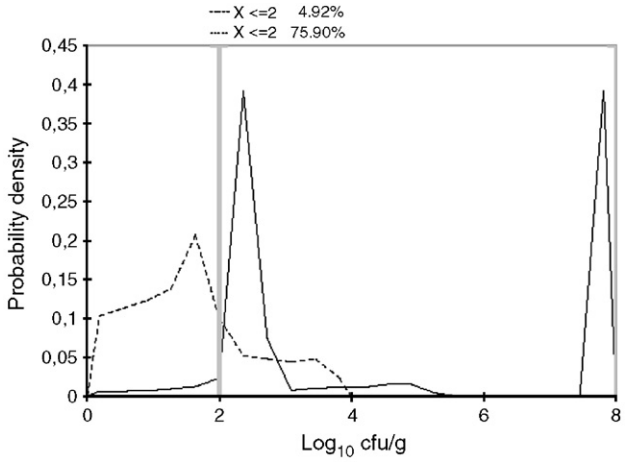

Fig. 1. Probability distribution of the level Y ($\log_{10}$ cfu/g) of *L. monocytogenes* at the end of the actual (——) and novel (- - - - -) shelf-life for commercial brand B.

(from 4.92% to 70.90%, and from 0.90% to 73.90% percentile, respectively); a probability distribution for the level of *L. monocytogenes* at the end of the actual and novel shelf-life for the commercial brand B, can be observed in Fig. 1. In the case of product A, the change in percentile was much slighter (14.51% percentile change). At first sight, a 100% probability of reaching levels below 100 cfu/g cannot be demonstrated with the assumptions and calculations performed; in fact, unless the actual initial concentration of cells is always below 100 cfu/g, there will always be a certain probability associated to levels above 100 cfu/g. In this case, the microbiological criterion of absence in 25 g should be achieved. However, in a hypothetical case of a meat industry reporting levels below 100 cfu/g at the stage when it is just manufactured, a demonstration like the one presented in this paper could be performed, and the percentile corresponding to 100 cfu/g at the end of the shelf-life could be very high, or even 100% (no samples exceeding 100 cfu/g). Probabilistic approaches usually yield a certain probability of having extreme values, which are often unacceptable, and, however, must be tolerated. The competent sanitary authority should decide which is tolerable (1%, 5%, 10%, etc.). This idea is in accordance with recent proposals (Havelaar et al., 2004; Whiting et al., 2006) of setting a margin of acceptability of defective product when applying a Food Safety Objective (FSO), a basis to develop microbiological criteria and other pathogens management strategies.

It is worthy to remark cross-contamination as the main source of contamination of cooked ham, responsible for the numbers of *L. monocytogenes* in the product (Bell and Kyriakides, 2003), being the assumption made in this paper. In this sense, the scope of manufacturers' responsibility in the safety of their product is of great importance. So, if the product was sliced at the factory, which is the case assumed in this study, the manufacturer should compile microbiological information related to presence/absence and concentration of *L. monocytogenes* in the product, and perform a demonstration as presented in this paper. However, when the cross-contamination event is beyond the control of manufacturers, such as the case of slicing at retail points, it seems to be a matter of other parts dealing with the product within the food chain, like retailers, to evaluate if the cross-contamination takes place, and, in such case, gather related information and include contamination levels of *L. monocytogenes* to perform a demonstration as in the present work.

The use of either deterministic or probabilistic approach leads to different results, and, consequently, different decisions could be derived from them. Competent sanitary authorities should agree in the approach selected for this demonstration. Beside this, some assumptions made in this paper, like predictive models used or temperature distribution, should also be approved and harmonized by the competent sanitary authority for the sake of, in a first step, comparison between different industries within a country, and, in a second step, comparison between industries in different countries. Also, if the competent sanitary authority was interested in comparing these results with national exposure assessments to biological hazards, every assumption should be carefully taken into account, and, if possible, make it equal to those made in national exposure assessments. It is not the intention of this paper to discuss the assumptions made here, but the practical application of emerging Regulations related to microbiological criteria in foods.

Beside this, this kind of studies is of great importance for establishing safety-based "use-by" date labels. In this sense, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (ILSI Research Foundation, 2005) was asked to provide advice on the requisite scientific parameters for establishing safety-based "use-by" dates for refrigerated RTE foods to help reduce the incidence of food-borne illness. More specifically, the Committee addressed the following question, among others: «Should safety-based "use-by" dates for refrigerated RTE foods be established using mathematical modelling techniques? If so, what modelling approaches are best suited to the development of safety-based "use-by" date labels for refrigerated RTE foods?». The Committee reviewed briefly some available predictive softwares, together with advantages and drawbacks, but no examples were given. In this paper, a direct application is shown with prepackaged cooked ham as a RTE product.

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