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Management of *Listeria monocytogenes* in fermented sausages using the Food Safety Objective concept underpinned by stochastic modeling and meta-analysis

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ABSTRACT

In the present work, a demonstration is made on how the risk from the presence of *Listeria monocytogenes* in fermented sausages can be managed using the concept of Food Safety Objective (*FSO*) aided by stochastic modeling (Bayesian analysis and Monte Carlo simulation) and meta-analysis. For this purpose, the ICMSF equation was used, which combines the initial level (H_0) of the hazard and its subsequent reduction (ΣR) and/or increase (ΣI) along the production chain. Each element of the equation was described by a distribution to investigate the effect not only of the level of the hazard, but also the effect of the accompanying variability. The distribution of each element was determined by Bayesian modeling (H_0) and meta-analysis (ΣR and ΣI). The output was a normal distribution $N(-5.36, 2.56)$ (log cfu/g) from which the percentage of the non-conforming products, i.e. the fraction above the *FSO* of 2 log cfu/g, was estimated at 0.202%. Different control measures were examined such as lowering initial *L. monocytogenes* level and inclusion of an additional killing step along the process resulting in reduction of the non-conforming products from 0.195% to 0.003% based on the mean and/or square-root change of the normal distribution, and 0.001%, respectively.

1. Introduction

Nowadays, sophisticated tools are available to Food Business Operators (FBOs) and risk managers in order to be in position to assess and control the safety of any food product (Perni et al., 2009). To achieve an Appropriate Level Of Protection (*ALOP*), a maximum frequency and/or concentration of a hazard in a food at the time of consumption is set, known as Food Safety Objective (*FSO*) (Codex Alimentarius Commission, 2004). Intermediate targets such as Performance Objectives (*PO*) are also set along the food chain contributing to meet the *FSO*. The equation proposed by the International Commission on Microbiological Specifications for Foods (ICMSF, 2002), that compares the microbiological level of the final product to the *FSO*, can be used to determine this intermediate targets and to evaluate whether they allow to meet the *FSO* or not. Then, FBOs and risk managers can investigate what control measures should be applied to meet the different targets.

For every food process, the elements of the first part of the ICMSF equation, namely initial level (H_0), total decrease (ΣR) and total increase (ΣI) of the microorganisms of interest, should not have a single value, but a range of values. Indeed, the use of a single value for the elements of the ICMSF equation to describe the microbial alterations during food process is not a representative approach because no consideration is taken for the possible variability and uncertainty of the output (Nauta, 2002; Pouillot and Lubran, 2011). Probability distributions and stochastic modeling are means of describing such variability and uncertainty. Bayesian analysis has been recently introduced in the field of exposure assessment for food safety to improve the accuracy of probabilistic models (Crepet et al., 2009; Delignette-Muller et al., 2006; Jaloustre et al., 2011; Pouillot et al., 2003). Probability distributions, assigned to the prior knowledge, are combined with experimental data to produce updated posterior probability distributions. Bayesian analysis is a powerful tool for reducing uncertainty of the estimated

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parameters and handle them in a probabilistic way (Lesaffre et al., 2007).

Meta-analysis is a statistical technique which combines the data from individual studies to provide a summary effect of a treatment or intervention. The power of this technique lies in its ability to combine different studies and provide a combined estimate with increased statistical power and broader applicability than an estimate originating from a single study (Borenstein et al., 2009). Recently, meta-analysis has been introduced in the field of food safety (Gonzales-Barron et al., 2008; Sanchez et al., 2007; Vialette et al., 2005).

The objective of this study was to demonstrate how fermented sausages safety can be managed with regards to *Listeria monocytogenes*, using the ICMSF equation and by taking into account variability and uncertainty of the parameters. The effectiveness of different control measures on the fraction of products exceeding the *FSO* is also illustrated. This allows FBOs to identify the stage(s) involved in the production chain with the greatest impact on the number of non-conforming products.

2. Materials and methods

2.1. Management of L. monocytogenes in fermented sausages using the FSO concept

The objective of the study was first to assess to what extent the ICMSF equation (ICMSF, 2002) applied to *L. monocytogenes* in fermented sausages was verified, by calculating the percentage of non-conforming products regarding the *FSO*, and then to determine what management options could be performed to reduce this percentage. The equation is as follows:

$$H_0 - \sum R + \sum I \leq FSO \quad (1)$$

where H_0, the initial level of *L. monocytogenes* in the meat batter of fermented sausages; ΣR, the total reduction of *L. monocytogenes* during the whole process; and ΣI, the total increase of *L. monocytogenes* (growth and/or recontamination) during the whole process. For the demonstration purposes of this study, the following assumptions were made:

i. Sliced air- or vacuum-packaged fermented sausages.
ii. Cold storage at refrigeration temperatures (4–5 °C).
iii. Shelf life equal to 30 days.
iv. *FSO* equal to 100 cfu/g or 2 log cfu/g referred to EC regulation 2073/2005 and its amendment 1441/2007 (Anonymous, 2005, 2007).
v. Random distribution of *L. monocytogenes* in the batter.
vi. All elements of the ICMSF equation are log normally distributed (Zwietering et al., 2010).
vii. Calculations are valid even for low *L. monocytogenes* counts (log cfu/g) (Zwietering et al., 2010).
viii. Recontamination of the fermented sausages during their selling is negligible since the products are vacuum-packaged.

Each element of the first part of Equation (1) was described by a normal distribution to include variability as explained in the following subsections. The resulting output is a normal distribution describing the level of *L. monocytogenes* in fermented sausages at the end of the shelf-life. It is characterized by a mean value (μ) and standard deviation (σ) equal to the sum of the means of H_0, ΣR and ΣI, and the square root of the sum of the squares of the elements standard deviations, respectively (Zwietering et al., 2010). Monte-Carlo simulations were performed using ten thousand iterations with the @Risk v4.5 software (Palisade Corp., Ithaca, NY, USA) to assess the final exposition to *L. monocytogenes*. From the final distribution the fraction of the products exceeding the *FSO* (non-conforming products) can be estimated with the use of the z value:

$$z = (X - \mu)/\sigma \quad (2)$$

where X is the *FSO*; μ and σ are the mean value and the standard deviation of the final distribution, respectively. From the calculated z value, the area of the normal distribution below the *FSO* can be determined from the respective tables of the normal distribution or using the Excel function NORMSDIST(z). Hence, the area exceeding the *FSO* limit representing the non-conforming products will be 1 − area below the *FSO* limit or 1 − NORMSDIST(z).

2.2. Determination of the initial population of L. monocytogenes in the batter of fermented sausages

Bayesian modeling was employed to calculate the posterior distribution of the initial level of *L. monocytogenes* (H_0) in the meat batter of fermented sausages from presence/absence data. The model was constructed in the Microsoft Excel 2007 (Microsoft, Redmond, WA, USA) and simulated with the @Risk v4.5 software according to Vose (2008) (Fig. 1). Details on the model construction and functions used are given in Andritsos et al. (2013). Very briefly, four columns were created: a) concentration of the pathogen (cfu/kg), b) prior, c) likelihood function and d) posterior. The concentration of the pathogen varied from 0.05 to 100, with a 0.05 step (Vose, 2008). The prior was equal to one since no prior information was available relative to *L. monocytogenes* concentration (uniformed prior) (Vose, 2008). The likelihood function was equal to a binomial distribution (number of successes, number of independent trials, and probability of success on each trial). For the first two parameters, the presence/absence data from the study of Martin et al. (2011), relative to detection of *L. monocytogenes* in the meat batter of fermented sausages, was used. Nineteen meat batter samples ($n = 19$) of 25 g (s) each were tested for *L. monocytogenes* presence. From the analyzed samples, 47.4% or 9 samples were tested positive. In this study, diluted meat samples were plated onto Agar *Listeria* according to Ottaviani and Agosti (ALOA) plates. Culture media, however, are not perfect in detecting the true prevalence of a pathogen, i.e. sensitivity (se) = 100% (Habib et al., 2008). Thus, to estimate the initial level of *L. monocytogenes*, the se of ALOA (67%) described by a beta distribution (se = beta(16,8) = 67%) taken from the study of Andritsos et al. (2013) was considered. The se of ALOA for the detection of *L. monocytogenes* in the meat batter of fermented sausages (Martin et al., 2011) was assumed to be similar to the se of the same culture medium for the detection of *L. monocytogenes* in minced pork meat (Andritsos et al., 2013). The third parameter was given by a Poisson probability mass function: $1 - \mathrm{EXP}(-\lambda \times s \times se)$, where λ is the concentration (first column) of *L. monocytogenes* (cfu/kg), s is the sample size analyzed (25 g or 0.025 kg) and se is the sensitivity of ALOA. Finally, the posterior distribution was equal to RiskMean(prior × likelihood). The @Risk v4.5 software was used to describe the shape of the resulting posterior distribution.

2.3. Meta-analysis of the in situ L. monocytogenes behavior during production and storage of fermented sausages

A literature search was performed in the databases of Sciencedirect, Scopus and PubMed to identify published papers written in English relative to *in situ* survival of *L. monocytogenes* in fermented sausages during their production and cold storage. "*L. monocytogenes*, prevalence, fermented sausages, fermented meats,