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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 *L. 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.

Keywords: Bayesian modeling, fermented meats, foodborne pathogens, Food Safety Objective, HACCP, meta-analysis

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 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 *L. 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:

$$H_0 - \Sigma R + \Sigma I \leq FSO \qquad (1)$$

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:

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

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: $1 - EXP(-\lambda \times s \times se)$, 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. "*Listeria monocytogenes*, prevalence, fermented sausages, fermented meats, salami,

non-thermal inactivation, survival, bacterial resistance, and cold storage" were the keywords used, during the search, alone or in combination. The keyword "growth" was not included since no additional papers relative to fermented sausages were found by using this keyword. The reference list of each paper found was searched as well to cover any additional publications of interest. The identified studies contained data related to different types of fermented sausages or schedules of fermentation and drying. Datasets for such papers were considered as independent investigations. Thus, more than one dataset per paper were included in the meta-analysis. The following information was extracted from each published work before fermentation (start of process) or cold storage at 4°C (start of storage) and after ripening (end of process) or 30 days of storage at 4°C (assumed shelf life): i) the mean value (*m*) of *L. monocytogenes* counts (log cfu/g), ii) the standard deviation (*sd*) of these measurements and iii) the number of samples of fermented sausages analyzed (*n*). After collecting the data, the effect size which allows the comparison and summation of the independent studies was determined. Because this kind of data was in a continuous form (*m* and *sd*) and the measurement scale was the same for all studies, the mean difference (*md*) was used which is the difference in the mean values between control (before fermentation or cold storage) and treated (after ripening or end of shelf life) groups (Borenstein et al., 2009). The final summary or combined effect was the average of the weighted effect sizes from each study. Weighting of the individual estimates was performed using the inverse variances method (Bax, 2011; Bax et al., 2006; Borenstein et al., 2009) accounting for the precision of each individual effect size as reflected by the sample size, quality of research design or other factors that may influence reliability and validity (Gonzales-Barron et al., 2008). To demonstrate a significant summary effect, a random-effects model was used

because it accounts for the between-study variability (heterogeneity). The heterogeneity between studies was assessed with the following statistics: Q, I^2 and τ^2. All these statistics reflect a certain dimension of the extent of heterogeneity between the studies in the data set (Borenstein et al., 2009).

The combined effect size is given in the form of a mean value with the accompanying 95% confidence (*ci*- and *ci*+) and prediction (*pi*- and *pi*+) interval from which the needed *sd* can be estimated using the following equation (Zwietering et al., 2010):

$$sd = [0.5 \times (pi+ - pi-)] / 1.96 \qquad (3)$$

where *pi*+ and *pi*- were the *max* and *min* values of the 95% prediction interval of the mean value of the summary effect.

Meta-analysis was performed by using the Excel add-in Mix Professional v2.0 (Bax, 2011; Bax et al., 2006). The *L. monocytogenes* counts were extracted from the corresponding Figures or Tables of the published works. For the extraction of the data from the published Figures the Ungraph 5 (Biosoft, Cambridge, UK) software was used.

3. Results and Discussion

3.1. Initial level of L. monocytogenes (H_0)

Initial level of *L. monocytogenes* (H_0) in the meat batter of fermented sausages was estimated at 45.03 cfu/kg (*sd* = 16.37 and 95% confidence interval = 20.00 to 83.50 cfu/kg) following a lognormal distribution or -1.43 log cfu/g (*sd* = 0.16 and 95% confidence interval = -1.74 to -1.11 log cfu/g) (Fig. 2). Initial level of *L. monocytogenes* was estimated using Bayesian modeling, which combines prior knowledge with the data available to give the updated posterior distributions. In this way the parameter of interest is handled in a probabilistic way (Lesaffre et al., 2007).

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

Nine and four studies reporting on *in situ L. monocytogenes* behavior during production (fermentation and ripening) and storage of fermented sausages, respectively were found (Table 1). Results showed non-thermal inactivation for the two process steps considered. Some of the studies contained more than one data set allowing the inclusion in the meta-analysis of 22 (for the production) and 7 (for the storage) data sets, in total, including 29 (for the production) and 16 (for the storage) different *L. monocytogenes* strains of different physiological state, and various fermented sausages with different technology of production and characteristics in terms of pH and a_w. The data were analyzed to derive the following parameters of interest: *m*, *sd* and *n* before fermentation or storage (control group) and after drying or end of shelf life (treated group) (Table 1).

The *L. monocytogenes* data from the challenge tests during production and storage of fermented sausages found in the literature were analyzed using meta-analysis. Since the inactivation of *L. monocytogenes* was not linear in all cases, the output considered was the *md* in the pathogen concentration between the starting (before fermentation or storage) and ending (after ripening or shelf life) point of the process. The results revealed a significant ($P < 0.001$) *L. monocytogenes* inactivation in the fermented sausages during production and post-process storage (Fig. 3). All studies displayed a negative *md*, which indicates that non-thermal inactivation occurs during fermentation-drying and cold storage. The *Q* statistics for the *md* were found to be significant ($P < 0.001$) in both cases, indicating that the true effects vary among studies. The variance (τ^2) of the true effect sizes, which reflects the amount of true

heterogeneity, was estimated to be 1.34 (1.07 to 1.67) and 2.46 (1.48 to 4.05) during process and cold storage of fermented sausages, respectively. Accordingly, the I^2 statistic, which reflects the proportion of the observed dispersion that is due to this heterogeneity, was 97.2% (96.5 to 97.7%) and 95.1% (92.1 to 97.0%), respectively. Such high value of the I^2 statistic indicates that most of the observed variance is real as explained below. These results support the choice of the random-effects model since there was no consistence across effect sizes of the studies (heterogeneity).

A random-effects model is more appropriate than a fixed-effects model considering the various sources of variation among studies such as sampling, measurement error, *L. monocytogenes* strains, physiological state of the inoculum, method and level of inoculation, type of fermented sausage, product characteristics in terms of pH and a_w, fermentation and ripening program, fermented sausages manufactured with starter cultures producing or not bacteriocins, and fermented sausages manufactured with the addition or not of antimicrobial agents such bacteriocins. The random-effects model compared to the fixed-effects includes an additional random error accounting for the extra variation which is supposed to be normally distributed. In random-effects meta-analysis, it is usually assumed that the true effects are normally distributed (Borenstein et al., 2009). Therefore, in the current study the resulting summary effect from the random-effects model can be described by a normal distribution $N(m, sd)$ (log cfu/g): $N(-1.89, 1.27)$ for the fermentation-drying step, and $N(-2.04, 2.22)$ for the cold storage step. To estimate the *sd* of the normal curve, the prediction interval was used in the equation 3 because it reflects the uncertainty of the combined effect size (Borenstein et al., 2009). The meta-analysis indicated that fermentation and ripening, and cold storage, on average, would be expected to reduce *L. monocytogenes*

concentration by approximately 2 logs each [*m*, -1.89 (-2.42 to -1.35) and -2.04 (-3.28 to -0.80) log cfu/g during process and storage of fermented sausages, respectively]. Evidence for the *L. monocytogenes* inactivation during production and cold storage of fermented sausages has been documented (Byelashov et al., 2009; Degenhardt and Sant' Anna, 2007; Drosinos et al., 2006; Farber et al., 1993; Foegeding et al., 1992; Gareis et al., 2012; Gounadaki et al., 2007; Lahti et al., 2001; Mataragas et al., 2015a, 2015b, 2015c, 2015d; Porto-Fett et al., 2008; Simpson et al., 2008; Thevenot et al., 2005) and the meta-analysis confirmed this effect, but it provided also a quantitative estimation of the overall effect of these processes on *L. monocytogenes* survival in the fermented sausages. Meta-analysis is a powerful statistical method analyzing a relatively large amount of data from different individual studies with different experimental designs to produce a more precise with greater statistical power estimate of a particular intervention/treatment or to identify sources of variation (Gonzales-Barron et al., 2008). The extent of *L. monocytogenes* inactivation, however, is influenced not only by the temperature applied during fermentation or storage but also the product characteristics in terms of pH and a_w (Byelashov et al., 2009; Drosinos et al., 2006; Gounadaki et al., 2007; Mataragas et al., 2015a, 2015b, 2015d; Simpson et al., 2008). These factors are considered in the meta-analysis approach since the data analyzed are coming only from *in situ* challenge tests. Thus, all the factors influencing the *L. monocytogenes* inactivation are 'included' in the model.

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

To determine if the *FSO* will be met, the equation 1 was used by combining the distributions of $H_0 = N(-1.43, 0.16)$, $\Sigma R = R1 + R2$ with $R1 = N(-1.89, 1.27)$ and $R2 = N(-2.04, 2.22)$ as no increase was observed during the process steps of interest

through the meta-analysis, i.e. $\Sigma I = 0$. The resulting distribution is normal, having as *m* the sum of the means of the above elements (*m* = -1.43 - 1.89 - 2.04 = -5.36 log cfu/g) and *sd* the square root of the sum of the variances [$sd = (0.16^2 + 1.27^2 + 2.22^2)^{1/2} = 2.56$] (Zwietering et al., 2010). The level of *L. monocytogenes* in the products [*N*(-5.36, 2.56)] and the fraction (0.202%) of the non-conforming products above the *FSO* (2 log cfu/g) can be calculated from the resulting normal distribution following the equation 2 (Fig. 4, Table 2). Analysis of 3357 samples of dry/semi-dry sausages at retail level revealed the presence of *L. monocytogenes* in 3 samples (0.089%) with contamination level above 2 log cfu/g (between 100-1000 cfu/g) (USDA, 2003).

Compared to this baseline estimation of the fraction of the non-conforming products, the effect of some interventions on that percentage were examined (Table 2):

i. Lowering initial level (H_0) of *L. monocytogenes* (*m* or *sd* or both) in the meat batter by a certain value by lowering the pathogen level in raw material, e.g. meat. This could be achieved, for example, through better microbiological testing and selection of raw materials at the reception stage, selecting certified supplier or changing specifications of the current supplier. In this case, the final average of *L. monocytogenes* level in the meat batter and/or the final standard deviation are reduced, leading to remarkably lower percentage of non-conforming products, especially when the *m* value of the H_0 distribution is changed.

ii. Inclusion of an additional killing step along the process such as a holding step of the final products at elevated temperatures before their distribution. It has been observed that the higher the storage temperature the greater is the *L. monocytogenes* inactivation (Byelashov et al., 2009; Gounadaki et al., 2007; Mataragas et al., 2015d; Simpson et al., 2008). Assuming, therefore, a holding step of the products at 25°C for

13-14 days, an additional reduction step of *L. monocytogenes* level could be achieved (approximately 4.00 ± 0.63). The overall effectiveness of the process, as reflected by the percentage of the non-conforming products, is greatly enhanced. Compared to the baseline estimation, the final average level of *L. monocytogenes* in fermented sausages is lower (from -5.36 to -9.36 log cfu/g) and the final standard deviation of the level increases (from 2.56 to 2.63), but the percentage of the non-conforming products decreases to 0.001% from 0.202%.

4. Conclusions

In this study the management of *Listeria monocytogenes* in the fermented sausages based on the concept of *FSO* has been illustrated using stochastic modeling and meta-analysis. Each element of the ICMSF equation was described by a probability distribution to consider the effect of the level and variability of each process stage on the number of non-conforming products, i.e. products with contamination level exceeding the *FSO*. Furthermore, control measures along the production process were examined such as lowering the level and/or variability of the initial *L. monocytogenes* contamination, and the introduction of an additional killing step by holding the final product at an elevated temperature for a certain time of period. In this way, a better understanding is achieved for the expected result of such interventions on the compliance of the products with the *FSO*. Food Business Operators can identify the stage(s) of the production chain with the highest benefit relative to the reduction of the non-conforming products.

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Figure legends

Fig. 1. Model for the determination of *L. monocytogenes* initial load (H_0) in the meat batter of fermented sausages created in an excel file and simulated with the @Risk program.

Fig. 2. The a) lognormal (cfu/kg) or b) normal (log cfu/g) distribution of the initial level of *L. monocytogenes* concentration (H_0) in the meat batter of fermented sausages according to the Bayesian analysis of the presence/absence data taken from Martin et al. (2011).

Fig. 3. Mean difference (*md*) of *L. monocytogenes* concentration (log cfu/g) a) before fermentation and after ripening, and b) before storage at 4-5°C and after 30 days of storage (end of shelf life). Solid squares, the *md* of each individual study; and open diamond, the combined summary effect according to the random-effects model. The left and right angles of the diamond symbol represent the 95% confidence interval of the summary effect while the accompanied solid lines represent the 95% prediction interval.

Fig. 4. Normal distribution of the *L. monocytogenes* level [$N(-5.36, 2.56)$] in fermented sausages at the end of their shelf life (30 days) using as inputs the distribution of the elements $H_0 = N(-1.43, 0.16)$, $\Sigma R = R1 + R2$ with $R1 = N(-1.89, 1.27)$ and $R2 = N(-2.04, 2.22)$ and $\Sigma I = 0$. The percentage of the non-conforming products, exceeding the *FSO* limit (2 log cfu/g), is represented by the area of the curve located at the right of the *FSO* (dashed line), which is equal to 0.202%.

Table 1

Studies, included in the meta-analysis, relative to the *in situ L. monocytogenes* behavior (mean, *m*; standard deviation, *sd*; and number of samples analyzed, *n*) during fermented sausages production (total number of samples considered = 281) and storage (total number of samples considered = 32) at 4-5°C for 30 days.

Reference	No. of strains	*L. monocytogenes* strain	Type of fermented sausage	After ripening or 30 days of storage at 4°C (Treated group)			Before fermentation or cold storage at 4°C (Control group)		
				m (log cfu/g)	*sd*	*n*	*m* (log cfu/g)	*sd*	*n*
Production									
Drosinos et al., 2006	1	NCTC 10527	Sremska (Serbia-Montenegro)	0.61	0.24	27	3.61	0.52	27
	1	NCTC 10527	Sudjuk (Bosnia-Herzegovina)	2.14	1.70	54	4.93	0.44	54
	1	NCTC 10527	Fermented dry sausage (Croatia)	1.49	1.79	63	4.43	0.59	63
	1	NCTC 10527	Fermented dry sausage (Hungary)	3.88	0.68	72	5.51	0.24	72
Degenhardt and Sant' Anna, 2007	1	ATCC 7644	Italian-style fermented sausage	-0.82	0.14	3	2.67	0.21	3
Foegeding et al., 1992	5	Scott A, F5069, ATCC 19115, NCF-U2K3, NCF-F1KK4	American-style fermented sausage	1.10	0.88	12	4.83	0.35	12
Gareis et al., 2012	3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)_PA_PrA[a]	2.51	0.15	2	3.48	1.02	2
	3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)_PA_PrB[a]	2.21	0.01	2	3.48	1.02	2
	3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)_PB_PrA[a]	1.46	0.35	2	3.00	1.17	2
	3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)_PB_PrB[a]	1.10	0.11	2	3.00	1.17	2
	3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)_PC_PrA[a]	2.59	0.81	2	4.08	1.17	2
	3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)_PC_PrB[a]	2.31	0.73	2	4.08	1.17	2
	3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)_PD_PrA[a]	2.57	1.78	2	4.04	1.40	2
	3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)_PD_PrB[a]	2.05	1.02	2	4.04	1.40	2
Farber et al., 1993	5	-	German- and American-style fermented sausages	-0.57	0.74	2	4.49	0.67	2
Thevenot et al., 2005	8	SR1, SR2, HC1, HC2, Saus1, Saus2, Equi1, Equi2	French-style fermented dry sausage	1.91	0.64	8	3.74	0.16	8
Lahti et al., 2001	1	EELA237	Finnish-style fermented dry sausage	3.14	1.16	4	4.43	1.07	4
Mataragas et al., 2015a	5		Cacciatore	5.64	0.31	4	6.17	0.10	4
	5		Felino	4.51	0.34	4	4.75	0.13	4
Mataragas et al., 2015b	5		Cacciatore	3.98	0.17	4	5.03	0.24	4
	5		Felino	4.25	0.13	4	4.88	0.10	4
	5		Milano	4.38	0.32	4	5.00	0.39	4
Storage									
Gounadaki et al., 2007	1	Scott A (serotype 4b)	Greek-style fermented sausage_Air[b]	3.26	0.57	2	5.55	1.34	2
	1	Scott A (serotype 4b)	Greek-style fermented sausage_Vacuum[b]	3.27	0.52	2	5.55	1.34	2
Simpson et al., 2008	10	N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, R2-765 (all serotype 4b), 558 (serotype 1/2), NA-1 (serotype 3b), N-7150 (serotype 3a)	Italian-style fermented sausage	0.77	0.92	4	4.18	0.07	4
Byelashov et al., 2009	10	N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, R2-765 (all serotype 4b), 558 (serotype 1/2), NA-1 (serotype 3b), N-7150 (serotype 3a)	Pepperoni (American-style)	-0.17	0.13	3	3.35	0.56	3
Porto-Fett et al., 2008	5	MFS2, MFS102, MFS104, MFS105, MFS110	Soudjouk-style fermented sausage_A[c]	6.55	0.84	6	6.65	0.43	6
	5	MFS2, MFS102, MFS104, MFS105, MFS110	Soudjouk-style fermented sausage_B[c]	5.80	0.13	6	6.06	0.50	6
	5	MFS2, MFS102, MFS104, MFS105, MFS110	Soudjouk-style fermented sausage_C[c]	3.17	0.68	9	5.77	0.16	9

[a] PA, PB, PC and PD, four different products were manufactured, i.e. product A, B, C and D, respectively; PrA and PrB, two different fermentation and ripening programs were applied for each product, i.e. program A and B, respectively

[b] Air, storage of the fermented sausage under air; Vacuum, storage of the fermented sausage under vacuum

[c] A, Experimentally manufactured fermented (pH 5.3) and dried soudjouk-style sausage; B, Experimentally manufactured fermented (pH 4.8) and dried soudjouk-style sausage; C, commercially manufactured soudjouk-style sausage

Table 2

The fraction (*f*) of non-conforming products, i.e. fermented sausages with *L. monocytogenes* level above 2 log cfu/g (*FSO*) at the end of shelf life (30 days), using as inputs the distributions estimated for each element (H_0 and $\Sigma R = R1 + R2$ since $\Sigma I = 0$) (baseline) and the effect of interventions 1 and 2 on that fraction.

Parameters	Baseline		Intervention 1[a]								Intervention 2[b]	
	m (log cfu/g)	*sd*	*m* (log cfu/g)	*sd*	*m* (log cfu/g)	*sd*	*m* (log cfu/g)	*sd*	*m* (log cfu/g)	*sd*	*m* (log cfu/g)	*sd*
H_0	-1.43	0.16	-2.43	0.16	-1.43	0.06	-2.43	0.06	-4.43	0.16	-1.43	0.16
R1	-1.89	1.27	-1.89	1.27	-1.89	1.27	-1.89	1.27	-1.89	1.27	-1.89	1.27
R2	-2.04	2.22	-2.04	2.22	-2.04	2.22	-2.04	2.22	-2.04	2.22	-2.04	2.22
Additional ΣR											-4.00	0.63
Final distribution[c]	-5.36	2.56	-6.36	2.56	-5.36	2.55	-6.36	2.55	-8.36	2.56	-9.36	2.63
f (%)[d]	0.202		0.055		0.195		0.052		0.003		0.001	

[a] Replacing the *m* (case 1 and 4) or *sd* (case 2) or both (case 3) of the distribution of *L. monocytogenes* initial level (H_0) in the meat batter by a certain value. From the left to right: Case 1, lowering *m* by 1 log cfu/g; Case 2, lowering *sd* by 0.10 units; Case 3, lowering *m* and *sd* by 1 log cfu/g and 0.10 units, respectively; and Case 4, lowering *m* by 3 log cfu/g

[b] Inclusion of an additional killing step before distribution of the final product by holding the fermented sausages at elevated temperatures for a certain period of time to achieve a further inactivation of the pathogen. After analyzing the data from Gounadaki et al., (2007), Simpson et al., (2008) and Byelashov et al., (2009), it was estimated that by holding the products at 25°C for 13-14 days an additional approximately 4.00 ± 0.63 log cfu/g reduction is achieved

[c] The parameters of the normal distribution were: *m* the sum of the means of the H_0 and $\Sigma R = R1 + R2$ elements since $\Sigma I = 0$ using the equation 1, i.e. for the baseline, *m* =

-1.43 - 1.89 - 2.04 = -5.36 log cfu/g, and *sd* the square root of the sum of the variances, i.e. for the baseline, $sd = (0.16^2 + 1.27^2 + 2.22^2)^{1/2} = 2.56$

[d] The percentage of the non-conforming products were estimated using the equation 2 with inputs the *m* and *sd* values of the final normal distribution, and the *FSO* limit of 2 log cfu/g, i.e. for the baseline, *f* = area of the normal curve above *FSO* = (1 - area of the normal curve below *FSO*) × 100 = [1 - NORMSDIST(*z*)] × 100 = {1 - NORMSDIST[(FSO - *m*) / *sd*]} × 100 = {1 - NORMSDIST[(2 - (-5.36)) / 2.56]} × 100 = 0.202%

Fig. 1

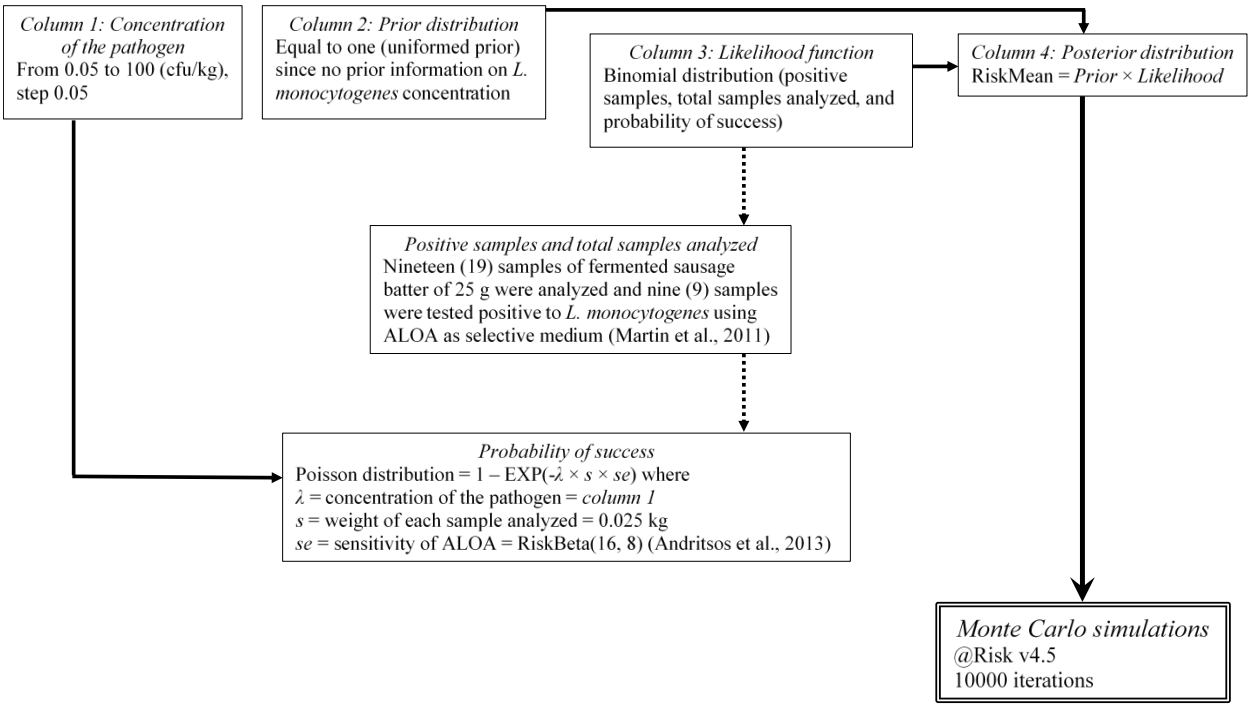

Column 1: Concentration of the pathogen
From 0.05 to 100 (cfu/kg), step 0.05
Column 2: Prior distribution
Equal to one (uniformed prior) since no prior information on L. monocytogenes concentration
Column 3: Likelihood function
Binomial distribution (positive samples, total samples analyzed, and probability of success)
Column 4: Posterior distribution
RiskMean = Prior × Likelihood
Positive samples and total samples analyzed
Nineteen (19) samples of fermented sausage batter of 25 g were analyzed and nine (9) samples were tested positive to L. monocytogenes using ALOA as selective medium (Martin et al., 2011)
Probability of success
Poisson distribution = 1 – EXP(-λ × s × se) where
λ = concentration of the pathogen = column 1
s = weight of each sample analyzed = 0.025 kg
se = sensitivity of ALOA = RiskBeta(16, 8) (Andritsos et al., 2013)
Monte Carlo simulations
@Risk v4.5
10000 iterations

Fig. 2

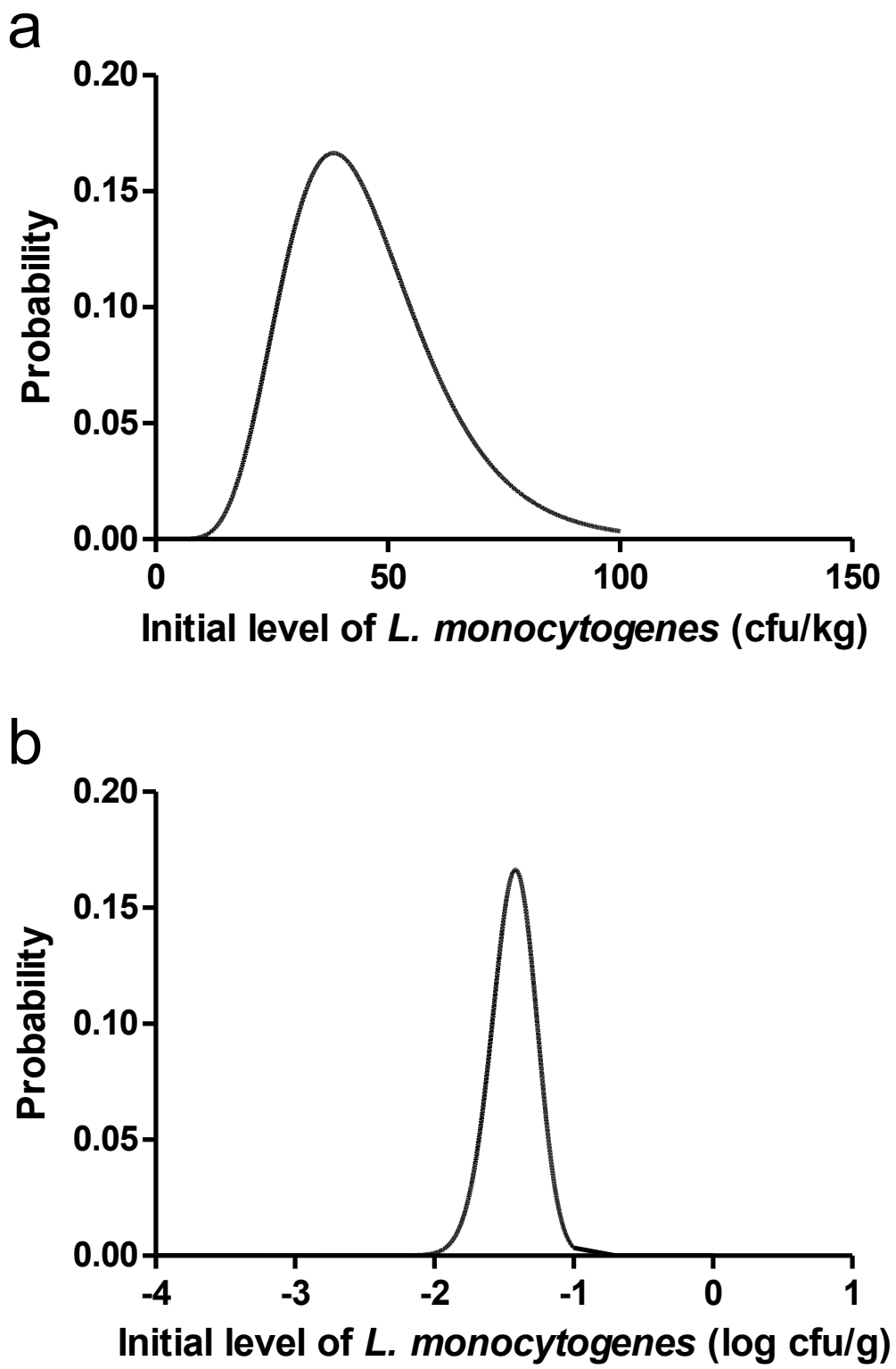
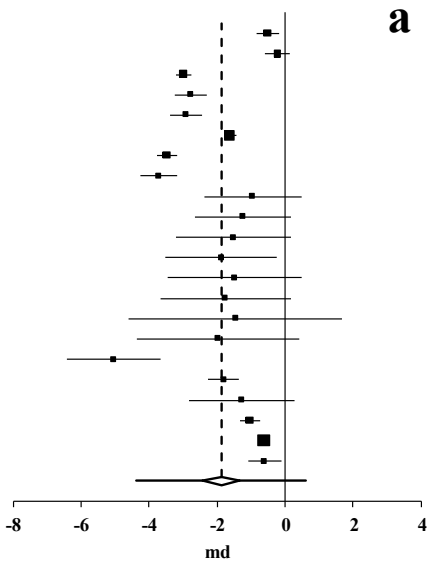

a
0.20
0.15
0.10
0.05
0.00
Probability
0
50
100
150
Initial level of *L. monocytogenes* (cfu/kg)
b
0.20
0.15
0.10
0.05
0.00
Probability
-4
-3
-2
-1
0
1
Initial level of *L. monocytogenes* (log cfu/g)

Fig. 3

ID	Author	Year	Measure	Weight %	P value
1	Mataragas et al.	2014a	-0.53	5.51%	<0.001
2	Mataragas et al.	2014a	-0.24	5.48%	0.19
3	Drosinos et al.	2006	-3.00	5.57%	<0.001
4	Drosinos et al.	2006	-2.79	5.39%	<0.001
5	Drosinos et al.	2006	-2.94	5.39%	<0.001
6	Drosinos et al.	2006	-1.63	5.59%	<0.001
7	Degenhardt and Sant'Anna	2007	-3.49	5.53%	<0.001
8	Foegeding et al.	1992	-3.73	5.32%	<0.001
9	Gareis et al.	2012	-0.97	4.02%	0.18
10	Gareis et al.	2012	-1.27	4.04%	0.08
11	Gareis et al.	2012	-1.54	3.61%	0.07
12	Gareis et al.	2012	-1.90	3.71%	0.02
13	Gareis et al.	2012	-1.49	3.20%	0.14
14	Gareis et al.	2012	-1.77	3.28%	0.07
15	Gareis et al.	2012	-1.47	1.93%	0.36
16	Gareis et al.	2012	-1.99	2.65%	0.10
17	Farber et al.	1993	-5.06	4.09%	<0.001
18	Thevenot et al.	2005	-1.83	5.40%	<0.001
19	Lahti et al.	2001	-1.29	3.83%	0.10
20	Mataragas et al.	2014b	-1.05	5.53%	<0.001
21	Mataragas et al.	2014b	-0.63	5.59%	<0.001
22	Mataragas et al.	2014b	-0.62	5.36%	0.01
Synthesis			**-1.89**	**100%**	**<0.001**



ID	Author	Year	Measure	Weight %	P value
1	Gounadaki et al.	2007	-2.30	11.28%	0.03
2	Gounadaki et al.	2007	-2.29	11.38%	0.02
3	Simpson et al.	2008	-3.42	14.89%	<0.001
4	Byelashov et al.	2009	-3.52	15.47%	<0.001
5	Porto-Fett et al.	2008	-0.10	15.26%	0.80
6	Porto-Fett et al.	2008	-0.26	15.89%	0.22
7	Porto-Fett et al.	2008	-2.60	15.83%	<0.001
Synthesis			**-2.04**	**100%**	**<0.001**

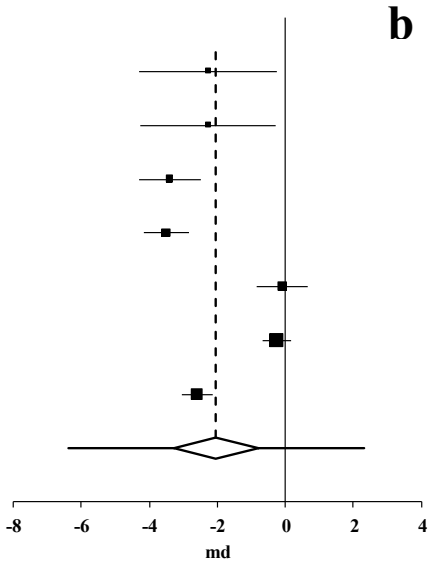
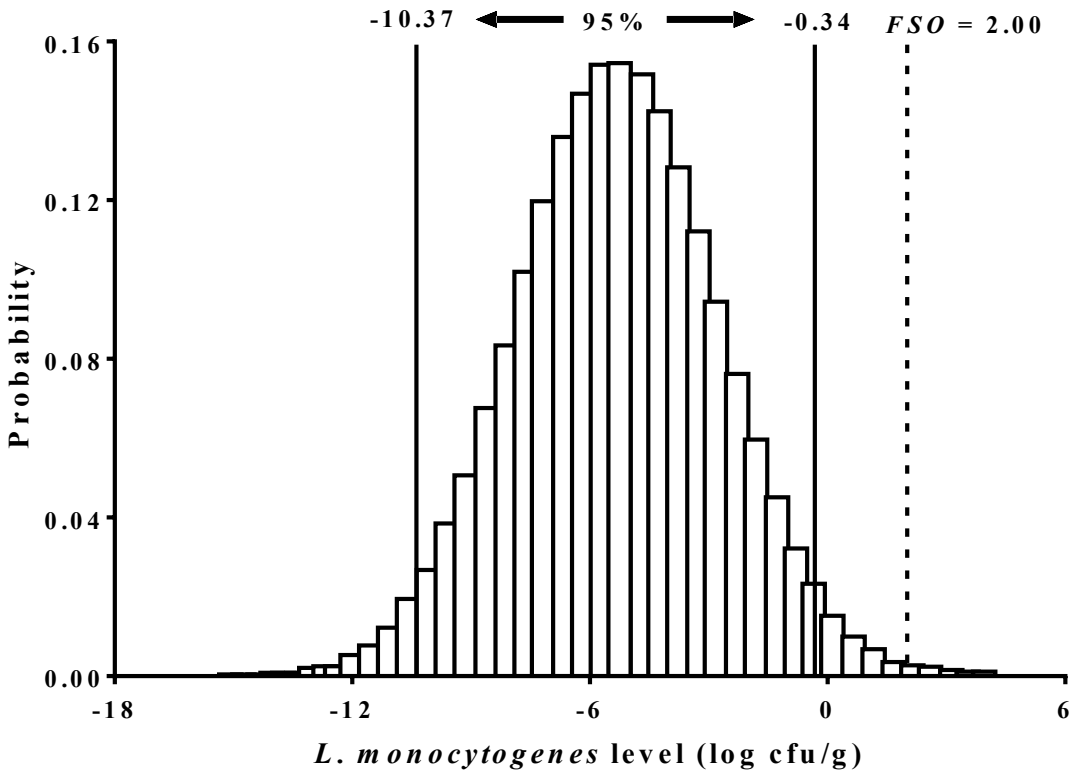


Fig. 4


-10.37
95%
-0.34
FSO = 2.00
0.16
0.12
0.08
0.04
0.00
Probability
-18
-12
-6
0
6
L. monocytogenes level (log cfu/g)