Estimating the non-thermal inactivation of Listeria monocytogenes in fermented sausages relative to temperature, pH and water activity

UNIVERSITÀ DEGLI STUDI DI TORINO

Estimating the non thermal inactivation of *Listeria monocytogenes* in fermented sausages relative to temperature, pH and water activity

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Abstract

Data relative to *in situ Listeria monocytogenes* inactivation in fermented sausages were collected from 13 individual studies found in the literature. Inactivation rates were extrapolated and used to develop a predictive model to evaluate the relative effects of pH, water activity (a_w) and temperature on *L. monocytogenes* fate during fermentation and ripening. Temperature explained *ca*. 60% of the data variability, while pH and a_w only a small part. Temperature alone may not be sufficient to cause pathogen's inactivation, but inactivation rate is dominated by temperature when pH and a_w are in the range which prevent *L. monocytogenes* growth. A predictive model based on two Arrhenius equations (ln[inactivation rate] = -25.71 - [-0.6829 / (8.314 × T)] for fermentation; and ln[inactivation rate] = -44.86 - [-1.219 / (8.314 × T)] for ripening) was developed. The model can be used to quantify the effect of temperature and/or time changes on fermented sausages safety. The advantages and limitations of the model are discussed.

Keywords: Fermented meats, food-borne pathogens, food safety, kinetic behavior model, inactivation, *Listeria monocytogenes*

1. Introduction

The safety of fermented sausages is warranted by the combined effect of different environmental factors, known as "hurdles" (Leistner, 2000). Nevertheless, some notable outbreaks of food-borne illness associated with fermented foods have occurred (Adams & Mitchell, 2002). Due to the wide spread of *Listeria monocytogenes*, the pathogen may contaminate raw materials and/or final products with numbers often exceeding the limit of 100 colony forming units (CFU)/g (De Cesare, Mioni, & Manfreda, 2007; Thevenot, Delignette-Muller, Christieans, & Vernozy-Rozand, 2005). Depending on the fermentation conditions and/or product characteristics, the pathogen may survive till the end of the process or during the storage and distribution of the product (Drosinos, Mataragas, Veskovic-Moracanin, Gasparik-Reichardt, Hadziosmanovic, & Alagic, 2006). Quantification of the kinetic behavior of *L. monocytogenes* relative to the environmental factors affecting its inactivation during sausage manufacture may produce valuable information, which may lead to better control of the pathogen in fermented sausages and finally to improved safety of the products.

Several studies (challenge tests) have been carried out to evaluate the effect of conditions prevailing during the manufacture of fermented sausages on the inactivation of *L. monocytogenes* (Tab. 1; Mataragas, Bellio, Rovetto, Astegiano, Decastelli, & Cocolin, 2014a, Mataragas, Bellio, Rovetto, Astegiano, Greci, Hertel, Decastelli, & Cocolin, 2014b). In each of these challenge tests a specific product (fermented sausage) has been used, making the extrapolation of the non-thermal inactivation of *L. monocytogenes* to other fermented sausages difficult, if not impossible. In other words, Food Business Operators (FBO) cannot use these

estimations to assess the safety of their own products, but new challenge tests should be carried out instead.

Therefore, the objectives of this study were: a) to develop a generic model to evaluate the extent of *L. monocytogenes* inactivation in fermented sausages, and b) to identify the main factors, which affect this inactivation. Thus, FBO may use this tool i) to obtain an indication of the pathogen inactivation in their own products and/or ii) to evaluate the safety of a process, especially when this is altered. The advantages and limitations of the developed model are also discussed. To achieve the above objectives, data from *in situ* studies found in the literature investigating *L. monocytogenes* inactivation in fermented sausages were gathered. The approach followed in the present study was greatly relied on the work of McQuestin, Shadbolt, & Ross (2009).

2. Materials and Methods

2.1. Data collection

Papers written in English and published until the execution of the present work were considered. Studies containing data on *in situ L. monocytogenes* survival in fermented sausages during fermentation and ripening, which allowed the determination of the pathogen inactivation rate (log CFU/g/day), were included. Each study contained different: a) type of sausages, b) level of inoculation with the pathogen, or c) fermentation-ripening programs, allowing the determination of one or more inactivation rates during each phase of the manufacture process. The search of the relevant studies was performed by consulting various literature databases, such as Sciencedirect, Scopus and PubMed. Keywords like "*Listeria monocytogenes*, non thermal inactivation, survival, bacterial resistance, cell viability, fermented sausages,

fermented meats and salami" were used during the search. Literature lists of the found relevant papers were also searched to uncover any additional publications.

As far as possible, the following information was extrapolated from the published works: number of *L. monocytogenes* strains inoculated in the sausages, type of fermented sausage, number of different inactivation rates determined, process (fermentation or ripening), process duration, applied temperatures of fermentation and ripening, pH and water activity (a_w) range of each process step, presence of additives such as nitrites, nitrates, lactic acid or other antimicrobial agents, and *L. monocytogenes* survival data. For each fermented sausage, representing a set of conditions, an inactivation rate of *L. monocytogenes* was determined by simple linear regression of pathogen's viability data (log CFU/g) versus time. This was made separately for each process step, i.e. fermentation and ripening for which *L. monocytogenes* survival was measured at least at the beginning and end of that process step, and during which at least the temperature was constant. The latter was the case for the ripening step, but not for the fermentation step, in which the temperature was changing over time. Thus, it was assumed that the inactivation rate determined during sausage fermentation corresponded to the average temperature of that step.

2.2. Statistical analysis

The statistical analysis of the collected data was based on the work of McQuestin et al. (2009). In order to discover potential predictor variables for *L. monocytogenes* inactivation during fermentation and ripening, the inactivation rate values determined were fitted to simple linear regression models based on the following predictors: temperature, pH and a_w. The influence of the temperature on the pathogen's

inactivation was evaluated by transforming the data to ln(inactivation rate) and the reciprocal of absolute temperature. The transformed data were analyzed by a simple linear regression using the Arrhenius model (Mataragas, Drosinos, Vaidanis & Metaxopoulos, 2006):

$$\ln[\text{inactivation rate (log CFU/g/day)}] = \ln(A)_{inactivation\ rate} - [E_a / (R \times T)]$$

where: $\ln(A)_{inactivation\ rate}$, is an additional factor to enable the model to fit the data of the inactivation rate (log CFU/g/day); E_a, is the (in)activation energy (J/mol); R, is the gas constant (8.314 J/mol × K); and T, is the absolute temperature (K). The strength of the relationship was assessed by the correlation coefficient (R^2). The root mean square error (RMSE), bias (B_f) and accuracy (A_f) factors were also determined (Ross, 1996). The effect of pH and a_w was investigated by plotting the log-transformed inactivation rate or the total amount of *L. monocytogenes* inactivation against the a) final pH and a_w, b) reduction in pH and a_w and c) rate of reduction in pH and a_w. Reduction and rate of reduction in pH and a_w were calculated during fermentation (start of process to end of fermentation process) and ripening (end of fermentation process to end of ripening), where significant decreases in pH and a_w occur, respectively. Regression analysis was applied by fitting a straight line to each log-transformed inactivation rate or total inactivation data set against the different aforementioned predictors, and R^2 was determined. Finally, the inactivation rate data were normalized for the effect of temperature as described in McQuestin et al. (2009) to remove the confounding effect of the influence of temperature. The results from one study (Mataragas et al., 2014a) were kept aside for validation purposes of the developed model. Statistical analysis was performed by using the software GraphPad Prism 5.04 (GraphPad Software, Inc., San Diego, CA, USA). The *L. monocytogenes* viability data (log CFU/g) 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

At the end of the search, 13 studies dealing with *in situ* non-thermal inactivation of *L. monocytogenes* in fermented sausages were found (Tab. 1). Most of them contained more than one data set allowing the determination of 86 inactivation rates, including 40 different *L. monocytogenes* strains, fermentation and ripening temperatures ranging from 12 to 38^{o}C and from 8 to 24^{o}C, respectively, pH ranging from 6.2 to 5.6 (initial values) and from 5.8 to 4.4 (final values), and a_w ranging from 0.98 to 0.92 (initial values) and from 0.94 to 0.80 (final values). Those inactivation rates were derived from linear inactivation curves or models. The data were analyzed to quantify *L. monocytogenes* inactivation in response to parameters relevant to fermented sausages. The effect of temperature on the *in situ L. monocytogenes* inactivation in fermented sausages was evaluated by fitting the data transformed to ln(inactivation rate) and the reciprocal of absolute temperature. Two distinct Arrhenius relationships for the temperatures applied during fermentation and ripening were used (Fig. 1). The Arrhenius equations fitted to all the data, irrespective of pH, a_w, *L. monocytogenes* strain, etc., for temperatures in the fermentation (from 17.7 to 38.0 ^{o}C, n = 36) (equation 1) and ripening (from 8.0 to 23.0 ^{o}C, n = 56) (equation 2) process step were:

$$\ln[\text{inactivation rate (log CFU/g/day)}] = -25.71 - [-0.6829 / (8.314 \times T)] \quad \text{(equation 1)}$$

$$\ln[\text{inactivation rate (log CFU/g/day)}] = -44.86 - [-1.219 / (8.314 \times T)] \quad \text{(equation 2)}$$

The R^2 was 0.603 (equation 1) and 0.580 (equation 2), indicating that temperature alone explains 60% of the variability in the fermentation and ripening data. Accordingly, the RMSE was 0.528 (equation 1) and 0.460 (equation 2).

The (in)activation energies estimated from equations 1 and 2 were different. McQuestin et al. (2009) also found different (in)activation energies of the two Arrhenius equations describing the effect of temperature (one for the range of 0 to 47^{o}C and the other for temperatures above 47^{o}C) on the *E. coli* inactivation in fermented meats and analogous aqueous systems. The authors explained that the different (in)activation energies indicate the different mechanisms of inactivation involved at temperatures above 47^{o}C and those in the range of 0^{o}C to 47^{o}C. In the present study, fermentation temperatures are much higher than those applied during ripening, reaching in some cases the 38^{o}C.

Observed inactivation rates were compared to the predicted ones by equation 1 and 2. For fermentation temperatures (equation 1), the B_f and A_f were determined at 0.999 and 1.50, respectively. The B_f of 0.999 indicates that, on average, the model very slightly under-predicts the observed inactivation rates. The A_f of 1.50 indicates that, on average, the predicted value differs from the observed by a factor of 1.5. For ripening temperatures (equation 2), the corresponding B_f and A_f values were 0.999 and 1.45, respectively.

The log-transformed inactivation rate and the total inactivation of *L. monocytogenes* in fermented sausages was plotted against the final pH, the reduction in pH during fermentation, and the rate of reduction in pH during fermentation. A linear regression was fitted to each plot indicating that the final pH, the reduction in pH during fermentation, and the rate of reduction in pH during fermentation explained less than 10% of the total amount of *L. monocytogenes* inactivation ($R^2 < 0.10$) (Tab. 2). A

better correlation was obtained between log-transformed inactivation rate and the final pH (R^2 = 0.12), the reduction in pH during fermentation (R^2 = 0.22), or the rate of reduction in pH during fermentation (R^2 = 0.18). When, however, the *L. monocytogenes* inactivation rate was normalized for the effect of temperature, using the Arrhenius model (equation 1), and plotted against the final pH, the reduction in pH during fermentation, and the rate of reduction in pH during fermentation, the correlation was reduced (Tab. 2), i.e. the relationship was weaker, indicating the dependence of the effect of pH on the temperature of the process. These relationships are shown in Figure 2.

Similarly, the effect of a_w on the *in situ L. monocytogenes* inactivation in fermented sausages was examined by plotting the log-transformed inactivation rate and the total inactivation of *L. monocytogenes* against the final a_w, the reduction in a_w during ripening, and the rate of reduction in a_w during ripening. Linear regression analysis showed that the effect of a_w accounted for a small part of the variation in the log-transformed *L. monocytogenes* inactivation rate (Tab. 3). Better correlation was observed between the total inactivation of *L. monocytogenes* and the various a_w variables. The effect of a_w was found to be less dependent on the temperature of the process, since the normalization of the inactivation data for the effect of temperature, using the Arrhenius model (equation 2), reduced slightly the R^2 values. This dependence was less strong compared to pH results after inspecting the reduction observed in the R^2 values.

The analysis showed that inactivation of *L. monocytogenes* is observed when pH and a_w values are within the range, which do not support growth of the pathogen (pH $\leq$ 5.0 and $a_w \leq 0.94$), but a further decrease in their values do not necessarily accelerate *L. monocytogenes* inactivation (relatively low R^2 values). On the other hand, the R^2

values for the effect of temperature indicate that this factor play an important role in the *in situ L. monocytogenes* inactivation: the higher the temperature the faster is the inactivation. McQuestin et al. (2009) observed a similar effect of temperature for the inactivation of *E. coli* in fermented meats and analogous aqueous systems. The authors noted that although the fermentation and ripening temperatures alone may not cause the destruction of the pathogen's cells, however the temperature applied is an important explanatory variable of the inactivation rate, and hence of the total inactivation.

The results from one study, regarding the *in situ* inactivation of *L. monocytogenes* in two Italian fermented sausages (Cacciatore and Felino) which contained starter cultures, nitrites and/or nitrates, were used for the validation of the developed model. The limits of the developed model relative to fermentation and ripening temperatures (based on the average values used in each processing step to develop the model), the initial and final pH, and the initial and final a_w were: 17.7-38.0^{o}C and 8.0-23.0^{o}C, 6.2-5.6 and 4.4-5.8, and 0.98-0.92 and 0.80-0.94, respectively. The corresponding values of the two Italian fermented sausages used for validation purposes were: for Cacciatore, 19.7^{o}C (fermentation) and 16.0^{o}C (ripening), on average, 5.7 and 4.7 (initial and final pH), and 0.98 and 0.92 (initial and final a_w); and for Felino, 18.7^{o}C (fermentation) and 13.0^{o}C (ripening), on average, 5.8 and 5.3 (initial and final pH), and 0.96 and 0.93 (initial and final a_w). The results from the validation of the model are shown in Table 4. The inactivation rate was predicted relatively well during fermentation and ripening of the sausages (the observed inactivation rate was within the 95% prediction bounds), except during fermentation of Felino in which the error of prediction was higher (the observed inactivation rate value was outside the 95%

prediction bounds), because no substantial inactivation was observed (inactivation rate < 0.001).

After normalizing data for the effect of temperature, the analysis showed a greater temperature dependence of the inactivation rate during the fermentation process (greater reduction in the R^2 values) in comparison with the ripening process. Furthermore, temperatures at or above 20°C are needed especially during the first 48h of fermentation for rapid inactivation of *L. monocytogenes* (Gounadaki, Skandamis, Drosinos, & Nychas, 2005), but in the Felino sausage the fermentation temperature was mainly below 20°C (18.7°C on average), while in the Cacciatore was almost at the limit of 20°C (19.7°C on average). Finally, a higher prediction error for the effect of temperature on the inactivation rate during fermentation (equation 1) compared to ripening (equation 2) of Felino was observed. Thus, the greater temperature dependence of the inactivation rate during the fermentation process and the lower fermentation temperatures applied in the Felino sausage may partly explain the measured very low *L. monocytogenes* inactivation (inactivation rate < 0.001 log CFU/g/day) and subsequently the higher prediction error (out of the 95% prediction bounds).

Although, the analysis contained studies with a) fermented sausages of pH ranging from 4.4 to 6.2, a_w ranging from 0.80 to 0.98, b) various *L. monocytogenes* serotypes and strains, c) differences in the physiological state of the bacterial cells, and d) product formulations, temperature explained *ca*. 60% of the variability in the data, while pH or a_w only a small part. Furthermore, the various pH and a_w variables tested seemed to be temperature dependent. The remaining unexplained variability is probably due to other factors, which may have a substantial effect on the *in situ L.*

monocytogenes inactivation in fermented sausages and mask any effect of pH and a_w (McQuestin et al., 2009).

In this work, the effect of temperature, pH and a_w on *in situ L. monocytogenes* inactivation in fermented sausages was assessed. The effect of other inhibitory agents such as nitrites, nitrates or lactic acid on *L. monocytogenes* inactivation was not possible to be determined because the relevant information, i.e. concentration, was not available in the majority of the studies. Such information could provide valuable information relative to the evaluation of their influence on *in situ L. monocytogenes* inactivation in fermented sausages, if relevant studies were carried out to cover these data gaps. The results revealed that fermentation and ripening temperatures play a significant role in the *in situ L. monocytogenes* inactivation in fermented sausages depicting higher pathogen's inactivation if these processes are being performed at higher temperatures for increased times, especially the fermentation step. Similar observations were made for other pathogens, as well as *E. coli* (McQuestin et al., 2009). Other factors such as pH and a_w are also important in *L. monocytogenes* inactivation, but increased holding times and temperatures will enhance pathogen's inactivation. The use of the model developed is limited for accurate predictions about the total *in situ* inactivation of *L. monocytogenes* in fermented sausages due to the wide prediction bounds of the fitted equations and the high prediction error occurred for fermentation temperatures mainly below 20^oC. The model, however, can be used for conservative predictions based on the range of temperatures used to develop the tool. In this context, it can be proven a useful and practical tool to estimate the relative changes in the safety of fermented sausages when temperature and time of fermentation and ripening are altered. In addition, its usefulness can be greatly

enhanced by incorporating data from additional studies relative to the *L. monocytogenes* inactivation at fermentation temperatures below 20^{o}C.

4. Conclusions

The current work provides information on the fate of *L. monocytogenes* in various conditions of sausage fermentation and ripening by the mean of meta-analysis. A predictive model based on two Arrhenius equations (equations 1 and 2), describing the influence of temperature on the *in situ L. monocytogenes* inactivation during the fermentation (equation 1) and ripening (equation 2) processes of fermented sausages, was developed. The usefulness or practicability of the developed predictive model may be extended by incorporating additional data from other challenge tests and/or by comprehensively validating the developed model with data (external validation) describing the *in situ* inactivation of *L. monocytogenes* in fermented sausages. The analysis revealed that more data should be incorporated in the developed model for fermentation temperatures mainly lower than 20^{o}C in order to have more accurate predictions on the fate of *L. monocytogenes*. In such cases, however, the lower 95% prediction bound can be taken into consideration for prediction purposes because this strategy coincide with a "worst-case" approach for model predictions. The FBO may obtain an indication of the pathogen inactivation in their products and/or evaluate the safety of a process. It should be noted, however, that practitioners should understand the limitations of the model in order to use model predictions and interpretation of its results with caution.

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Figure captions

Fig. 1. Arrhenius plot [ln(inactivation rate) vs. 1/absolute temperature] showing the effect of temperature on *in situ L. monocytogenes* inactivation rate in fermented sausages. The solid line is the Arrhenius model fitted to the data (solid circles) for a) fermentation (17.7-38.0 ^{o}C) and b) ripening (8.0-23.0 ^{o}C) temperatures (solid circles) and fits the equations a) $y = -25.71 - [-0.6829 / (8.314 \times T)]$ ($R^2 = 0.603$) and b) $y = -44.86 - [-1.219 / (8.314 \times T)]$ ($R^2 = 0.580$). The dashed lines depict the 95% prediction bands of the regression line.

Fig. 2. Effect of a) final pH, b) reduction in pH during fermentation and c) rate of reduction in pH during fermentation on I) log-transformed inactivation rate and II) inactivation rate normalized for temperature, using equation 1. Solid circles, observed data.

FIG. 1

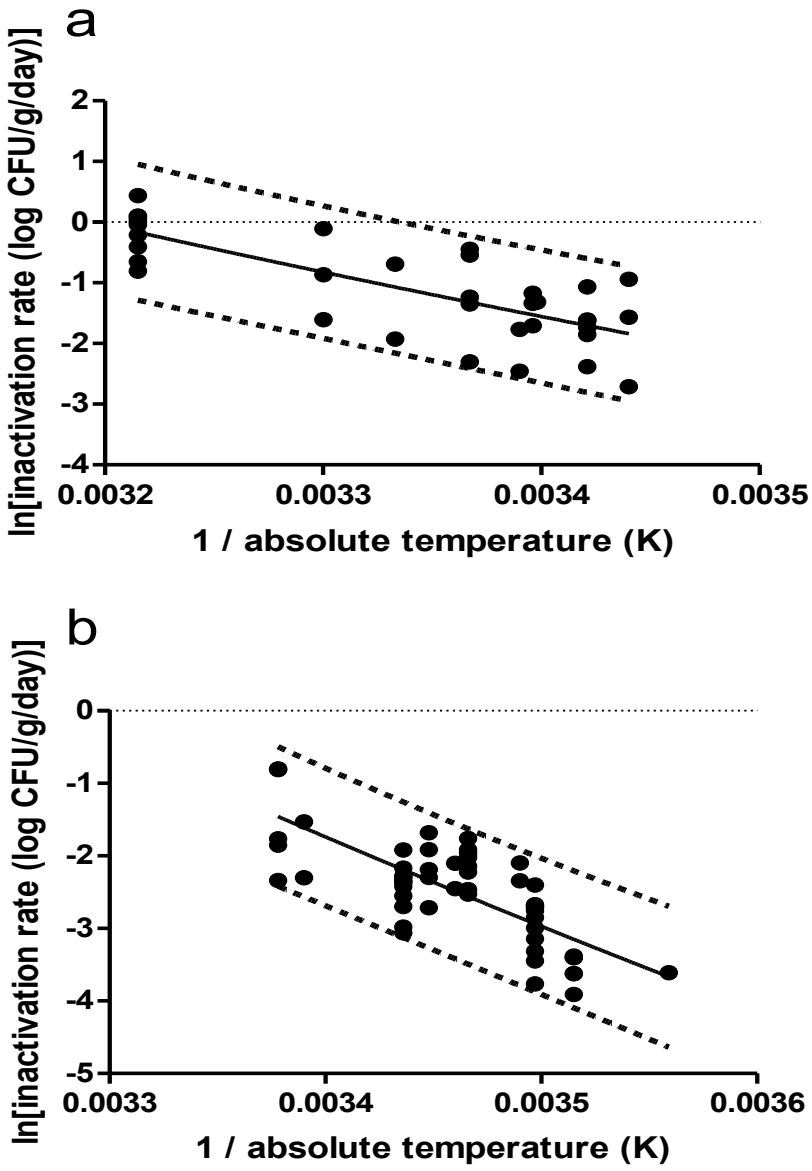
a
ln[inactivation rate (log CFU/g/day)]
2
1
0
-1
-2
-3
-4
0.0032
0.0033
0.0034
0.0035
1 / absolute temperature (K)
b
ln[inactivation rate (log CFU/g/day)]
0
-1
-2
-3
-4
-5
0.0033
0.0034
0.0035
0.0036
1 / absolute temperature (K)

FIG. 2

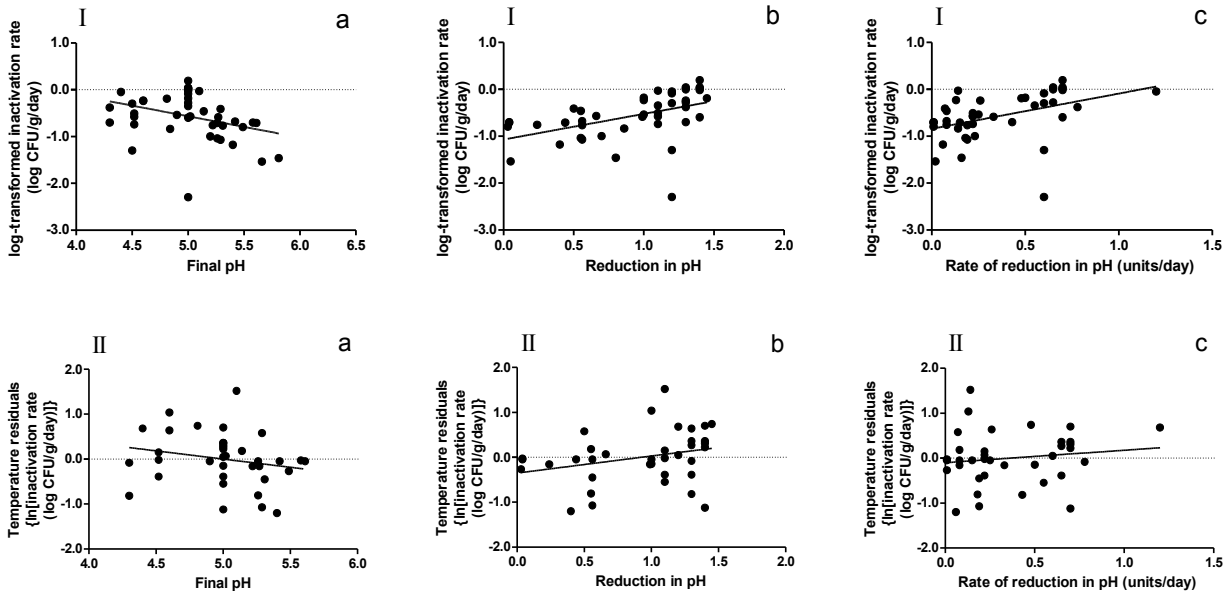
I
a
log-transformed inactivation rate (log CFU/g/day)
Final pH
I
b
log-transformed inactivation rate (log CFU/g/day)
Reduction in pH
I
c
log-transformed inactivation rate (log CFU/g/day)
Rate of reduction in pH (units/day)
II
a
Temperature residuals {ln[inactivation rate (log CFU/g/day)]}
Final pH
II
b
Temperature residuals {ln[inactivation rate (log CFU/g/day)]}
Reduction in pH
II
c
Temperature residuals {ln[inactivation rate (log CFU/g/day)]}
Rate of reduction in pH (units/day)

Table 1

Studies included in the analysis relative to *in situ L. monocytogenes* inactivation in fermented sausages.

a/a	Reference	No. of strains	*L. monocytogenes* strain	Type of fermented sausage	No. of rates[a]	Range			Comments
						Temperature (^{o}C)[b]	pH[c]	a_w[c]	
1	Drosinos et al., 2006	1	NCTC 10527	Sremska (Serbia-Montenegro)	3	14-20 (F), 14-16 (R)	6.0-5.4	0.92-0.80	Sausages contained nitrites and starters. The experimental conditions investigated included sausages inoculated with a) starters producing or not bacterions and b) bacteriocins. All sausages received smoking during fermentation. *Lactobacillus sakei* used as starters producing bacteriocins.
		1	NCTC 10527	Sudjuk (Bosnia-Herzegovina)	6	15-24 (F), 22-24 (R)	6.0-5.0	-[d]	
		1	NCTC 10527	Fermented dry sausage (Croatia)	7	18-20 (F), 16-18 (R)	5.7-5.3	-	
		1	NCTC 10527	Fermented dry sausage (Hungary)	8	17-20 (F), 15-16 (R)	5.8-5.4	0.96-0.85	
2	Degenhardt and Sant' Anna, 2007	1	ATCC 7644	Italian-style fermented sausage	3	12-24 (F), 12-14 (R)	5.6-5.1	0.96-0.89	Sausages contained nitrites, nitrates and starters. The experimental conditions investigated included a) a standard recipe with no inhibitor additive, b) a batch with bioprotective culture (lactic acid bacteria) and c) a batch with sodium lactate added.
3	Barazi and Erkmen, 2008	1	ATCC 13932	Sucuk	1	20-22 (F), 17-18 (R)	5.7-5.0	-	Sausages contained nitrites, nitrates and starters.
4	Foegeding et al., 1992	5	Scott A, F5069, ATCC 19115, NCF-U2K3, NCF-F1KK4	American-style fermented sausage	12	38 (F), 13 (R)	6.2-5.0	-	Sausages contained nitrites and starters. *Pediococcus acidilactici* used as starters produced bacteriocins. The experimental conditions investigated included sausages inoculated with starters producing or not bacteriocins. Sausages received smoking during fermentation.
5	Gareis et	3	SLCC 6139	Minisalami	8	20-24 (F)[e],	5.9-	0.97-	Sausages contained

	al., 2012		Li127, NTCC 10527 Li2, Li 135	(German-style)		18 (R)	5.5	0.85	nitrites and starters. The experimental conditions investigated included sausages made from a) pork, b) pork and beef, c) poultry and d) air-dried minisalami. Two levels (high and low) of inoculation were used. Two fermentation programs were investigated. All sausages received smoking during fermentation.
		3	SLCC 6139 Li127, NTCC 10527 Li2, Li 135	Minisalami (German-style)	8	19-22 (F)[e], 18 (R)	5.9-5.5	0.97-0.84	
6	Nissen and Holck, 1998	3	2230/92, 167, 187	Norwegian-style fermented dry sausage	1	27 (F), 14 (R)	5.8-4.8	0.95-0.89	Sausages contained nitrites and starters. Sausage received smoking during fermentation.
7	Farber et al., 1993[f]	5	-	German-style fermented sausage	1	16-20 (F), 16 (R)	5.6-4.6	0.97-0.81	Sausages contained nitrites and starters. Sausage received smoking during fermentation. In the German-style fermented sausage a non bacteriocin producing starter culture of *Pediococcus acidilactici* was used, but in the American style fermented sausage a bacteriocin producing starter of the same strain was used.
		5	-	American-style fermented sausage	1	16-20 (F), 16 (R)	5.7-5.1	0.96-0.89	
8	Porto-Fett et al., 2008	5	MFS2, MFS102, MFS104, MFS105, MFS110	Soudjouk-style fermented semi-dry sausage	1	24 (F), 22 (R)	5.9-4.8	0.97-0.92	Sausages contained nitrites and starters.
		5	MFS2, MFS102, MFS104, MFS105, MFS110	Soudjouk-style fermented semi-dry sausage	1	24 (F), 22 (R)	5.9-5.3	0.97-0.92	
9	Nightingale et al., 2006	5	ATCC 19116, ATCC 19113, 101M, 109, 108M	Italian-style salami	1	30 (F) for 24h, 10-13 (R)[g]	5.6-4.4	0.98-0.94	Sausages contained nitrites and starters. Sausage received smoking during fermentation.
		5	ATCC 19116, ATCC 19113, 101M, 109, 108M	Italian-style salami	1	30 (F) for 24h, 10-13 (R)[h]	5.6-4.8	0.98-0.87	
		5	ATCC 19116, ATCC 19113, 101M, 109, 108M	Italian-style salami	1	30 (F) for 40h, 10-13 (R)[g]	5.6-4.5	0.98-0.93	
		5	ATCC 19116,	Italian-style	1	30 (F) for	5.6-	0.98-	

			ATCC 19113, 101M, 109, 108M	salami		40h, 10-13 (R)[h]	4.8	0.88	
		5	ATCC 19116, ATCC 19113, 101M, 109, 108M	Italian-style salami	1	30 (F) for 72h, 10-13 (R)[g]	5.6-4.5	0.98-0.93	
		5	ATCC 19116, ATCC 19113, 101M, 109, 108M	Italian-style salami	1	30 (F) for 72h, 10-13 (R)[h]	5.6-4.8	0.98-0.88	
10	Hwang et al., 2009	5	MFS2, H7776, Scott A, 101M, F6854	Soudjouk-style fermented semi-dry sausage	1	24 (F), 22 (R)	5.9-5.2	0.97-0.92	Sausages contained nitrites and starters.
		5	MFS2, H7776, Scott A, 101M, F6854	Soudjouk-style fermented semi-dry sausage	1	24 (F), 22 (R)	5.9-4.9	0.97-0.89	
		5	MFS2, H7776, Scott A, 101M, F6854	Soudjouk-style fermented semi-dry sausage	1	24 (F), 22 (R)	5.9-4.6	0.97-0.86	
11	Lindqvist and Lindblad, 2009	1	L8	Swedish-style fermented sausage	1	27 (F), 8 (R)	5.7-4.5	0.97-0.81	Sausages contained nitrites and starters. Sausage received smoking during fermentation.
		1	L8	Swedish-style fermented sausage	1	27 (F), 22 (R)	5.7-4.5	0.97-0.81	
12	Thevenot et al., 2005	8	SR1, SR2, HC1, HC2, Saus1, Saus2, Equi1, Equi2	French-style fermented dry sausage	8	20-24 (F), 13-14 (R)	5.9-5.8	0.96-0.81	Sausages contained nitrates and starters. Each batch was inoculated with one strain only.
13	Lahti et al., 2001	1	EELA237	Finnish-style fermented dry sausage	4	20-23 (F), 17 (R)	5.6-4.7	0.95-0.93	Sausages contained nitrites and starters. Sausage received smoking during fermentation. Two different starters in two different levels (low and high) were used. One starter contained a bacteriocin producing *Pediococcus acidilactici* and the other a non bacteriocin-producing *Lactobacillus curvatus*.

[a] No of rates determined for each process step, i.e. fermentation and ripening
[b] Temperatures applied during each process step, which were different for fermentation (F) and ripening (R)
[c] The range represents the values at the beginning and end of the production process
[d] -, not described
[e] Two different fermentation programs were studied
[f] Data from Italian-style fermented sausage were not used because no starters were used during its production
[g] Dried to moisture/protein ratio (MPR) of 1.9:1
[h] Dried to moisture/protein ratio (MPR) of 1.4:1

Table 2

Effect of pH on *in situ L. monocytogenes* inactivation in fermented sausages from linear regression analysis of inactivation against pH variables.

y axis	*x* axis	No. of data points	Linear regression equation	R^2
Total inactivation (log CFU/g)	Final pH	78	$y = 0.7096x - 1.6976$	0.0445
	Reduction in pH[a]	78	$y = -0.531x + 2.3739$	0.0233
	Rate of reduction in pH[a, b]	78	$y = -1.4553x + 2.3645$	0.0678
log-transformed inactivation rate (log CFU/g/day)	Final pH	43	$y = -0.4545x + 1.7075$	0.1156
	Reduction in pH[a]	43	$y = 0.5444x - 1.0688$	0.2233
	Rate of reduction in pH[a, b]	43	$y = 0.7488x - 0.8404$	0.1792
Temperature residuals[c]	Final pH	36	$y = -0.3679x + 1.8406$	0.0465
	Reduction in pH[a]	36	$y = 0.3795x - 0.3507$	0.0802
	Rate of reduction in pH[a, b]	36	$y = 0.2769x - 0.1007$	0.0185

[a] During fermentation

[b] pH units/day

[c] Data were normalized for the effect of temperature by using the Arrhenius equation:

$\ln[\text{inactivation rate (log CFU/g/day)}] = -25.71 - [-0.6829 / (8.314 \times T)]$ (equation 1)

Table 3

Effect of a_w on *in situ L. monocytogenes* inactivation in fermented sausages from linear regression analysis of inactivation against a_w variables.

y axis	*x* axis	No. of data points	Linear regression equation	R^2
Total inactivation (log CFU/g)	Final a_w	56	$y = -13.572x + 13.311$	0.2368
	Reduction in a_w[a]	56	$y = 11.171x + 0.6712$	0.1021
	Rate of reduction in a_w[a, b]	56	$y = -74.281x + 2.0593$	0.0957
log-transformed inactivation rate (log CFU/g/day)	Final a_w	55	$y = -3.5911x + 1.9684$	0.1617
	Reduction in a_w[a]	55	$y = 2.1139x - 1.2968$	0.0346
	Rate of reduction in a_w[a, b]	55	$y = -17.934x - 1.0115$	0.0571
Temperature residuals[c]	Final a_w	42	$y = -6.1405x + 5.236$	0.2011
	Reduction in a_w[a]	42	$y = 5.3576x - 0.494$	0.1029
	Rate of reduction in a_w[a, b]	42	$y = 91.948x - 0.3205$	0.0501

[a] During ripening

[b] a_w units/day

[c] Data were normalized for the effect of temperature by using the Arrhenius equation:

$\ln[\text{inactivation rate (log CFU/g/day)}] = -44.86 - [-1.219 / (8.314 \times T)]$ (equation 2)

Table 4

Validation of the developed predictive model for the *in situ* fate of *L. monocytogenes* in fermented sausages.

Fermented sausage and process stage	Observed inactivation rate (log CFU/g/day)[a]	Predicted inactivation rate (log CFU/g/day)	95% prediction bands	
			Upper	Lower
Cacciatore fermentation[b]	0.16	0.19	0.57	0.06
Cacciatore ripening[c]	0.03	0.08	0.21	0.03
Felino fermentation[b]	<0.001	0.17	0.52	0.06
Felino ripening[c]	0.02	0.05	0.14	0.02

[a] Data were taken from Mataragas et al. (2014a)

[b] The Arrhenius equation: ln[inactivation rate (log CFU/g/day)] = -25.71 - [-0.6829 / (8.314 × T)] (equation 1) and an average fermentation temperature of 19.7^{o}C (Cacciatore) and 18.7^{o}C (Felino) for the parameter T were used for model predictions. T is in K, i.e. 1 / (273 + ^{o}C)

[c] The Arrhenius equation: ln[inactivation rate (log CFU/g/day)] = -44.86 - [-1.219 / (8.314 × T)] (equation 2) average fermentation and ripening temperature of 16^{o}C (Cacciatore) and 13^{o}C (Felino) for the parameter T were used for model predictions. T is in K, i.e. 1 / (273 + ^{o}C)