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Evaluation of the *Listeria monocytogenes* inactivation during post-process storage of fermented sausages: A basis for the development of a decision support tool

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ABSTRACT

In situ quantitative data on *Listeria monocytogenes* survival during storage of vacuum-packaged fermented sausages at various temperatures were collected from the literature to develop a generic predictive model regarding its fate at a specific storage temperature. The development of the tool was based on the z-concept. The time needed for 4D reduction of the pathogen was estimated and its influence by the temperature was further described by linear regression. A secondary model was developed for describing the effect of sausage water activity on the z-concept parameters at the reference temperature. The decision support tool was successfully validated against the studies not used during the development of the model. Based on the model predictions, a decision can be made about the required time of product storage before its distribution to achieve an additional pathogen inactivation. Such tools can be incorporated in a HACCP plan of a food-producing company to assure food safety.

1. Introduction

The role of the processes of fermentation and ripening of fermented meats is twofold, contributing to the organoleptic characteristics of the products, but also to its stability. Insufficient fermentation and/or maturation of the product may lead to an unsafe final product because pathogenic bacteria can survive in the process. There are instances, however, where fermentation and ripening are successful, but the conditions (pH and water activity in combination with fermentation temperature) prevailing during manufacturing facilitate the adaptation and better survival of pathogenic bacteria such as *Listeria monocytogenes* and *Salmonella enterica* (Mataragas, Bellio, Rovetto, Astegiano, Decastelli, et al., 2014, Submitted for publication; Mataragas, Bellio, Rovetto, Astegiano, Greci, et al., 2015). In those situations where the desired reduction of the pathogen is not induced by the current practices of fermentation and ripening, the existence of a mechanism which would allow further reduction of the pathogen in the finished product in order to enhance consumer safety would be highly beneficial for Food Business Operators (FBOs).

L. monocytogenes may contaminate food products at different steps of the manufacturing process since the organism is able to survive on equipment and in production facilities (Samelis & Metaxopoulos, 1999). The presence of the pathogen has been reported throughout the process of manufacturing fermented sausages (Buncic, Paunovic, & Radisic, 1991; Glass & Doyle, 1989; Hew, Hajmeer, Farver, Glover, & Cliver, 2005). It has not unambiguously been shown to have caused listeriosis from the consumption of fermented sausages, and pathogens like salmonellae and STEC/VTEC are much more relevant to these products (Adams & Mitchell, 2002). Nevertheless, the detection of *L. monocytogenes* in fermented sausages may cause considerable problems to the manufacturer, and therefore, there is much interest in eliminating this bacterium, and the present study is topical.

Studies have shown that *L. monocytogenes* inactivation in fermented sausage is higher during its storage at ambient temperature (25 °C) compared to chilled temperatures (4 °C) (Gounadaki, Skandamis, Drosinos, & Nychas, 2007). The aim of this work was to gather from the literature the available quantitative data referred to *in situ* survival of *L. monocytogenes*, during storage of vacuum-packaged fermented sausage at various temperatures, and develop a generic model predicting the fate of the pathogen at a

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specific storage temperature. Based on the product characteristics, a suggestion is made about the storage conditions to be applied to achieve the additional reduction required. The tool will allow FBOs to introduce into their production process an additional step of *L. monocytogenes* inactivation before the final distribution of the product by keeping it at a specific temperature to ensure that the desired inactivation of *L. monocytogenes* is attained.

2. Materials and methods

2.1. Collection of data

Papers written in English and published until the execution of the current work were considered. Studies included were those reporting quantitative data relative to *in situ* survival of *L. monocytogenes* during storage of vacuum-packaged fermented sausages at various temperatures, and furthermore allowed the determination of the inactivation rate (per day). This meta-analysis involved fermented sausages inoculated with different *L. monocytogenes* strains being in different physiological state at the time of inoculation facilitating the determination of one or more inactivation rates in some cases. In addition, all fermented sausages were manufactured with the addition of starter cultures and nitrite. The search of the relevant studies was performed by consulting various literature databases, such as Sciencedirect, Scopus and PubMed. Keywords used alone or in combination were "*L. monocytogenes*, non-thermal inactivation, survival, storage, fermented sausages, fermented meats and salami". Literature lists of the found relevant papers were also searched to uncover any additional publications.

As far as possible, the following information was extracted from the published works: number of *L. monocytogenes* strains inoculated in the sausages, type of fermented sausage, number of different inactivation rates determined, storage temperature conditions, storage duration, pH and water activity (a_w) range of each fermented sausage, and *L. monocytogenes* survival data.

2.2. Modeling L. monocytogenes inactivation during storage and parameters estimation

For each experimental case, representing a set of conditions (fermented sausage, storage temperature condition and physiological state of the inoculums), an inactivation rate of *L. monocytogenes* was determined by plotting pathogen's viability data (log CFU/g or cm^2) versus time. Two models describing the *L. monocytogenes* inactivation pattern observed in the studies were used, the log-linear (Bigelow & Esty, 1920) and biphasic (Cerf, 1977). The log-linear model is.

Table 1
Published studies included in the meta-analysis of the *in situ L. monocytogenes* inactivation during storage of fermented sausages[a] at various temperatures.

Reference	No. of strains	*L. monocytogenes* strain	Type of fermented sausage	No. of rates	Range			Comments
					Temperature (°C)[b]	pH[c]	a_w[c]	
Model development								
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)	9	4, 12 and 25	4.5–4.8	0.83–0.85	Three different physiological states of the inoculated *L. monocyogenes*: acid-adapted, extract-habituated and non habituated.
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	12	4, 12 and 25	4.6–4.9	0.89–0.92	Four different physiological states of the inoculated *L. monocytogenes*: non acid-adapted, acid-adapted, partially acid-adapted and habituated.
Gounadaki et al., 2007	1	Scott A (serotype 4b)	Greek-style fermented sausage	3	5[d], 15[d] and 25	4.5–4.6	0.87–0.90	The data from the low inoculum and the aerobic storage of the inoculated fermented sausage were not considered.
Porto-Fett et al., 2008	5	MFS2, MFS102, MFS104, MFS105, MFS110	Soudjouk-style fermented sausage	4	4, 10[d], 21[d] and 30[d]	4.8–5.0	0.82–0.85	–
Model validation								
Barazi & Erkmen, 2008	2	ATCC 13932 and a strain of serotype 4a	Sucuk	1	4	5.0	0.84	The data from the fermented sausage stored under air or various modified atmospheres or made without starter cultures and stored under air were not considered.
Lahti, Johansson, Honkanen-Buzalski, Hill, & Nurmi, 2001	1	A strain of serotype 4b	Dry fermented sausage	1	15	4.7–4.8	0.92	–
Lindqvist & Lindblad, 2009	3	L8, L58, L67	Dry fermented sausage	2	8 and 22	4.5–4.7	0.96–0.97	The data from the inactivation in broths were not considered.

[a] Only vacuum-packaged fermented sausages and manufactured with the addition of starter cultures and nitrite were considered.
[b] Storage temperature.
[c] Values of the final commercial product.
[d] Temperatures used for validation of the decision support tool.