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Chapter 3

Modeling the Behavior of *Listeria monocytogenes* in Meat

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Abstract

This work was conducted to present some studies that show the behavior of *Listeria monocytogenes* in meat, according to intrinsic and extrinsic factors. The understanding of factors that affect the survival and growth of *L. monocytogenes* in meat, such as temperature, pH, acid, salt, water activity or modified atmosphere packaging, is crucial to develop strategies for food operators to reduce and prevent *Listeria* contamination and growth. The knowledge of *L. monocytogenes* behavior according to its physiological and ecological characteristics, under all probable conditions, will support risk assessors to find strategies to control this ubiquitous bacteria in food industry and food service. The Regulation (EC) No 2073/2005, reviewed by the Regulation (EC) No 1441/2007, does not establish the limits for *L. monocytogenes* in fresh meat. However, it is generally accepted a level of 100 cells on fresh meat, except for some risk groups. Food business operators and authorities can use predictive microbiology models as important tools to model bacterial growth in quantitative microbial risk assessments.

Keywords: *Listeria monocytogenes*, meat, modified atmosphere packaging (MAP), antimicrobial agents, predictive microbiology

1. Introduction

Meat is a protein food commodity with a significant water content that makes it a great matrix susceptible to bacterial growth [1]. Since meat forms part of the dietary habits of consumers, several strategies to improve its safety, shelf-life and quality have been studied in the recent years.

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The genus *Listeria* includes a group of Gram-positive psychrotrophic bacteria that can be isolated from a large variety of environmental sources such as water, soil, foodstuffs, animals or humans [2, 3]. Also, *Listeria* can colonize various inert surfaces (e.g., surfaces of food machinery) [4]. Genus *Listeria* includes nonsporulating, catalase positive, Voges-Proskauer positive, indol and oxidase negative, facultative anaerobic rods that show motility at 25°C. *Listeria* can also grow in a large variety of conditions like high salt concentrations, low water activity, broad pH range (pH 4.5–9) and broad range of temperature (0–45°C, optimum 30–37°C) [3, 5]. This genus *Listeria* includes several species such as *L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri, L. grayi, L. marthii, L. rocourtiae, L. leichmannii, L. weihenstephanensis, L. floridensis, L. aquatica, L. cornellensis, L. riparia and L. grandensis* [6]. Among them, *L. monocytogenes* is the most important due to its pathogenicity that affects animals and humans. The ingestion of contaminated foods is the most important source of human infection. According to somatic (O) and flagellar (H) antigens, 13 serotypes of *L. monocytogenes* have been recognized and identified alphanumerically as 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. Serotypes 1/2a, 1/2b and 1/2c are the most frequently isolated from both foodstuffs and food processing areas [6, 7]. Concerning the different kind of implicated food vehicles of listeriosis outbreaks, cooled meats, ready-to-eat foods, cheese, smoked fish and seafood seem to be more susceptible to *L. monocytogenes* development [8–10]. According to [9], in 2016, a total of 0.47 cases of listeriosis per 100,000 population was reported with an incidence of about 10% compared to the previous year. In addition, mortality achieved 16% among the confirmed cases. For most healthy people, listeriosis does not imply more than a threat, limited to gastrointestinal symptomatology ended in 36–48 hours. However, life-threatening infections mainly occur in high-risk populations, including pregnant women, neonates, infants, elderly and individuals with compromised immune systems [10]. Clinical features of listeriosis have considerable variability and can be confused with other infections. Sometimes, gastrointestinal manifestations as primary infection are observed. These digestive manifestations are usually self-limited and spontaneously resolved [11]. *L. monocytogenes* has a tropism for the central nervous system causing meningitis. Sepsis without a localized infection is the most common presentation in patients with deficient immune systems [12]. Since *L. monocytogenes* is a microorganism of ubiquitous nature, meat and meat products may become contaminated throughout contact with raw materials, processing environment and at retail markets [4], *L. monocytogenes* can adhere to the surfaces forming biofilms [13], which consist of cells and extracellular polymeric materials that protect bacteria and lead to its survival and growth. Indeed, *L. monocytogenes* showed some resistance against biocides [13] and temperature-dependent resistance to phages. Therefore, finding alternative methodologies to avoid the contamination and further survival and growth of *L. monocytogenes* are important requests of meat industry [14].

The Regulation (EC) No 2073/2005, reviewed by the Regulation (EC) No 1441/2007, does not establish the limits for *L. monocytogenes* in fresh meat. However, a level of 100 cells on fresh meat, except for some risk groups, is generally accepted.

This chapter provides a brief background on *L. monocytogenes* as an important foodborne pathogen and describes the main factors, such as temperature, pH, acid, salt, water activity or packaging, that influence its behavior in meats. There will be referred some control strategies for control of survival and growth of *L. monocytogenes* and the advantageous use of predictive microbiology programs.

2. Factors related to survival and growth of *L. monocytogenes* in meat

2.1. Influence of pH

The optimal pH growth of *L. monocytogenes* is between 6 and 8 [15]. However, *L. monocytogenes* can adapt, grow and survive in acid environments. Its resistance depends on other ecological factors and its physiological condition. The influence of the pH in the growth of *L. monocytogenes* was largely studied [1, 16]. With reference to [1], it was shown that ultimate pH (normal and DFD meats) influenced significantly the growth of *L. monocytogenes* inoculated on beef samples stored at two temperatures (4 and 9°C). The growth of *L. monocytogenes* was higher on DFD meat, revealing the effect of the ultimate pH with evident dependence of the storage temperature. In fact, at 4°C, no growth of *L. monocytogenes* was observed on meat with normal ultimate pH, but on DFD meat, this bacteria achieved levels of 5.5 log CFU/g in vacuum-packed samples at day 14 of storage. At this time, levels of 8 log CFU/g was obtained in vacuum-packed meats stored at abusive temperature (9° C) [1].

It has been shown that tolerance to low pH can be induced in *L. monocytogenes* by exposure to sublethal pH conditions. During the adaptation period, *L. monocytogenes* synthesizes a set of proteins that allows it to survive under stress conditions. Thus, according to [17], it was evidenced the existence of proteins, using two-dimensional gel electrophoresis, which are only present in acid stress conditions. The activation of several genes responsible for the codification of the proteins that confer resistance in pH stress conditions has been discussed by several authors [18, 19]. The stress sigma factor (σ^B) has been referred as responsible for *L. monocytogenes* resistance, although other genes are also involved in the resistance mechanisms [19, 20].

The resistance of *L. monocytogenes* to acid conditions may compromise the safety of several foodstuffs with low pH. It should be taken into consideration especially in foodstuffs with a long shelf-life in which survival microorganisms might be associated to an outbreak.

2.2. Osmotic stress

The resistance of *L. monocytogenes* at low aW values depends on environmental factors as well as its physiological condition. Most of the reports assessed the osmotic resistance together with other factors such as temperature or pH. [21] reported that minimum aW value required for *L. monocytogenes* growth generally increased with the reduction of temperature. The range of aW of *L. monocytogenes* that allows growth is variable. According to [22], it was showed that aW resistance depends on the initial counts of *L. monocytogenes* in broth. Thus, at low contamination (between 1 and 20 CFU/ml), an inhibition of *L. monocytogenes* was observed at aW values above 0.975. However, the critical aW for *L. monocytogenes* growth was set at 0.965 at high contamination levels (between 500 and 1000 CFU/ml). With reference to [23], the authors inoculated 10^7 CFU/ml in Mueller-Hinton broth at three levels of aW (0.91, 0.95 and 0.97) to study the effect of aW factor. Although a reduction, of about 1 log CFU/ml, was observed after incubation for 4 hours at 0.97 of aW, *L. monocytogenes* survivors developed after 24 hours of *lag* phase, showing that can adapt to the osmotic stress condition. *L. monocytogenes* is tolerant to NaCl, and it was capable to grow in 25.5% and survived for 1 year in 16% NaCl [24].

The adaption of *L. monocytogenes* to osmotic stress is associated to three main mechanisms: induction of proteins, accumulation of solutes as osmoprotectants and the stress sigma factor. The induction of proteins has been observed by [25] throughout electrophoresis analysis. Although the mechanisms are not clearly understood, some genetic interference has been reported by [26]. The accumulation of osmoprotectants to maintain the osmotic balance such as glycine betaine, proline betaine, acetyl carnitine or carnitine was described by [27]. The amount of each accumulated osmolyte by the cell appeared to be dependent of the growth media osmolarity. All of them play an important role in the osmoprotection, although with reference to [28] observed that carnitine is not as effective as glycine betaine in contributing to either salt or chill stress responses of *L. monocytogenes*. The stress sigma factor is induced upon exposure of *Listeria* to several stress conditions and improves the resistance of *Listeria* by regulation the production of protective substances [29].

2.3. Temperature

L. monocytogenes is capable to survive and to multiply over a wide range of temperatures. The lower limit for the growth of *L. monocytogenes* in food matrices with a high content of nutrients and neutral pH is around 0°C. With reference to [1], the final storage of air and vacuum-packed beef samples stored at abusive temperatures (9°C) produced higher (2–3 log CFU/g) counts of *L. monocytogenes* than observed in samples stored at 4°C. The presence of *L. monocytogenes* in refrigerated meat products during the product shelf-life has been reported by [30]. Although refrigeration is a common conservation method, the indiscriminate use of cold, that is, in sliced dry-cured meat products [23] may improve the survival of *L. monocytogenes* [31] reported that *L. monocytogenes* has grown in air-packed beef stored at 5°C up to 16 days. The *lag* phase is variable according to the environment temperature and may be associated to the physiological modification of *Listeria* to survive at low temperatures. The changes in the membrane composition at low temperatures may lead to a change in the membrane lipid composition in order to maintain the fluidity required for proper enzymatic activity and solute transport. Growth at low temperatures also results in an increase in the percentage of unsaturated fatty acids to improve the membrane fluidity [32].

Low temperatures lead to changes in gene expression and induction of proteins named cold shock proteins in response to temperature shocks. In consequence, this adaptation of *Listeria* implies changes in its gene expression [33]. As previously discussed, the accumulation of solutes such as glycine, betaine and carnitine acts as a cryoprotectant. Moreover, the role of the alternative sigma factor B (σ^B) is associated to the resistance of *L. monocytogenes* at low temperatures as it may be involved in the stimulation of the genes responsible for the synthesis and accumulation of the cryoprotectant solutes [34].

2.4. Packaging

The growth of *L. monocytogenes* is scarcely affected by anaerobic or oxygen-reduced atmosphere. According to [16, 35], modified atmosphere packaging (MAP) systems may reduce the survival and growth of *L. monocytogenes* by the presence of carbon dioxide in modified atmosphere packaging (MAP). In fresh beef, MAP with 60% CO_2: 30% O_2: 10% N_2 prevented growth at 4°C

for more than 2 weeks of storage. Although regarding vacuum packaging, this preservation methodology seems not affecting the growth of *L. monocytogenes* as observed by [16]. With reference to [35], it was showed that *L. monocytogenes* survives better in vacuum packaging than in air-packed beef samples. According to [36], neither *L. monocytogenes* grow after 42 days of storage nor significant reductions were observed in inoculated vacuum-packed beef stored at 4°C [37] observed *L. monocytogenes* growth in vacuum-packaged beef stored at 0 and 5°C. They indicated that growth of this bacteria on beef depends on the storage temperature, pH and the type of tissue (fat or lean). Although *L. monocytogenes* grows at both temperatures, a scarce *lag* period was observed in beef stored at 5°C. Similarly, [35] observed an increase of *lag* phase of *L. monocytogenes* in beef samples stored at 4°C compared to those stored at 9°C. In a study with pork cuts [38] stored at mean refrigerator temperatures did not increase the populations of *L. monocytogenes* over 2 log CFU/g in the end of product shelf-life. However, at abusive temperatures, microbial counts were higher than 3 log CFU/g for some cases, which required a more severe heat inactivation treatment before consumption. According to the *lag* phase of *L. monocytogenes* in vacuum-packed beef at 0°C, it was extended until 60 days. Regarding the type of the tissue, a faster growth of *L. monocytogenes* was observed in fat than in lean that may be associated to the differences of pH of both tissues. In consequence, to improve the beef safety against *L. monocytogenes*, the storage at low temperatures and vacuum packaging must be associated to other barrier systems such as bacteriocins or essential oils [39].

3. Strategies for *L. monocytogenes* growth control in meat

Classically, the main methodologies for fresh meat preservation are chilling and freezing, but technologies such as packaging systems like modified atmosphere packaging (MAP) and active packaging (AP) or use of natural antimicrobial compounds have arisen to improve its safety and quality.

Currently, consumers' growing concern about chemical hazard in foods reflects an increased awareness about the harmful effects that they may have on human health. In consequence, the consumers' demand on more healthy and natural foods, leading food industry to use natural substances such as plant extracts, essential oils, chitosan and organic acids to satisfy this green consumering tendency.

The use of essential oils (EOs) to control *L. monocytogenes* has been studied by several authors. With reference to [40], the antimicrobial effect of thyme EO against *L. monocytogenes* in minced beef during 12 days of storage at 4°C was studied, and about 2 log CFU/g reduction of *L. monocytogenes* counts was observed after 2 days of storage. Although an increase of *L. monocytogenes* counts was found after 6 days of storage, indicating a potential of its adaptation to the EO. Similar results were observed by [41] in minced meat inoculated with thymus EO, although concentrations of thymus EO at 0.25 and 1.25% decreased progressively the counts of *L. monocytogenes* up to 15 days of storage at 7°C.

In a study of [39] rosemary EO sprayed in beef samples presented a greater inhibitory effect against *L. monocytogenes* compared to thymus EO was reported. This fact can be related to the

chemical composition of this EO since the concentration of phenolic compounds (i.e., thymol) was lower than the obtained in rosemary EO. With reference to [42], an antimicrobial effect of oregano, cinnamomum, rosmarinus, salvia and thymus EO against *L. monocytogenes* in meatballs stored at 4°C was observed, while the extension of the antimicrobial effect varied according to the added EO and its concentration. A reduction of 1 and 2 log CFU/g, on average, was observed when concentrations of about 1 and 2% were added, respectively. It indicates that the antimicrobial effect of EO in foodstuffs is not enough to guarantee the safety of meat in case of high contamination. In addition, the negative impact on sensory acceptance was also indicated by the authors.

Regarding active packaging, the addiction of several substances with antimicrobial effect such as organic acids, chitosan or nisin among others has also been studied to improve meat safety against *L. monocytogenes*. With reference to [43], the authors observed that the use of chitosan diluted in acetic acid or lactic acid as coating in highly contaminated ready-to-eat roast beef (6.5 log/CFU) is useful to control *L. monocytogenes*. The use of sodium lactate and sodium diacetate in edible coating in combination with polysaccharide-based edible coatings have been studied by [44] in chilled and frozen roasted turkey. Although organic acids decreased the counts of *L. monocytogenes*, its combination with chitosan increased the antimicrobial effect. With reference to [45] who evaluated the decontaminating efficacy of lactic acid (2%), potassium sorbate (1%), sodium hypochlorite (200 ppm) and ethanol (10%) sprayed on the surface of meat previously inoculated with 100 μL of a suspension of *L. monocytogenes* (1.5×10^4 CFU/g), the authors observed that samples treated with lactic acid showed significantly lower counts than the controls and other treatments. Lactic acid was shown to be promising in the control of *L. monocytogenes* presenting an early bactericidal effect.

The use of *Lactobacillus sakei* to control *L. monocytogenes* in fresh beef was reported by [46]. Incorporation of lactic acid bacteria into sodium-caseinate films protected beef by lowering the growth of *L. monocytogenes* during storage under abusive temperatures. This strategy could be useful to guarantee the safety of fresh beef along the food chain in which temperature fluctuations may occur.

Bacteriophages harmless to human cells are considered natural biocontrol agents against foodborne pathogens [47]. Bacteriophages are bacterial viruses with host specificity and lysis activities and can be used as preservatives or for pathogens rapid detection [48].

Phages used for biocontrol purposes should be virulent and feature abroad host range, that is, infect and kill as many target strains as possible [49, 50]. Virulent myoviruses closely related to P100 and A511 are the most popular and have been isolated from the sources in Europe, the US and New Zealand [51–53]. Commercially, "ListexTM P100" is available that was generally recognized as safe (GRAS) by FDA and USDA in 2007 for use in all food products. Several studies have showed its efficacy in foods such as ready-to-eat (RTE) meats and poultry [50, 51]. The phage A511, closely related to P100, also showed efficacy in various RTE foods [54]. According to [55], the direct immobilization of the viral particles in the cellulose membranes of the packaging materials can be used in alternative to the phage suspension as a possible intervention strategy against *Listeria*.

4. Predictive microbiology models of *L. monocytogenes*

Predictive microbiology models are used to infer about the evolution of microbial population considering the initial contamination and food environment, as the responses of microorganisms populations in a specific environment are reproducible [56]. Mathematical models may be generally categorized into three types: primary, secondary and tertiary models. The primary models are used to estimate the changes in the microbial population as a function of time, under a single set of conditions [57, 58]. The secondary models describe the microorganisms' responses to environmental conditions, according to one or more parameters of a primary model [59]. The tertiary models were defined by [60] as algorithms incorporated into software to integrate the effect of environmental variables on microbial responses and to provide predictions of the outcomes.

The increasing interest in the behavior of hazards such as *L. monocytogenes* promoted important advances in predictive microbiology, and it started to use the food matrix, instead of culture media [61]. Traditional strategies using fast-growing strains in optimal growth conditions usually overestimate the bacterial growth in a food product. This can lead to safe results but may also conduct to unnecessarily safety measures. A stochastic (or probabilistic) approach take into account the variability and uncertainty of various factors that affecting microbial behavior by using probability distributions of the input data. This provides safe enough predictions to avoid unacceptable health risks for consumers [62].

Predictive microbiology models are important tools to model bacterial growth in quantitative microbial risk assessments (MQRA) [63]. In this context, food business operators and authorities can use accessible predictive models, such as Pathogen Modeling Program [64], SymPrevius [65] and ComBase [66]. The incorporation of predictive microbiology models in MQRA must follow some guidelines [56]. The complexity of the predictive microbiology model elected in a MQRA depends on different factors, namely the needs of risk assessment, available model and data availability [63].

For an assessment of microorganisms' behavior in naturally contaminated foods, biological factors, food characteristics and storage conditions must be considered [67]. These authors emphasize the variability of *L. monocytogenes* growth in foods. According to [61], the role of microbial competition in models is now taken into consideration. Some studies were published regarding the survival of *L. monocytogenes* in fresh beef stored at two temperatures and different packaging systems as modified atmosphere packaging (MAP), using omnibus model based on the Weibull Equation [35]. Besides the increase of studies using predictive models, there are few data referred to the application of predictive models to composite foods containing raw and cooked ingredients [67].

According to [63], it is challenging for a risk assessor to choose an applicable predictive microbiology model in the abundant literature. This author suggests that the choice of a model should be done with the closed cooperation between microbiologists, mathematicians and risk managers.

5. Conclusions

Besides the Regulation (EC) No 2073/2005, reviewed by the Regulation (EC) No 1441/2007 does not establish limits for *L. monocytogenes* in fresh meat, it is generally accepted a level of 100 cells on fresh meat, except for high-risk populations. Thus, the implementation of control procedures during processing and at retail level is important. These measures are closely dependent on intrinsic and extrinsic meat factors that could influence microbial growth, namely pH and storage temperature.

Several strategies to improve meat safety and shelf-life have been studied in the latest years. From those, the use of alternative meat packaging systems has been strongly studied to obtain an attractive meat with a higher shelf-life. However, in some cases, these strategies associated to refrigerated storage can promote the survival and growth of some pathogenic microorganisms such as *L. monocytogenes*. However, some authors referred that independently of the refrigeration temperature, the presence of CO_2 in the package atmosphere exerted a bactericidal effect on *L. monocytogenes* cells.

Food business operators and authorities can use predictive microbiology models as important tools to model bacterial survival or growth in quantitative microbial risk assessments. There are several mathematical models to predict the behavior of microorganisms in meat and meat products. However, predictive microbiological models must be carefully used and by whom who is expertise and has an understanding of their limitations and conditions of use.

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Conflict of interest

The authors declare no conflict of interests.

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