DOCTORAL PhD DISSERTATION

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Heat stress adaptation of *Listeria* spp. after sub-lethal heat treatment

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LIST OF ABBREVIATIONS

ACP - Automated Cleaning Programme

ANOVA - Analysis of variance

BRC - British Retail Consortium

CCP - Critical Control Point

CDC - Centers for Disease Control and Prevention

CFU - Colony Forming Unit

CIP - Clean-in-Place

DAPC - Discriminant Analysis of Principal. Components

DC - Detection Criterion

E. - Escherichia

EC - European Commission

ECDC - European Centre for Disease Prevention and Control

EFSA - European Food Safety Authority

EN ISO - European Standards International Organization for Standardization

EU - European Union

FDA - United States Food and Drug Administration

GHP - Good Hygiene Practice

GMP - Good Manufacturing Practice

GSP - Good Sanitation Practice

HACCP - Hazard Analysis Critical Control Point

HPP - High-Pressure Processing

HSPs - Heat shock proteins

L. - Listeria

MALDI-TOF MS - Matrix-assisted laser desorption/ionization-time of flight mass spectrometry

mV - millivolts

nm - nanometer

OD - Optical Density

PCR - Polymerase Chain Reaction

PEF - Pulsed Electric Field

RMSE - Root Mean Square Error

RTE - Ready-to-Eat

SMEs - Small and medium-sized enterprises

TSA - Tryptic Soy Agar

TSB - Tryptic Soy Broth

TTD - Time to Detection

TVC - Total Viable Count

VBNC - Viable but nonculturable

1. INTRODUCTION

Listeria monocytogenes can be found in various environments such as soil, water, and food processing equipment. Listeriosis is one of the most common foodborne diseases worldwide. Its high case-fatality rate and psychrotrophic features make *L. monocytogenes* a great concern in food safety.

Heat treatment is still the most common preservation technique used in the food industry. The thermal resistance in bacteria is often characterized by two metrics, namely the D-value and the z-value. Applying precise time-temperature combinations is crucial to effectively reduce or eliminate microorganisms during thermal processing.

Stress adaptation is a response of microorganisms to stresses caused by hurdle technologies, including heat treatment. Certain stress responses can lead to enhanced survival, and increased virulence, and provide resistance against multiple stressors. Prior exposures to temperatures over the optimal growth range can make a microorganism more resistant to subsequent heat treatment. Microorganisms can survive heat treatment conditions that normally are lethal because of this thermo-tolerance response. Therefore, the process of adapting to heat stress can lead to an increase in D value.

The enumeration of microorganisms is still commonly performed using conventional plate counting methods. However, they are time-consuming and labour-intensive. Therefore, alternative enumeration approaches have been developed for rapid and accurate enumeration. Redox potential measurements and optical density measurements, for instance, are two indirect techniques for quantification. Their ability to provide faster results renders these two techniques advantageous for doing more comprehensive research.

In predictive microbiology, microbial inactivation characteristics are represented by mathematical models. These models predict inactivation parameters, which are applied in various disciplines such as microbial risk assessment. There are accessible tools that allow users to apply their data to various models without requiring a thorough comprehension of the mathematical principles underlying such models.

Vending machines become small food production facilities due to technological developments in robotics. Ensuring adequate hygiene of the vending machines is crucial to maintain food safety. Vending machines are generally cleaned manually. The implementation of Clean-in-Place (CIP) technology in vending machines can minimize the need for human intervention.

2. OBJECTIVES

The primary objective of my dissertation research was to gain a deeper comprehension of the heat stress adaptation response in *Listeria* spp. and assess the suitability of redox potential measurement and optical density measurement for quantifying *Listeria* spp. In addition to this study, I also examined the use of a CIP application for cleaning a vending machine that produces smoothies and hot soups. *L. innocua* is frequently employed as a surrogate for *L. monocytogenes*, hence it is also included in these investigations.

The specific objectives of my research were:

- to understand the physiological responses of *L. innocua* and *L. monocytogenes* to sub-lethal temperature stress conditions;
- to compare the effect of strain variability, different sub-lethal exposure conditions, and different heat destruction methods on stress adaptation;
- to evaluate the applicability of redox potential measurement and optical density measurement as an enumeration of *L. monocytogenes* and compare with plate counting method;
- to compare different mathematical models of heat inactivation of *L. monocytogenes*.

A variety of conventional and rapid microbiological techniques, molecular microbiological approaches, and different software tools were applied to reveal the underlying questions. Besides, a case study was conducted on vending machines to broaden knowledge on CIP cleaning and its validation, with a specific focus on examining the existence of *L. monocytogenes* and implementing the use of thermal processing in cleaning protocols to ensure microbial safety and efficacy.

3. LITERATURE OVERVIEW

3.1. *Listeria monocytogenes*

Listeria monocytogenes is a bacterium that is Gram-positive, rod-shaped and belongs to the genus *Listeria* (Duze et al. 2021). *L. monocytogenes*, out of more than 15 species in the *Listeria* genus, is primarily responsible for causing infections in humans (Orsi and Wiedmann 2016). *L. ivanovii* has the ability to infect animals, although it rarely causes harm to humans. The other species of *Listeria* are not known to cause disease in either humans or animals (EFSA 2022). It is facultatively anaerobic so it can thrive in both oxygenated and oxygen-depleted conditions. As a persistent hazard in food processing and storage facilities, one of the most alarming characteristics of *L. monocytogenes* is its capacity to grow and replicate at refrigerated temperatures as being psychrotrophic (Newell et al. 2010, Carpentier and Cerf 2011).

L. monocytogenes is well-known for causing a serious illness called listeriosis (Shamloo et al. 2019). Listeriosis can cause severe risks to susceptible groups such as pregnant, newborns, the elderly, and immuno-compromised. Individuals belonging to those groups are at high risk of developing severe complications like meningitis, septicemia, miscarriage, or fatality as a result of infection (Schlech 2019). Healthy individuals may have moderate symptoms such as fever, muscle pain, and gastrointestinal problems.

Multiple factors have been identified as influencing the growth and survival of *L. monocytogenes*. It has the ability to multiply rapidly throughout a broad range of temperatures, with the most favourable growth happening from 30 °C to 37 °C. Nevertheless, it is noteworthy for its capacity to thrive at low temperatures typically found in refrigeration (-0.1 – 4 °C) (Walker et al. 1990). It exhibits a wide pH tolerance, ranging from acidic environment (pH 3.5) to mildly alkaline conditions (pH 9.4) (O'Driscoll et al. 1996, Engstrom et al. 2020). The ability to adapt enables it to thrive in various food matrices, including acidic meals like fruit juices and fermented products. *L. monocytogenes* can flourish in conditions characterized by low water activity levels, reaching

as low as 0.92, which are typical of dry foods (Pérez-Baltar et al. 2020). It has resistance to modest concentrations of salt (NaCl). The microorganism may thrive in salt concentrations of up to 10%, while survival in higher concentrations was also reported (Boyer et al. 2009, Schirmer et al. 2014, Wiktorczyk-Kapischke et al. 2023). *L. monocytogenes* has the ability to create biofilms, which are bacterial communities. These biofilms make the bacteria resistant to disinfectants and environmental challenges like drying out and lack of food (van der Veen and Abee 2011, Bonsaglia et al. 2014, Ferreira et al. 2014, Pang et al. 2019, Fagerlund et al. 2021, Mazaheri et al. 2021). It can survive and reproduce at cold temperatures, although it is vulnerable to heat treatment. However, exposure to heat stress that does not immediately cause mortality can stimulate adaptative responses. Therefore, this response can lead to an improved capacity to endure elevated temperatures.

L. monocytogenes is ubiquitous in the environment, meaning it can be found in various sources like soil, water, and animals. The food processing conditions provide an optimal habitat for *L. monocytogenes* (Buchanan et al. 2017). Listeriosis is the fifth most common zoonotic disease in the EU (EFSA 2022). It is one of the most serious foodborne diseases with a high rate of hospitalizations, especially in infants and the elderly (Cassini et al. 2018). According to estimates, listeriosis killed 5463 people around the world in 2010 (De Noordhout et al. 2014, World Health Organization 2015). In the EU, 335 people died in 2023 because of listeriosis with the fatality rate at 19.7 %, with a notification rate of 0.66 cases per 100000 people. These numbers were the highest recorded since EU surveillance began in 2007 (ECDC and EFSA 2024). Meat products, fruits and vegetables, dairy products, fish and seafood, and ready-to-eat (RTE) foods have all been linked to listeriosis (Ziegler et al. 2019).

In the EU, Commission Regulation (EC) No 2073/2005, as amended by Commission Regulation (EC) No 1441/2007, has established microbiological criteria for *L. monocytogenes* in foods (European Commission, 2007, 2005). For *L. monocytogenes* in RTE foods that support its growth, the proposed microbiological criteria are: levels must be below 100 CFU/g throughout the shelf

life of the product, and there must be an absence of *L. monocytogenes* in 25 g of the product before it leaves the control of the food business operator (European Commission 2005). Although food safety laws and regulations have improved, *Listeria* outbreaks have occurred in the EU. Most outbreaks were linked to RTE foods such as cold smoked salmon (ECDC and EFSA 2024), meat products (CDC 2023), dairy products (FDA and CDC 2024), and frozen vegetables (EFSA and ECDC 2018), primarily due to household consumption rather than social or community events.

In 2018, *L. monocytogenes* occurred in multiple countries in the EU leading to 47 cases and 9 deaths. A frozen vegetable-producing factory in Hungary was found as the source of contamination. Microbial analysis showed that the bacteria had been in the plant for years, showing its persistence in food-processing environments (EFSA and ECDC 2018).

A comprehensive strategy is needed to prevent *Listeria* contamination. This strategy must include all the stages of food production and the distribution chain. Good Manufacturing Practices (GMP), Good Storage Practices (GSP), and Good Hygiene Practices (GHP) are the standards used in the industry as key preventive measures (Disanto et al. 2021). Strict sanitation protocols, refrigeration and temperature control, surveillance, and traceability are some important procedures included in those standards (de Oliveira et al. 2016).

3.2. Microbial enumeration methods

To assess food safety and implement right control measures, accurate enumeration of *L. monocytogenes* is essential. Fields of predictive microbiology, epidemiology, quantitative risk assessment, and monitoring programs in food processing facilities all rely on reliable data from the enumeration (Auvolat and Besse 2016). Many different approaches, from simple culture-based methods to more complex molecular and spectroscopic methods have been developed over the years (Besse and Colin 2004).

The primary technique for the enumeration of *L. monocytogenes* is conventional plate counting. The new available version of the EN ISO 11290-2 recognized as the European and International Standard is the established method for quantifying *L. monocytogenes* (ISO 11290-2, 2017). It is the standard method used to determine the quantitative criteria for *L. monocytogenes* as outlined in EC Regulation No. 2073/2005 (ISO 11290-2:1998, 2004). This method involves spreading decimally diluted samples onto selective agar plates. Plate counting provides quantitative data. However, it is time-consuming as it requires several days to obtain results. Therefore, faster alternative methods have been developed (Chikhi et al. 2024).

Indirect methods provide alternative approaches to evaluating microbial activity. Redox potential measurement-based method and optical density measurement-based methods are two examples of indirect methods. Evaluating the electron acceptor capacity of a system by measuring the redox potential gives insights into microbial growth dynamics. In liquid cultures, optical density measurements monitor changes in turbidity as a surrogate for bacterial growth. Even though these methods are rapid and cost-effective, they have some disadvantages. For example, not all of them are specific for *L. monocytogenes* and may not quantify only the target microorganism (Vasavada et al. 2020).

Recent advancements in molecular methods have revolutionized *L. monocytogenes* enumeration. Techniques such as polymerase chain reaction (PCR) enable rapid and specific detection of pathogens (Jasson et al. 2010). However, they may require specialized equipment and expertise, limiting their accessibility and cost-effectiveness, particularly in resource-limited settings (Vasavada et al. 2020).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) represents another innovation in microbial identification, including *L. monocytogenes* (Jadhav et al. 2015). MALDI-TOF allows for the rapid and accurate identification of microbial isolates based on their mass spectra (Barbuddhe et al. 2008). While MALDI-TOF offers rapid identification of

L. monocytogenes, its utility for enumeration purposes is limited, as it primarily provides qualitative rather than quantitative data. Currently, there are plenty of test kits available in the market that provide insights on the detection and enumeration of *Listeria* spp. (Välimaa et al., 2015).

There are detailed review articles comparing various new techniques developed for counting *L. monocytogenes* (Law et al. 2015, Ripolles-Avila et al. 2020, Rohde et al. 2017, Chikhi et al. 2024). In comparing these enumeration methods, several factors must be considered. Traditional culture-based methods offer quantitative data but are slow and labour-intensive. Indirect methods provide rapid results but have some disadvantages. Molecular methods offer high sensitivity and specificity but may be cost-prohibitive. MALDI-TOF enables rapid identification but is not suited for enumeration. Ultimately, the choice of method depends on factors such as the desired level of sensitivity, resources available, and specific requirements of the application.

3.2.1. Redox potential measurement-based method

Redox potential indicates the electron transfer capability of a system, measured in millivolts (mV). In microbial environments, redox potential is influenced by the metabolic activities of microorganisms (Szakmár et al. 2014). When the bacteria proliferate and thus metabolize substrates, they reduce the redox potential of the environment. This decrease is due to the consumption of oxidants and the production of reductants. Therefore, redox potential measurement can be used to predict the microbial activity of a system. It is used in various fields such as wastewater treatment (Goncharuk et al. 2010), fermentation processes (Lin et al. 2011, Liu et al. 2012, Nelson et al. 2023), and food microbiology (Brasca et al. 2007, Yakdhane et al. 2024).

Erdősi et al. (2012) stated that the total count determination of microbial contamination on model surfaces and in the sanitary control of a food manufacturing pilot plant took just 10-16 hours using the redox potential measurement, in contrast to the 72 hours required for plate counting. The same

research group reported a maximum of 34 hours of measuring time with redox potential measurement for *Listeria* species (Erdősi et al. 2014).

Reichart et al. (2007) developed and validated a redox potential measurement-based method for the detection and enumeration of microorganisms. When bacteria multiply, redox potential decreases. The redox potential curve for each type of microorganism is different. The rate of change in redox potential (dE/dt) is proportional to the concentration of microbial cells. Thanks to this relationship, it is possible to determine the microbial concentration with the calibration curve. Impedance microbiology is an effective method that facilitates both qualitative and quantitative analysis of microorganisms through the measurement of variations in electrical conductivity (Wawerla et al. 1999). Like impedimetric measurements, the logarithm of the initial concentration of a microorganism and time to detection (TTD) show a linear relationship. TTD is a threshold value where the redox change passes the detection criterion (DC). The fundamentals of the redox potential measurement-based method are shown in Figure 1 (Engelhardt et al. 2018).

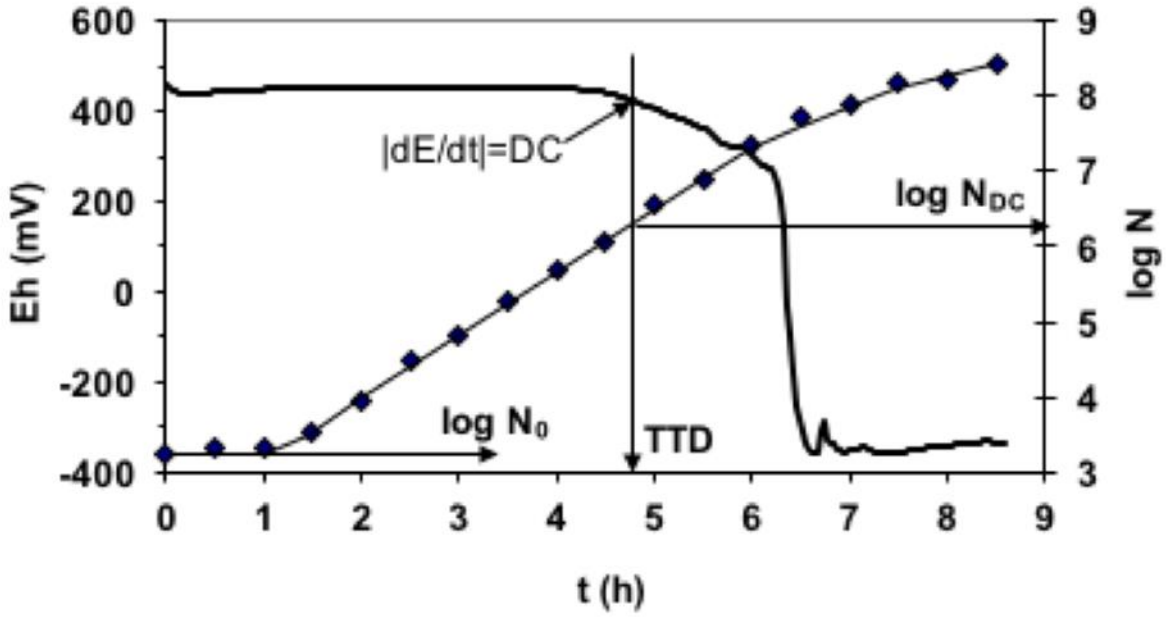


Figure 1. The Fundamentals of TTD in the Redox Potential Measuring System (Engelhardt et al. 2018)

Note. Eh: redox potential of the medium; N: viable cell concentration; N_0 initial viable cell concentration in the medium; DC: detection criterium; N_{DC} the threshold value of the viable cell concentration at the time of TTD.

The method can provide results faster compared to traditional plate counting methods. While conventional culture methods require several days for the results of the enumeration procedure, it is much faster with the redox method, depending on the initial concentration (Erdősi et al. 2012). The method is non-destructive so it is possible to monitor microbial activity without compromising the integrity of the sample. It also allows high-throughput screening and continuous monitoring.

Redox potential indicates microbial activity but does not provide information on the type of microorganism. A combination of the method with different methods can be used to overcome this problem. For example, combining the redox potential method with real-time PCR is used for the detection of *L. monocytogenes* and *Salmonella* in different food products (Erdősi et al. 2014a, Erdősi et al. 2014b).

3.2.2. Optical density measurement-based method

Optical density (OD) measurements are widely used in enumerating microorganisms. It has different applications like examining microbial growth under various stress conditions (Stevenson et al. 2016). With this method, the density of the microbial population is estimated by measuring the absorbance of a culture at a specific wavelength, typically around 600 nm for bacteria (McGoverin et al. 2021).

The OD measurement is based on the Beer-Lambert law. The absorbance of a sample is directly proportional to the concentration of the population (Mira et al. 2022). As microorganisms multiply, the culture becomes more turbid, leading to higher absorbance readings. The standard curve is obtained by the known cell concentration and their corresponding absorbance values (Stevenson et al. 2016). After that, microbial concentration can be calculated using the standard curve.

Enumeration of microorganisms with OD measurements has some advantages. It is known as a rapid method, providing real-time data. It is possible to use it for continuous monitoring without disturbing the sample. It is simple to use and requires minimal sample preparation. This reduces the possibility of contamination and experimental errors (Beal et al. 2020).

Time to detection (TTD) values can be used in an application of enumeration employing OD measurement to evaluate microbial development. TTD is the time takes for a suspension to attain a given turbidity level. A particular cellular concentration is indicated by this point. By applying a standard curve, this method allows the estimation of the unknown cell content of a microbial growth. This approach is applied in different fields, including food business quality control, study of microbial kinetics, and stress condition analysis (Baka et al. 2015).

Nevertheless, there are some constraints associated with OD measurements. A notable limitation is that OD measurements are indirect and susceptible to impact from several factors, including the morphology, size, and composition of different substances in the medium (Myers et al. 2013). These variables can result in discrepancies in the association between absorbance and cell

concentration. In addition, OD measurements exhibit reduced efficacy when used in cell densities that are either low or high (McGoverin et al. 2021). At low concentrations, the level of absorbance may be insufficient to accurately identify. In contrast, the readings may plateau at high densities due to light scattering, leading to an underestimation of cell concentration. Another drawback is that optical density measurements can be biased by viable but non-cultivable (VBNC) cells, intact non-viable cells, and dividing cells (Hazan et al. 2012, Haaber et al. 2016). Another limitation of OD measurements is that they count both live and dead cells as both types provide an optical sign (Kesisoglou et al., 2022).

3.3. Heat stress adaptation of *L. monocytogenes*

L. monocytogenes is one of the pathogens known for its endurance in various environmental conditions, including its thermal resistance (Chan and Wiedmann 2008, Chaturongakul et al. 2008). Thermal resistance in bacteria is generally defined by two parameters, D- and z-value (Juneja et al. 2011). Despite ongoing arguments over this assumption, the D- and z-values are derived under the assumption that the heat inactivation kinetics of microorganisms follow a logarithmic pattern (Augustin et al. 1998, Peleg 2021). The D-value, or decimal reduction time, is defined as the time required for reducing the population by one log cycle at a specific temperature. D-values of *L. monocytogenes* at 60 ℃ range from 0.5 to 3 minutes (O'Bryan et al. 2006). D-value can vary depending on the strain, the matrix, and environmental conditions (Lianou and Koutsoumanis 2013). This variability shows the ability of *L. monocytogenes* to survive under different thermal processing conditions.

The z-value represents the temperature change to achieve a tenfold reduction in the D-value. The z-value of *L. monocytogenes* can vary between 5 ℃ and 10 ℃ (O'Bryan et al. 2006). D- and z-values indicate the sensitivity of bacteria to heat. They are essential for designing thermal processing parameters in the food industry. They must be sufficient to inactivate *L. monocytogenes* without compromising food quality.

Microbial stress is any physical, chemical, or biological adverse effect that influences microbial growth or survival (Schimel et al. 2007). Microbial stressors include traditional or novel food preservation techniques such as thermal processing, pulsed electric fields, and high hydrostatic pressure. All of these preservative factors are called hurdles (Leistner, 2000). These hurdles have destructive effects on the physiology, function, and activity of bacteria. Multiple factors establish the type and quantity of hurdles required. For instance, nutrient- and vitamin-rich food will promote microbial growth (booster or trampoline effect), necessitating an increase in the quantity and intensity of hurdles. The knowledge of the hurdle effect has led to the development of the hurdle technology (Leistner, 2000).

Stress may be classified as lethal/severe or sublethal depending on the intensity. Sublethal stress causes microbial injury that leads to either inhibition or retarded growth due to modifications in cellular metabolic activity (Wesche et al. 2009). In contrast, when the cells are subjected to lethal or extreme stress, the damage is irreversible (Arvaniti et al. 2021).

Exposure to sublethal stress can result in the adaptation of microorganisms to the subsequent lethal levels of the same stress, which is called stress adaptation or stress hardening (Dawan and Ahn 2022). Bacteria exposed to sublethal stress can adapt to following the same or different stress by becoming more resilient (Lou and Yousef 1997). Thus, microorganisms can survive under the conditions that would normally inactivate them.

As it was stated earlier, the optimal growth temperature of *L. monocytogenes* is between 30 °C to 37 °C (Jamshidi and Zeinali 2019). When subjected to temperatures above the optimum range, any thermal treatment causes stress. Microbial cells exhibit distinct physiological reactions such as the production of heat shock proteins (HSPs) that lead to thermotolerance. The degree of observed thermotolerance is influenced by the extent of temperature exposure above optimum limits and the specific matrix in which the cells are exposed.

L. monocytogenes raises its D-value for the subsequent heat treatment as a stress response to sublethal heat (Wesche et al. 2009). Temperature and duration of sublethal heat exposure, growth phase of bacteria, and composition of food matrix affect the degree of D-value increase (Jorgensen et al. 1999, Shen et al. 2014). Increased sublethal temperature or extended exposures, cells in stationary phase, and protective nutritional components like fats and proteins can all contribute to enhanced thermal resistance (Dawoud et al. 2017, Zhang et al. 2022).

Stress adaptation is an essential survival mechanism for *L. monocytogenes*. Various defensive mechanisms are triggered in response to mild heat stress. Heat stress adaptation involves increasing the expression of HSPs and other molecular chaperones. HSPs ensure the proper functioning of cells during periods of stress by reconfiguring unfolded proteins and breaking down the proteins that have been damaged. The stress response network allows *L. monocytogenes* to endure unfavourable environments (Bucur et al. 2018).

The heat stress adaptation mechanism involves several regulatory systems (Sibanda and Buys 2022). A key component of the stress response of *L. monocytogenes* is the alternative sigma factor, σ^B (Bucur et al. 2018). The activity of σ^B increases under heat stress. It triggers a defensive response which improves the ability of the pathogen to gain stress resistance (Moorhead and Dykes 2003). It controls the transcription of genes associated with stress resistance, including those that encode HSPs (Wiktorczyk-Kapischke et al. 2021). In addition, the CtsR regulon, which regulates the expression of heat shock genes, also plays a role in the adaptive response (Osek et al. 2022).

De Angelis and Gobbetti (2004) divide adaptive reaction into two classes. In the first class, when microbes are exposed to sub-lethal concentrations of physical, chemical, or biological stress, they develop a response which shields the cells from further lethal treatments of the same stress. In the second class, microorganisms possess the ability to adjust to stresses that they have not before come across. The second class comprises more universal systems, and cells that have already adapted to one stressor may be less susceptible to subsequent stressors. This kind of cross-

protection is produced in response to low pH, excessive osmolarity, exposure to extremes in temperature, and nutritional deficiency (Wesche et al. 2009). Exposure to one type of sublethal stress can cause adaptation to another stress (Tasara and Stephan 2006, Lianou and Sofos 2007, Schmid et al. 2009). This cross-adaptation presents issues in the food industry since different hurdles are used in food processing and storage. Exposure to sublethal heat, acid, osmotic, and oxidative stress can result in the development of resistance to different types of stress in *L. monocytogenes* (Acuff et al. 2023).

In recent years, fresh, natural, and minimally processed foods are getting popular. Consumers are increasingly looking for original flavours, textures, and nutritional profiles when they buy a product (Li and Gänzle 2016). Fresh juices, fruits and vegetables, and RTE meals are examples where maintaining the sensory and nutritional attributes are paramount. This trend opened a discussion in the food industry by questioning the high temperatures in conventional thermal processing (Arioli et al. 2019). Therefore, there is a need to balance food safety with the preservation of food quality (Pratap Singh et al. 2018).

Alternative processing technologies have emerged to inactivate pathogens without a negative effect on the nutritional and sensory characteristics of food (Gandhi and Chikindas 2007). However, thermal processing remains the most common method in the food industry because of its efficacy and established protocols (Dlusskaya et al. 2011). Pasteurization, canning, and cooking are examples of thermal destruction methods to ensure food safety (Li and Gänzle 2016, Tsai et al. 2019). Pasteurization is widely used in liquid foods like milk and juices. Instead, canning and cooking are standard practices for solid and semi-solid foods (Awuah et al. 2007). These processes are designed for a high degree of lethality against pathogens such as *L. monocytogenes* (Juneja et al. 2011).

Heat stress can occur in the natural environment of microorganisms as well as during food processing (Ruiz et al. 2017). It is important to provide conditions to inactivate microorganisms,

but also prevent them from developing heat resistance. Sublethal heat stress is the condition where the cells are exposed to a temperature above the optimum range and below the lethal level. Even a short period of sublethal heat exposure can be enough to cause enhanced heat resistance (Shen et al. 2014).

The thermotolerance of *L. monocytogenes* exhibits significant variation (Taylor et al. 2019). Age of culture, strain variation, growth conditions, test conditions, food matrix, and environmental stresses are parameters that affect the heat resistance of *L. monocytogenes* (Doyle et al. 2001). Similarly, enhanced heat resistance ability is influenced by different parameters such as strain variation, heating conditions, prior growth conditions, pH, and media (Bucur et al. 2018). For instance, nutritional components, like fats and proteins can have protective effects against the inactivation of pathogens. Thus, careful consideration is needed for foods that contain high levels of those compounds like dairy products, processed meats, and RTE meals (Verheyen et al. 2020). Time and temperature together have an impact on how much pathogen reduction is accomplished in heated treatment methods (McCann et al. 2009, Espinosa et al. 2020). Higher temperatures and longer processing times during thermal destruction may be necessary to ensure the safety of those products. However, this solution may in turn affect the sensory and nutritional quality.

Non-thermal technologies like high-pressure processing (HPP) and pulsed electric fields (PEF) are developed as potential solutions to these challenges (Gandhi and Chikindas 2007, Bucur et al. 2018, Meloni 2019). HPP disrupt the cellular structures of microorganisms without the need for high temperatures. PEF permeabilize bacterial cell membranes. Thus, both methods can enhance microbial safety with minimal impact on food quality. These technologies can provide a synergistic effect when used in combination with mild heat treatments or other novel technologies (Patterson et al. 2011, Meloni 2019). These synergistic combinations may overcome the limitations of conventional thermal processing techniques (Gurtler et al. 2019).

Understanding the sublethal heat stress adaptation of *L. monocytogenes* is crucial for food safety (Wiktorczyk-Kapischke et al. 2023). The efficacy of heat treatment may be adversely affected by heat stress adaptation. Foods that are subjected to sublethal heat treatments such as the sous-vide technique are particularly at concern. Foods are cooked at relatively low temperatures (typically between 55 ℃ and 65 ℃) for long periods with sous-vide style. This method is used for enhancing the sensory characteristics of food. However, it may not achieve sufficient lethality against heat-adapted *L. monocytogenes*. An *L. monocytogenes* outbreak linked to beef stew cooked under sous-vide conditions was reported in Denmark in 2009 (Ricci et al. 2018). Slow heating rates during thermal treatments can also cause enhanced heat resistance of *L. monocytogenes* (Stephens et al. 1994). *L. monocytogenes* can survive under refrigerated conditions (Saunders et al. 2016). The ability of the pathogen to survive and potentially grow during subsequent storage or distribution phases underscores the need for stringent control measures. The development of efficient thermal processing strategies requires an understanding of adaptive processes and the factors influencing increased D-values (Ricci et al. 2021).

3.4. Mathematical modelling of thermal inactivation

Predictive microbiology can be defined as using mathematical models to represent microbial kinetic behaviour (Gil et al. 2017). Environmental factors affect microbial growth, survival, and inactivation. Predictive microbiology focuses on expressing these responses mathematically. Factors like temperature, pH, nutrient levels, and humidity affect the inactivation of microorganisms. Predictive models can predict the behaviour of microorganisms under different conditions (Baranyi and Buss da Silva 2017).

Predictive microbiology has become a valuable tool for food safety. Microbial response can be predicted with mathematical models at any step during the food production and distribution (Peñalver-Soto et al. 2019). This allows us to compare different treatments, risk assessment, and shelf-life estimation (Tarlak 2023). It is useful to evaluate the survival of microorganisms under different environmental conditions like temperature. With mathematical models, it is possible to

change the heat inactivation parameters when there is any change in conditions. Therefore, mathematical modelling can be utilized in food safety and quality control like modifying Critical Control Point (CCP) in Hazard Analysis and Critical Control Points (HACCP) system (Gil et al. 2017).

When the mathematical models describe the number of survivals over time under specific conditions like temperature, they are called primary models. Historically, it has been assumed that the survival of microorganisms varies log-linearly with time (Peleg 2021). However, research has shown that there are deviations from this log-linear behaviour depending on the thermal resistance of microorganisms, media, prior stress conditions (Gil et al. 2017). Three phases, initial shoulder, log-linear, and lastly tail are the phases that describe the microbial inactivation behaviour during the inactivation (Gil et al. 2017). Parameters that represent those phases are called primary model parameters. The parameters obtained from primary models are influenced by different factors such as temperature in heat inactivation. The secondary models show how the primary model parameters vary in response to environmental factors such as temperature, pH, and water activity. Tertiary models are software programs that combine primary and secondary models into a user-friendly interface.

Different software programs, packages, and websites are available to predict microbial growth, survival, and inactivation. The objective of these tools is to provide a connection between those who develop predictive modelling techniques and the end-users in the food business who are either unfamiliar with or lack proficiency with mathematical models (Geeraerd et al. 2005). Some examples are GInaFit (Geeraerd et al. 2005), Combase (https://www.combase.cc/), Bioinactivation (Garre et al. 2017) and GrowthCurver (Sprouffske and Wagner 2016). Microbial inactivation tools include generally used mathematical models for inactivation. Users can provide the data from their heat inactivation experiments, and obtain model parameters as well as statistical measures for the prediction of those parameters. These tools are user-friendly, meaning they are easy to use, and you do not need a deep knowledge of programming or mathematical concepts of the models.

3.5. Microbial assessment of vending machines

Developments in food robotics transformed the vending machine industry from pre-packaged food sales to small food production plants. Nowadays, consumers can enjoy various products such as fresh drinks like smoothies, soups, and even pizza produced by vending machines. This transformation came with a new challenge for the industry which is the hygienic-sanitary requirements of the vending machines.

Vending machines are generally cleaned manually (Hall et al. 2007). Machine parts are disassembled and cleaned by the operators with detergents and cold/hot water, and finished by the disinfection section (Saltmarsh 2023). Microbial survival and contamination may occur in vending machines, which can be difficult to maintain temperature and hygiene. In the catering sector, 97% of foodborne illnesses are caused by food mishandling (Egan et al. 2007). Similarly, Hunter (1992) holds vending machine operators responsible for the high number of total viable counts (TVC) and coliforms due to inadequate cleaning. Due to incorrect cleaning and refrigeration, microorganisms like *Listeria* spp. can survive and adapt under varied stressors, emphasizing the need for robust thermal sanitation standards to reduce these potential hazards. The first vending machine-related case was that *Bacillus cereus* was found in a hot drink vending machine and made people sich who drank hot chocolate from that machine (Nelms et al. 1997).

Research on vending machines focused on their accessibility, product availability, and healthfulness so far (Matthews and Horacek 2015). Limited research has been conducted on the hygienic-sanitary quality of vending machines (Raposo et al. 2015, Cardaci et al. 2016, Cossu et al. 2016, Godic Torkar et al. 2017, Caggiano et al. 2023). Like other food businesses, implementing the HACCP system has been advised for the vending machine sector to guarantee food safety (Hunter 1992). The HACCP system is predicated on the adoption of the GMP and GHP procedures. However, there is an inadequate amount of data and guidelines regarding the GHPs for food vending machines.

Clean-in-place (CIP) technique is widely used in the food industry. CIP systems can be fully automated, thus it is possible to track each step of the cleaning procedure (Moerman et al. 2013). The primary benefit of vending machines is their ability to provide food to consumers without the presence of a human being. The same idea can be applied during the cleaning process of those vending machines. It is possible to use CIP systems and clean machines without the need for human assistance. Since the on-site food processing vending machine industry is relatively new, more research is needed for competent cleaning procedures.

4. MATERIALS AND METHODS

4.1. Bacterial Strains

The four different *Listeria* strains used in this study are shown in Table 1. All strains were from the strain collection of the Department of Food Microbiology, Hygiene and Safety, Hungarian University of Agriculture and Life Sciences. Strains were stored in Tryptic Soy Broth (TSB, Biokar, France) containing 30% (v/v) glycerol at -80 °C and sub-cultured twice before short-term storage. For working stock, strains were stored on Tryptic Soy Agar (TSA, Biokar, France) slants at 4 °C. For the experiments, overnight cultures were prepared by inoculating 10 mL of TSB with one loopful of stock cultures and incubated for 24 hours at 37 °C to obtain stationary-phase cultures.

Table 1. Bacterial strains used in this study

Species Name with Collection Number	Serotype	Origin	Code
Listeria innocua T1		Not Known	T1
Listeria monocytogenes L2	1/2a	Dairy	L2
Listeria monocytogenes L4	3a	Cheese	L4
Listeria monocytogenes L7	3b	Cheese	L7

4.2. Indirect enumeration methods

4.2.1. Redox potential measurement-based method

The redox potential measurements were conducted using the MicroTester apparatus, a 16-channel redox potential measuring instrument (Microtest Ltd, Hungary).

To establish a standard curve for *Listeria* strains, test tubes containing 9 mL of ½ concentration TSB (as recommended by the instrument provider) were inoculated with 1 mL of various dilution

members, and the Time to Detection (TTD) values were determined. The detection criterion (DC) was set to -0.5 mV/min. Viable counts of each dilution were enumerated by plate counting on TSA. A standard curve was constructed using the initial viable cell numbers ($\log_{10}$N in CFU/mL) determined by plate counting and the TTD values (hours) obtained from the instrument, employing linear regression. This equation was then uploaded into the computer. Each experiment was performed in triplicate on three different days.

In the following sections, all parameters were subjected to arithmetic mean computations using the following formula when working with replicates.

$$X = \sum \frac{X_i}{n}$$

X is the arithmetic mean, n is the number of replicates, where i varies from 1 to n.

Standard curves were established via linear regression. Standard curve slopes were analyzed using one-way ANOVA and Student's t-test for post-hoc analysis. All statistical analyses were performed utilizing Microsoft Excel 2021.

4.2.2. Optical Density Measurement Method

The optical density (OD) measurements were conducted in TSA at 37 ℃. Turbidity of the samples was measured every 30 minutes for 24 hours at 595 nm using a Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States). Absorbance readings were taken in 96-well plates with 300 µL of volume per well in triplicates.

Similar to redox potential measurement, linear curves of $\log_{10}$N-TTD values were created to establish standard curves for *L. monocytogenes* strains. For $\log_{10}$N values, different dilution members of the strains were enumerated on TSA. TTD values were calculated from the analysis of absorbance readings from turbidity measurements, using the R Package Growthcurver (Sprouffske and Wagner 2016). The package facilitated the summarization of microbial growth

curve characteristics, including determining the doubling time for each dilution member to be used as TTD.

Standard curves were established via linear regression. Standard curve slopes were analyzed using one-way ANOVA and Student's t-test for post-hoc analysis. All statistical analyses were performed utilizing Microsoft Excel 2021.

4.3. **Heat Stress Adaptation of *L. innocua***

4.3.1. Isothermal heat destruction of *L. innocua*, studied by conventional plate counting

Sub-lethal heat treatment experiments were conducted following the protocol outlined by Ágoston et al. (2010). Briefly, 1 mL of microbial suspensions were placed in 1.5 mL Eppendorf tubes (Molecular BioProducts, San Diego, CA) and subjected to sub-lethal heat stress in a water bath (Haake, Germany), with the water level adjusted to cover the samples. The sub-lethal heat treatment conditions were 46 ℃ for 30 and 60 minutes. The samples were immediately subjected to heat destruction treatments after the sub-lethal heat treatments. Control samples were included in the heat destruction experiments without prior sub-lethal heat treatment.

After exposure to sub-lethal heat stress treatment, the samples were promptly transferred to a water bath set at 60 ℃. Isothermal heat destruction temperature was chosen concerning the protocol by Ágoston et al. (2010). Samples were collected at 3-minute intervals for 9 minutes. Subsequently, the samples were placed in an ice bath prior to the enumeration procedure. Each experiment was performed in triplicate on three different days.

Following the heat destruction experiments, samples underwent serial dilution using 9 mL of peptone-NaCl (0.1%-0.85%) diluent. These serially diluted samples were then plated onto TSA plates and incubated for 24 hours at 37 ℃. Colonies were enumerated in triplicates and recorded as Colony Forming Unit (CFU) per mL. Cell counts were analyzed after the logarithmic transformation.

D-values at 60 °C, representing the time needed to inactivate 90% of the target microorganism, were determined using a first-order inactivation model (Bigelow and Esty 1920), as described by Yesil et al. (2024). Survivor curves were constructed by plotting $\log_{10}$N populations against time, and the negative reciprocal of the slope of the equation yielded the D-value.

Furthermore, isothermal heat destruction data were analyzed using one-way ANOVA and Student's t-test for post-hoc analysis, utilizing Microsoft Excel 2021.

4.3.2. Sample preparation and MALDI-TOF MS analysis

For MALDI-TOF MS analysis, both control and samples subjected to sub-lethal heat treatment at 46 °C for 30 minutes were utilized for *L. innocua*. Sample preparation for cluster analysis of MALDI-TOF MS peaks was conducted with modifications based on the method outlined by Schott et al. (2016). All samples were harvested by centrifugation (12 470 g, 5 min), the supernatant was disposed, and the sample was taken from the pellet by sterile toothpick and transferred onto a stainless target. Formic acid (FA, 70%) and acetonitrile (ACN) (50:50, v/v) were applied to the target for protein extraction. Lastly, samples were overlaid with 1 µL of matrix solution (10 mg/mL alpha-cyano-4-hydroxy-cinnamic acid in ACN, dH_2O and trifluoroacetic acid (TFA) 50:47.5:2.5, v/v). Mass spectra within the 2-20 kDa mass range were obtained using MALDI-TOF MS equipment (Bruker Daltonics, Bremen, Germany) with 280 accumulated laser shots. All experiments were performed in triplicate on three separate days.

All exported mass spectra underwent baseline subtraction as a preprocessing step. Subsequently, peak-based cluster analysis was applied to understand stress response dynamics. Dendrogram visualization was conducted using the KNIME Analytics Platform (Version 4.2.1) (Berthold et al. 2008) in collaboration with R (R Core Team 2024). Discriminant analysis of principal components (DAPC) was performed using the adegenet (Jombart 2008) package. DAPC produced a barplot of eigenvalues and a scatterplot representing individuals as dots and groups as inertia ellipses (Haykir et al. 2022).

4.3.3. Dynamic heat destruction of *L. innocua*, quantified by redox potential measurement-based method

Sub-lethal heat treatment experiments were conducted as explained in Section 4.3.1. The sub-lethal heat treatment conditions applied in this study included temperatures of 46, 48, and 50 ℃ for 30, 60, and 90 minutes, respectively. The samples were immediately subjected to dynamic heat destruction treatments after the sub-lethal heat treatments. Control samples were included in the heat destruction experiments without prior sub-lethal heat treatment.

Following exposure to sub-lethal heat stress, dynamic heat destruction experiments were performed in accordance with the procedure established by Török and Reichart (1983). Briefly, a heating spiral set to 80 ℃ was utilized to heat 400 mL of peptone water in a beaker, serving as the heating medium. Upon reaching 50 ℃, 1 mL of microbial suspension was poured into the beaker to initiate dynamic heat destruction experiments. Temperature readings were recorded, and 1 mL samples were collected at 0.5-minute intervals over 9 minutes. Each sample was directly transferred via pipette into tubes containing 9 mL of ½ concentration TSB for subsequent redox potential measurement. For each experiment, 18 samples were collected and enumerated with one replicate. Using the standard curve, the redox potential measurement instrument automatically calculated the cell concentration after the heat destruction experiments.

To evaluate the dynamic heat destruction of *L. innocua*, the D-values were employed to compute the z-value for each treatment. This involved determining the negative reciprocal of the linear regression slope between the $\log_{10}$D values (comprising the 18 values per treatment) and the corresponding temperatures.

The dynamic heat destruction data of *L. innocua* underwent analysis through repeated measures analysis of variance (ANOVA) and paired t-test for subsequent post-hoc analysis aimed at discerning differences among various treatments. All statistical analyses were performed utilizing Microsoft Excel 2021.

4.4. **Heat stress adaptation of *L. monocytogenes***

4.4.1. Isothermal heat destruction of *L. monocytogenes*, evaluated by conventional plate counting

All three *L. monocytogenes* strains from Table 1 were utilized. The sub-lethal heat treatment conditions were 46 ℃ for 30, 60, and 90 minutes. The samples were immediately subjected to isothermal heat destruction treatments after the sub-lethal heat treatments. Control samples were included in the heat destruction experiments without prior sub-lethal heat treatment.

Following exposure to sub-lethal heat stress, the samples were promptly transferred to a water bath set at 60 ℃. Samples were obtained every minute during a 5-minute heat destruction period. Subsequently, the samples were placed in an ice bath prior to the enumeration procedure. The enumeration procedure was done with plate counting, D_{60} values were determined and statistical analyses were done as explained in Section 4.3.1. Each experiment was performed in triplicate on three different days.

4.4.2. Isothermal heat destruction of *L. monocytogenes*, determined by optical density measurement-based method

The same sublethal treatment conditions in Section 4.4.1 were applied to the same strains, but microbial loads were determined using the optical density measurement. Enumeration was done with OD measurement as explained in Section 4.2.2. Microbial loads of the three *L. monocytogenes* strains after heat destruction were determined using the standard curve. D_{60} values were determined and statistical analyses were done as explained in Section 4.3.1. Each experiment was performed in triplicate on three different days.

4.5. **Predictive modelling of thermal inactivation parameters with GInaFiT**

The isothermal heat destruction data of three *L. monocytogenes* strains from Section 4.4.1. underwent fitting to nine survival models using the Microsoft Excel add-in tool GInaFiT (Geeraerd et al. 2005). Alongside parameter estimation, this tool furnished various statistical metrics for each model.

Isothermal heat destruction data and the statistical metrics were analyzed using one-way ANOVA and Student's t-test for post-hoc analysis. All statistical analyses were performed utilizing Microsoft Excel 2021.

4.6. **Cleaning program of the vending machine**

To plan and implement the automated cleaning programs (ACPs), a brand-new vending machine that produces smoothies was used as a pilot plant. Study phases were decided according to the work of Fernández-Segovia et al. (2014). The process involved three sequential stages: establishing microbiological thresholds, developing ACPs, and validating the effectiveness of the cleaning protocols using microbial assessments (Haykir et al. 2023). A certified laboratory conducted sampling and microbiological analysis to carry out independent studies.

5. RESULTS AND DISCUSSION

5.1. Indirect enumeration methods

5.1.1. Redox potential measurement-based method

Figure 2 displays the standard curves of four *Listeria* strains. Each standard curve represents the average from three replicates conducted on different days.

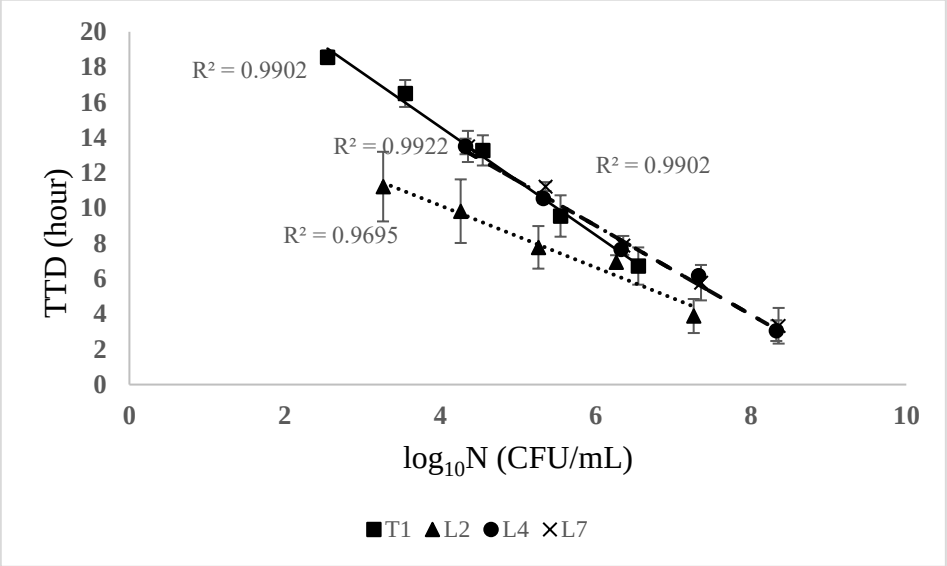


Figure 2. Standard curves of redox potential measurements for four *Listeria* spp. Each standard curve represents the average from three replicates.

Standard curves enable the conversion of redox potential measurement into the microbial concentration due to an inversely proportional relationship between TTD and $\log_{10}$N (Reichart et al. 2007). Therefore, creating a standard curve is of great importance for the quantitative accuracy of the redox potential measurement method. Validation characteristics such as linearity, detection limit, and method repeatability are assessed by analyzing the standard curves. The regression equations for the standard curves are presented in Table 2 and Table 3.

Table 2. Sensitivity of the standard curves for the redox potential method

Strain	Regression equation TTD = f(log_{10}N)	Sensitivity* (h/log_{10} unit)
T1	TTD (h) = -3.06 x log_{10}N + 26.85	3.06 (0.21)**[a]
L2	TTD (h) = -1.75 x log_{10}N + 17.18	1.75 (0.36)[b]
L4	TTD (h) = -2.53 x log_{10}N + 24.18	2.53 (0.22)[c]
L7	TTD (h) = -2.58 x log_{10}N + 24.73	2.58 (0.11)[c]

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

The sensitivity of the method was evaluated by examining the slopes of the TTD-log_{10}N curves, and the findings are outlined in Table 2. A one-unit increase in the logarithm of the initial cell concentration led to TTD reductions ranging from 1.75 to 3.06 hours across various strains. Statistical analysis revealed that the slopes of L4 and L7 were similar ($P > 0.05$), whereas T1 and L2 exhibited significant differences ($P < 0.05$).

The detection limit of the method can be ascertained from the intercept of the standard curves. TTD values corresponding to $log_{10}N=0$, indicating a single cell, varied from 17.18 to 26.85 hours. In the study conducted by Erdősi et al. (2014), three *L. monocytogenes* curves resulted in a single standard curve, distinct from *L. innocua*. A two-sided Student's t-test at the 0.95 confidence level showed a significant difference in the slopes between my study and their study, highlighting the importance of creating strain-specific standard curves.

In traditional plate counting, the enumeration process generally takes 48-72 hours (Reichart et al., 2007). In my study, the redox potential measurement method's detection limit for a single cell fell from 17.18 to 26.85 hours. Therefore, the method provided a faster quantification of *Listeria* spp. Additionally, it is less labor-intensive than conventional methods as it eliminates preparing the dilution series when a standard curve is present.

The repeatability of a method is quantified through the standard deviation of $\log_{10}N$ determinations, computed as the square root of the residual mean square from the variance analysis of regression. The standard deviations for the determinations are presented in Table 3, ranging from 0.13 to 0.32 $\log_{10}$ units, which were within the range of the repeatability of the standard conventional method of enumerating *L. monocytogenes* in food (Auvolat and Besse, 2016).

Table 3. Results of the regression analysis of the standard curves of redox potential measurement

Strain	Regression Equation $\log_{10}N = f(TTD)$	R^2	$SE_{\lg N}$
T1	$\log_{10}N = -0.32 \times TTD + 8.74$	0.992	0.161
L2	$\log_{10}N = -0.55 \times TTD + 9.64$	0.969	0.318
L4	$\log_{10}N = -0.39 \times TTD + 9.53$	0.990	0.180
L7	$\log_{10}N = -0.39 \times TTD + 9.58$	0.995	0.126

R^2: coefficient of determination, $SE_{\lg N}$: repeatability

5.1.2. Optical density measurement-based method

Figure 3, Table 4, and Table 5 present the standard curves and regression equations derived from optical density measurements. TTD values were determined using the Growthcurver package across various dilution levels, while $\log_{10}N$ values were obtained through plate counting on TSA plates. The optical density measurement method was not utilized in the enumeration of T1. Thus, standard curve analysis was not done for T1 in this section.

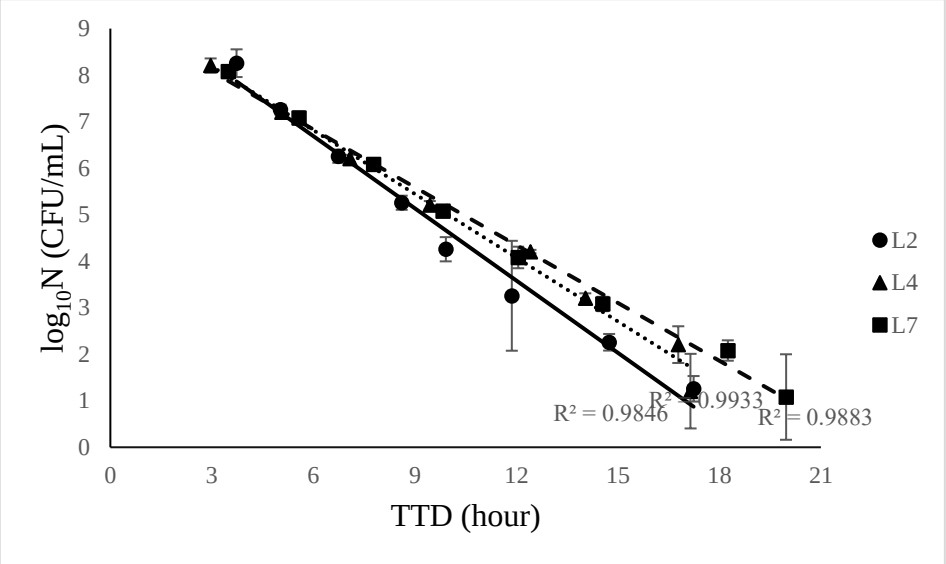


Figure 3. Standard curves of three *L. monocytogenes* from optical density measurement. Each standard curve represents the average from three replicates.

The standard curve slopes for L2, L4, and L7 were calculated as 1.90, 2.16, and 2.40, respectively, with statistical comparison revealing similarities between L4 and L7 slopes ($P > 0.05$), while significant differences were observed for L2 ($P < 0.05$). Sensitivity assessment of the method involved analyzing TTD-$\log_{10}$N curve slopes, detailed in Table 4, demonstrating TTD reductions of 1.90, 2.16, and 2.40 hours per unit increase in initial cell concentration for L2, L4, and L7, respectively. Detection limits were determined from intercepts of standard curves, yielding 18.78, 20.80, and 22.41 hours for TTD values corresponding to $\log_{10}N=0$, indicating a single cell. Notably, L2 exhibited the highest growth rate, while L7 displayed the lowest growth rate.

Table 4. Sensitivity of the standard curves for the optical density method

Strain	Regression equation TTD = f(log_{10}N)	Sensitivity* (h/log_{10} unit)
L2	TTD (h) = -1.90 x log_{10}N + 18.78	1.90 (0.07)**[a]
L4	TTD (h) = -2.16 x log_{10}N + 20.80	2.16 (0.09)[b]
L7	TTD (h) = -2.40 x log_{10}N + 22.41	2.40 (0.08)[b]

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

The repeatability of the method, evaluated similarly to the redox potential measurement method, showed standard errors of regression analysis for L2, L4, and L7 at 0.33, 0.29, and 0.22, respectively.

Table 5. Results of the regression analysis of the standard curves of optical density measurement

Strain	Regression Equation logN = f (TTD)	Slope*	R^2	$SE_{lg\ N}$
L2	log_{10}N = -0.52 x TTD + 9.80	0.52 (0.02)**[a]	0.985	0.328
L4	log_{10}N = -0.45 x TTD + 9.56	0.45 (0.02)[b]	0.988	0.286
L7	log_{10}N = -0.42 x TTD + 9.32	0.42 (0.02)[b]	0.993	0.217

R^2: coefficient of determination, SE_{lgN}: standard error of the regression

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

Different direct and indirect methods for assessing microbial culture outcomes are thoroughly developed. Conventional plate count methods retain their foundational role in studies concerning growth, inhibition, and activation. For indirect methods of microbial analysis, optical density (OD) measurements have been commonly used in the last decades. Several studies have utilized OD

measurements to analyze the growth of *L. innocua* in foods (Nyhan et al. 2020), to estimate the growth parameters of *L. monocytogenes* following sublethal heat treatment (Xuan et al. 2017), and to model the growth of *L. monocytogenes* in fish (Bolívar et al. 2018). Generally, growth parameters like growth rate and lag time are used to assess the effectiveness of microbial inactivation treatments. In this work, I used TTD measurements based on absorbance values to quantify microbial inactivation of thermal destruction. Absorbance measurements offer a rapid, non-invasive, cost-effective, and easily automatable means of tracking bacterial growth, compared to traditional viable count methods. After analyzing the standard curve of TTD-$\log_{10}$N graphs, the standard errors of the regression analysis were within the range of the repeatability of the standard conventional method of enumerating *L. monocytogenes* in food, similar to the redox potential measurement method. Detection limits ranged from 18.78 to 22.41 hours in optical density measurements, which provides a faster solution to the quantification of *L. monocytogenes* compared to the plate counting method.

According to Dalgaard and Koutsoumanis (2001), absorbance techniques are best suited for situations where high cell densities are attained, such as those typical of spoilage bacteria growth in food environments. Despite the acknowledged limitations of absorbance in constructing growth curves, it can still serve a valuable purpose. While it may not yield extremely precise growth kinetic parameters, it can be employed to compare the growth of various cultures or the same cultures under different conditions (Pla et al. 2015), as I analyzed microbial inactivation. TTD data demonstrate a linear relationship with the logarithm of the initial inoculum (Mytilinaios et al. 2015). Besides the problem of linearity, if the initial cell count is low, the main contributor to the variability in detection times closely parallels the variability observed in the individual lag times (Métris et al. 2003). The key determinants influencing the output of the TTD method include the calibration curve, the mathematical methodology employed to derive TTD values from the OD data, the detection limit, and the range of inoculum associated with TTD values and microbial

counts (Baka et al. 2015). There are studies concentrating on advancing the TTD method based on OD measurements (Baranyi and Pin 1999, Mytilinaios et al. 2012, Jánosity et al. 2022).

5.2. **Heat Stress Adaptation of *L. innocua***

5.2.1. Isothermal heat destruction of *L. innocua*, studied by conventional plate counting

Figure 4 and Figure 5 represent the inactivation kinetics of T1 with sublethal heat at 46 ℃ for 30 and 60 minutes and control samples.

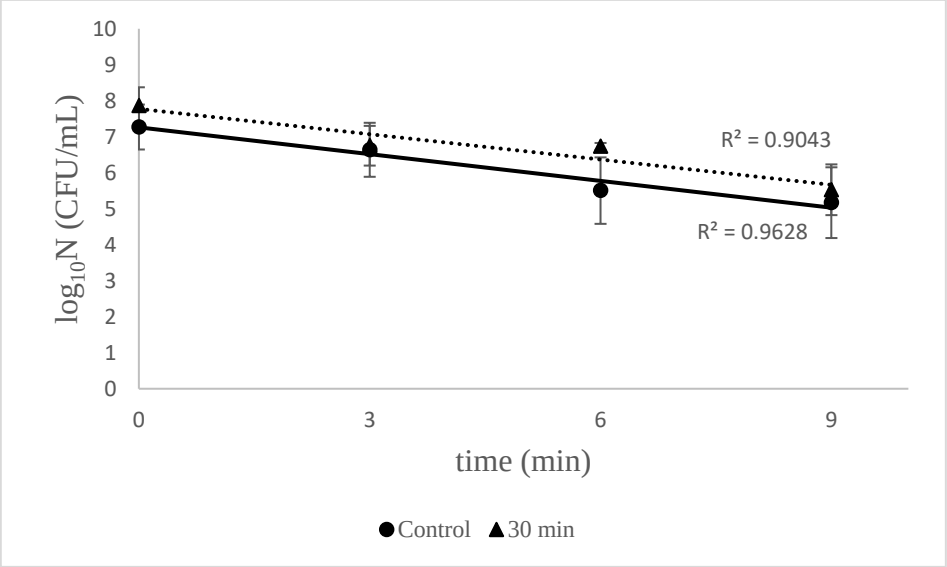


Figure 4. Inactivation of T1 at 60 °C of control and sublethal exposed samples at 46 °C for 30 minutes. After sub-lethal heat treatment at 46 °C for 30 minutes, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

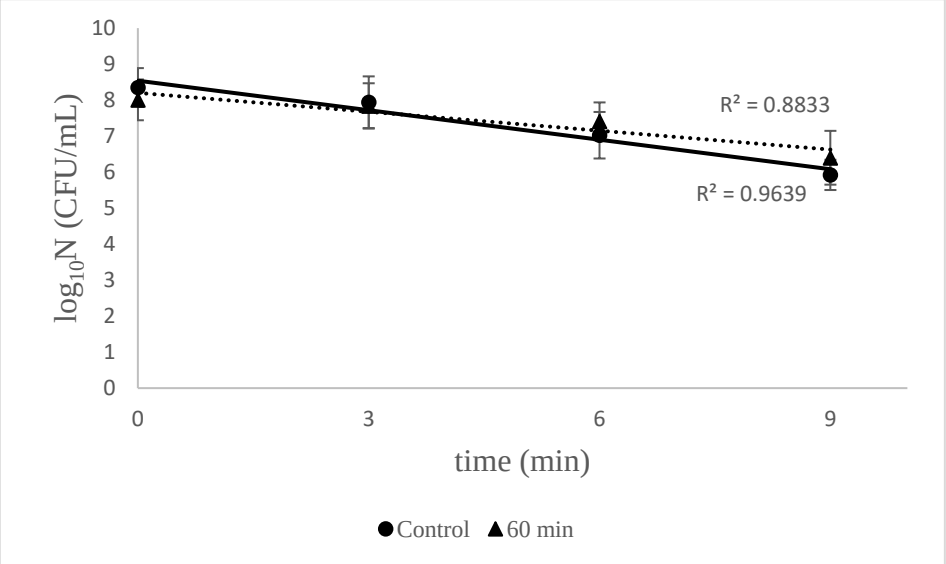


Figure 5. Inactivation of T1 at 60 °C of control and sublethal exposed samples at 46 °C for 60 minutes. After sub-lethal heat treatment at 46 °C for 60 minutes, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

Table 6. displays the D values for both control samples and those exposed to sublethal heat (Haykir et al. 2022). For samples treated at 46 °C for 30 minutes, the D values were 4.04 minutes for the control and 4.26 minutes for the pre-treated samples. Meanwhile, for samples treated at 46 °C for 60 minutes, the D values were 3.66 minutes for the control and 5.71 minutes for the treated samples. The sublethal heat treatment of 30 minutes at 46 °C did not significantly alter the D values at 60 °C when compared to the control ($P > 0.05$). However, a 60-minute sublethal treatment at 46 °C significantly improved the survival at 60 °C ($P < 0.05$).

Table 6. Effect of pre-exposure to sub-lethal temperatures of 46 °C for 30 and 60 minutes on the D_{60} values for T1. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

Exposure time to sub-lethal temperature (min)	D-value (min)*	
	Control	Pre-treated
30	4.03 (1.06)**[a]	4.26 (0.36)[a]
60	3.66 (0.47)[a]	5.71 (0.85)[b]

*Value within the bracket is the standard deviation.

** For each row, different superscripts denote statistically significant differences ($P < 0.05$).

In order to assess differences at the proteome level, analysis of mass spectra obtained from MALDI-TOF MS was examined on 34 samples, including control samples and sublethal heat-exposed samples at 46 °C for 30 minutes. Among these, 18 samples belonged to sublethal heat-exposed samples, while the remaining 16 were the control samples. Because of procedural error, two control samples were excluded due to the absence of mass spectra. These 34 samples were analyzed by discriminant analysis of principal components (DAPC), resulting in three distinct clusters (Figure 6). Cluster 1 included 12 samples, Cluster 2 contained four, and Cluster 3 comprised 18. Among the control samples, seven were in Cluster 1, two in Cluster 2, and seven in Cluster 3. For the sublethal heat-exposed samples, five were in Cluster 1, two in Cluster 2, and eleven in Cluster 3. Eigenvalues in Figure 6. highlight how the discriminant functions contribute to the group separation. Here, the first axis dominates, which aligns with the clear separation between Cluster 1 and the other two clusters. The analysis displayed an expressive separation of samples into different groups. However, the distribution of the samples into different groups did not show a meaningful pattern.

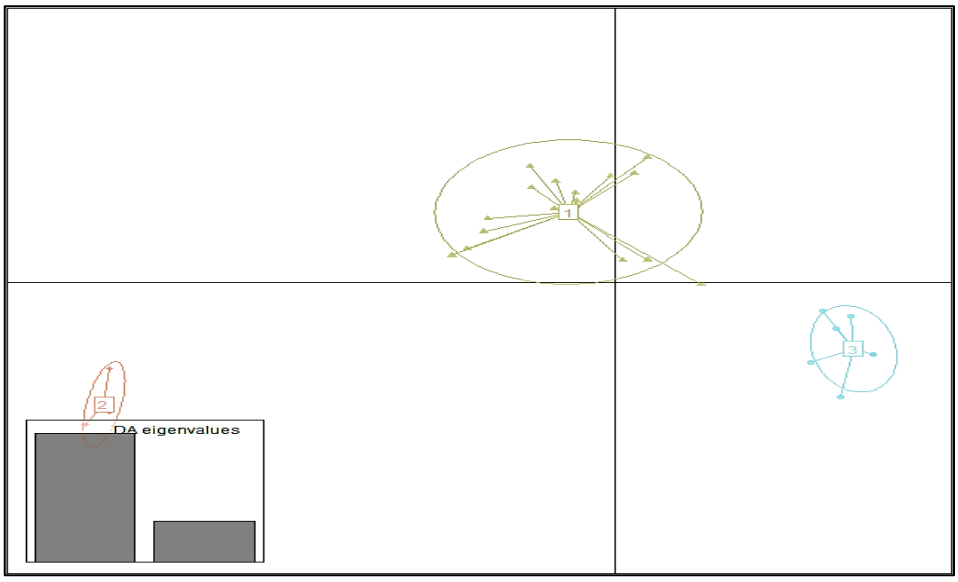


Figure 6. Clusters of the MALDI-TOF MS peaks, obtained from the DAPC analysis of the peaks from control and pre-heat exposed samples at 46 °C for 30 minutes.

van der Veen et al. (2007) demonstrated differential gene expression of *L. monocytogenes* after sublethal heat exposure at 48 °C. Similarly, Ágoston et al. (2009) showed differentially expressed proteins in *L. monocytogenes* after sublethal heat exposure at 48 °C for 30 minutes. To investigate molecular changes in *L. innocua* after sublethal heat treatment, mass spectra of control and prior heat-treated samples obtained from MALDI-TOF MS were analyzed. The hypothesis was that alterations in the proteome might cause differentiation of control and sublethal-treated samples into distinct clusters or display differentiation based on the heat destruction time needed for proteomic changes.

In my study, the outcome of different clusters did not show a comprehensive result, indicating these three clusters did not show a meaningful pattern. This verifies our heat destruction findings, showing no significant difference ($P > 0.05$) in D-values between control and pre-treated samples at 46 °C for 30 minutes. According to my knowledge, this study was the first peak-based cluster analysis applied to heat-stressed samples of *L. innocua* or *L. monocytogenes* (Haykir et al. 2022).

5.2.2. Dynamic heat destruction of *L. innocua*, quantified by redox potential measurement-based method

The redox potential measurement-based method was employed to evaluate the heat resistance of *L. innocua* with and without sublethal heat treatments. This study represents the first application of this method to investigate heat destruction of *Listeria* spp. Instrument-detected $log_{10}N$ values were determined for each sampling point, and thermal inactivation curves were subsequently generated. The thermal inactivation curves are presented in Figures 7, 8, and 9.

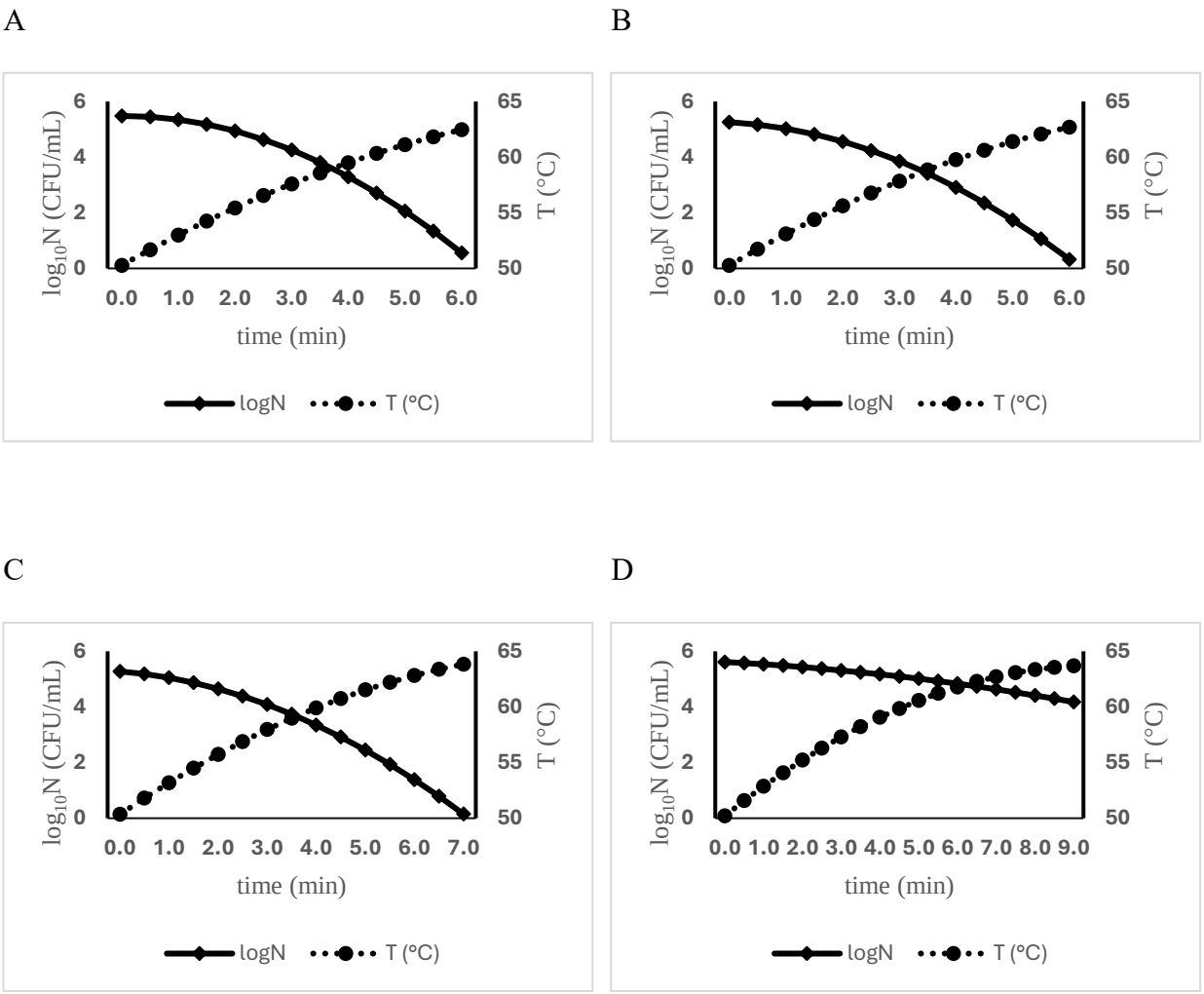


Figure 7. Dynamic heat destruction of T1 with and without sublethal heat treatment at 46 °C for control (A), 30 min (B), 60 min (C), and 90 min (D). After sub-lethal heat treatment at 46 °C for different durations, including the control, samples were subjected to dynamic heat treatment with a heating spiral at 80 °C with one replicate. Microbial loads were enumerated using the redox potential measurement method.

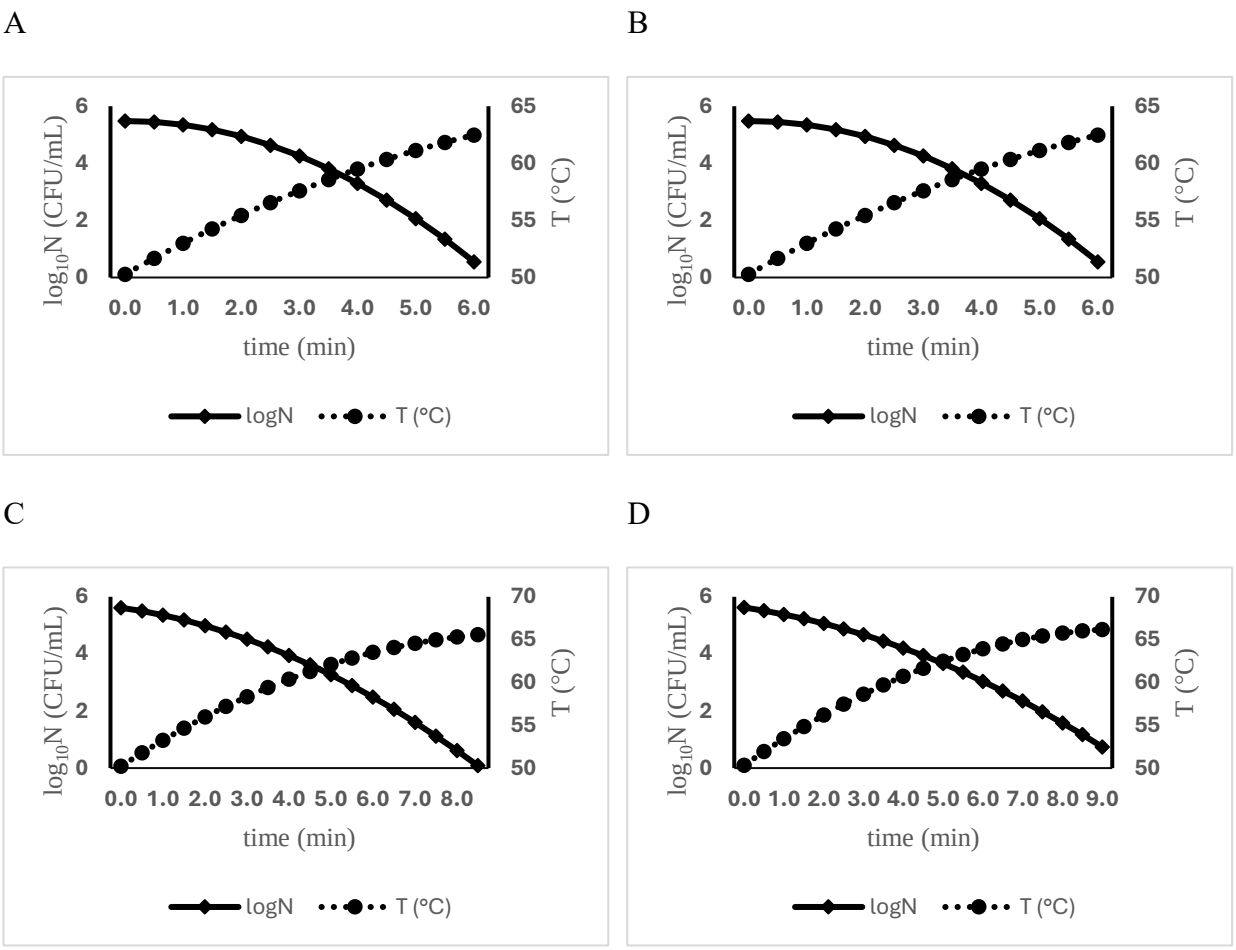
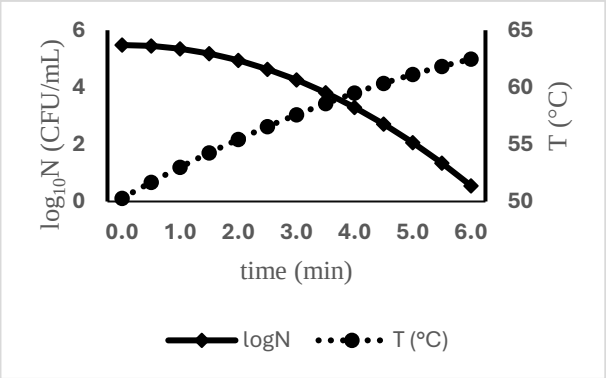
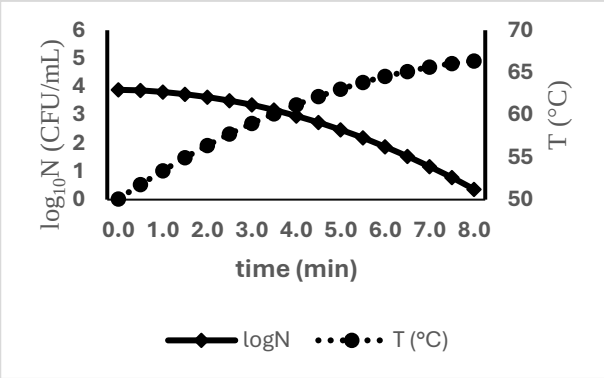


Figure 8. Dynamic heat destruction of T1 with and without sublethal heat treatment at 48 °C for control (A), 30 min (B), 60 min (C), and 90 min (D). After sub-lethal heat treatment at 48 °C for different durations, including the control, samples were subjected to dynamic heat treatment with a heating spiral at 80 °C with one replicate. Microbial loads were enumerated using the redox potential measurement method.

A



B



C

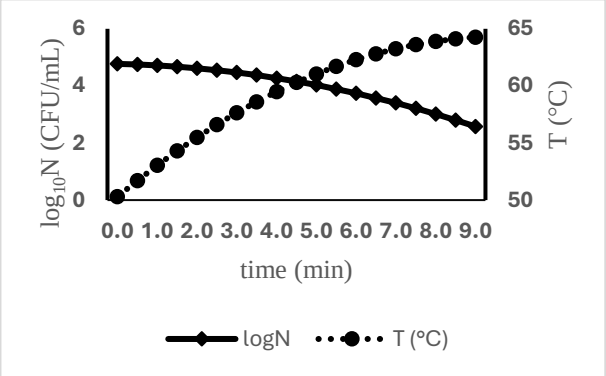
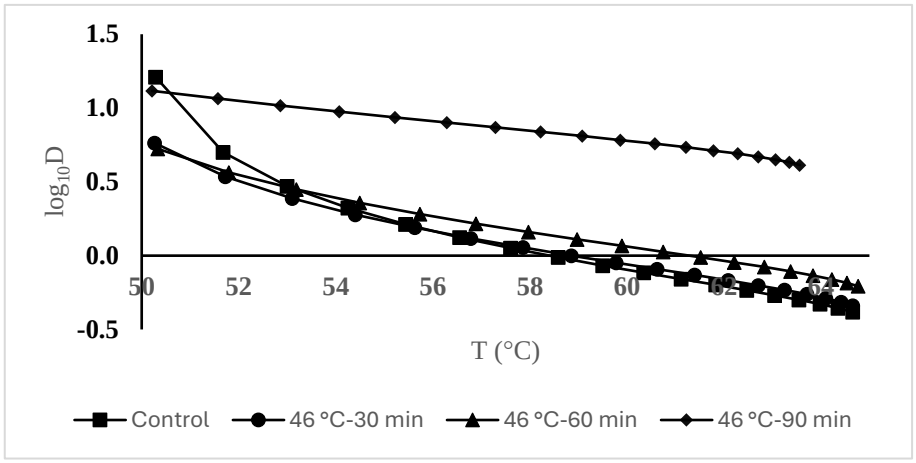


Figure 9. Dynamic heat destruction of T1 with and without sublethal heat treatment at 50 °C for control (A), 30 min (B), and 60 min (C). After sub-lethal heat treatment at 50 °C for different durations, including the control, samples were subjected to dynamic heat treatment with a heating spiral at 80 °C with one replicate. Microbial loads were enumerated using the redox potential measurement method.

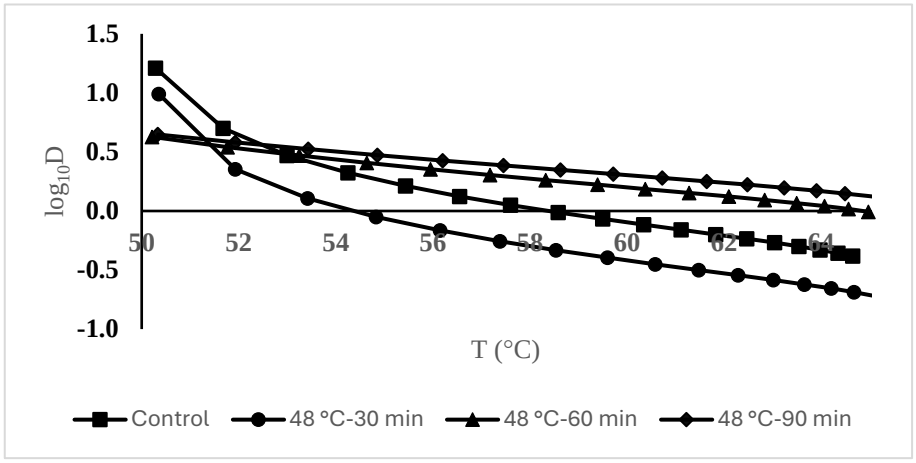
Figure 10 and Table 7 demonstrate the thermal death curves and equations for control and previously sub-lethally heat-treated samples. Three distinct sub-lethal heat stress conditions were implemented, involving temperatures of 46, 48, and 50 °C for 30, 60, and 90 minutes. Samples from pre-heated at 50 °C for 90 minutes were excluded due to defective TTD values. The effect of sub-lethal heat treatment was evaluated by comparing $\log_{10}D$ values using repeated measures ANOVA and paired t-tests for post-hoc analysis. The findings reveal no significant difference between the control and sub-lethal heat treatment at 46 °C for 30 and 60 minutes ($P > 0.05$), while all other treatments exhibited statistical significance from the control ($P < 0.05$). Skandamis et al.

(2008) noted an extended time for the first decimal reduction at 46 °C for 30 and 90 minutes compared to control samples. In my isothermal study using the same strain (Section 5.2.1.), D_{60} values exhibited no significant difference after 30 minutes of sublethal heat treatment at 46 °C but differed after 60 minutes compared to control samples. Factors such as strain variability, test conditions, and statistical models contribute to the variability in thermal resistance (Aryani et al. 2015, Soni et al. 2022).

A



B



C

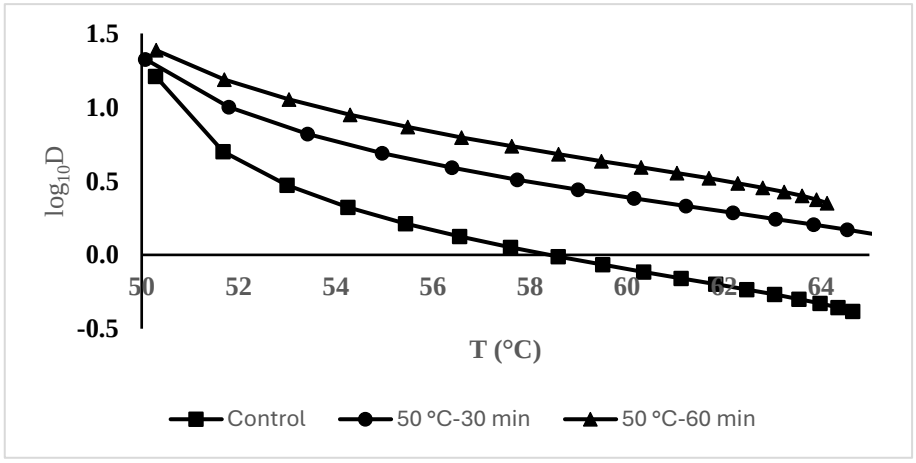


Figure 10. Thermal death curves of T1of control and sub-lethal heat treatment at 46 °C (A), 48 °C (B), and 50 °C (C). After sub-lethal heat treatment at different conditions, including the control, samples were subjected to dynamic heat treatment with a heating spiral at 80 °C with one replicate. Microbial loads were enumerated using the redox potential measurement method.

Clemente-Carazo et al. (2020), stated that dynamic heat treatments offer valuable insights into microbial adaptive responses to diverse thermal conditions after investigation of heat resistance of *L. monocytogenes* under both isothermal and dynamic conditions. Under isothermal conditions, strain differences did not yield significant variations. On the other hand, under dynamic conditions, strain differences became crucial, highlighting the distinct capacities of the strains in adapting to stress.

The thermal history of a cell population is a crucial factor in determining its sensitivity to heat. Stephens et al. (1994) found that slower heating rates from 5.0 to 0.7 °C/min led to proportional increases in thermotolerance. In contrast, Miller et al. (2011) observed that as the heating rate increases, the shoulder decreases, and the tail effect disappears in the heat destruction of *L. innocua*. These findings suggest that the thermal resistance of pathogens during subsequent processes is influenced by their previous handling or treatment, highlighting the path-dependent nature of thermal inactivation.

Z-values were computed for nine treatments, spanning a range of 11.5 to 28.4 °C (Table 7). The control samples displayed the lowest z-value, while the sub-lethal heat treatment at 48 °C for 90 minutes exposed the highest. Across all temperatures, extended durations of sub-lethal heat treatment consistently resulted in elevated z-values. However, no conclusive trend appeared when comparing treatments at different temperatures.

O'Bryan et al. (2006) provided a summary of the z-values for *L. monocytogenes* and *L. innocua* M1 in meat and poultry samples. The z-values for *L. monocytogenes* varied from 3.50 to 7.39, whereas for *L. innocua*, it ranged between 4.86 and 8.67. Wang et al. (2024) reported that z-values varied from 10.58 to 13.80 °C during the dynamic heat treatment of *L. monocytogenes* in milk. The z-value of control samples in my study was 11.5 °C, and sub-lethal heat treatments displayed z-values up to 2.5-fold higher than the control. Strain variability and test conditions may be the

reasons for variations between different studies. Aryani et al. (2015) underlined the significance of employing a broad temperature range for estimating the z-values, a practice also implemented in my study (50 to 65 °C).

Table 7. Thermal Inactivation Parameters for T1. After sub-lethal heat treatment at different conditions, including the control, samples were subjected to dynamic heat treatment with a heating spiral at 80 °C with one replicate. Microbial loads were enumerated using the redox potential measurement method.

Treatment	Thermal death curve equation*	R^2	z-value (°C)**
Control	$\log_{10}D = -0.09 \times T_i + 5.18^a$	0.90	11.5 (9.8-13.8)
46 °C, 30 mins	$\log_{10}D = -0.07 \times T_i + 3.97^a$	0.97	15.0 (13.8-16.4)
46 °C, 60 mins	$\log_{10}D = -0.06 \times T_i + 3.56^a$	0.99	17.2 (16.2-18-3)
46 °C, 90 mins	$\log_{10}D = -0.04 \times T_i + 2.92^b$	0.99	27.9 (27.0-28.9)
48 °C, 30 mins	$\log_{10}D = -0.08 \times T_i + 4.45^c$	0.97	12.6 (11.6-13.9)
48 °C, 60 mins	$\log_{10}D = -0.04 \times T_i + 2.68^d$	0.99	24.2 (23.5-24.9)
48 °C, 90 mins	$\log_{10}D = -0.04 \times T_i + 2.41^e$	0.99	28.4 (27.6-29.2)
50 °C, 30 mins	$\log_{10}D = -0.07 \times T_i + 4.39^f$	0.96	15.2 (13.7-17.1)
50 °C, 60 mins	$\log_{10}D = -0.07 \times T_i + 4.59^g$	0.98	15.1 (14.0-16.3)

D: decimal reduction time; T_i: temperature; R^2: coefficient of determination

*Different letters are significantly different $\log_{10}D$ values within each column based on paired t-test ($P < 0.05$).

**Value within bracket is the 95% confidence interval.

A comprehensive understanding of bacterial thermal resistance is essential for ensuring the safety of thermally processed foods. D and z-values signify the treatment time necessary at a specific temperature to reduce bacterial populations to acceptable levels (Soni et al. 2022). My results, consistent with existing literature, underscore the significance of enhanced heat resistance in modelling heat destruction parameters, with increased D and z values following prior treatment at milder temperatures. Stress acclimation can occur during dynamic heat treatments, even without sub-lethal heat exposure (Clemente-Carazo et al. 2020). Recent advancements include the development of new mathematical models that take stress acclimation into account (Garre et al.

2019). Further research is necessary to address the limited database on *L. monocytogenes* heat resistance in diverse matrices, including buffers and food, particularly under dynamic heat conditions.

5.3. Heat stress adaptation of *L. monocytogenes*

5.3.1. Isothermal heat destruction of *L. monocytogenes*, evaluated by conventional plate counting

In order to evaluate the heat stress adaptation of *L. monocytogenes*, three strains (L2, L4, and L7) were exposed to isothermal heat inactivation at 60 °C, with and without sublethal heat treatment. Sub-lethal heat treatment conditions were at 46 °C for 30, 60, and 90 minutes.

Thermal inactivation of control and sublethal heat-treated samples of L2 was demonstrated in Figure 11. D-values for each treatment were presented in Table 8.

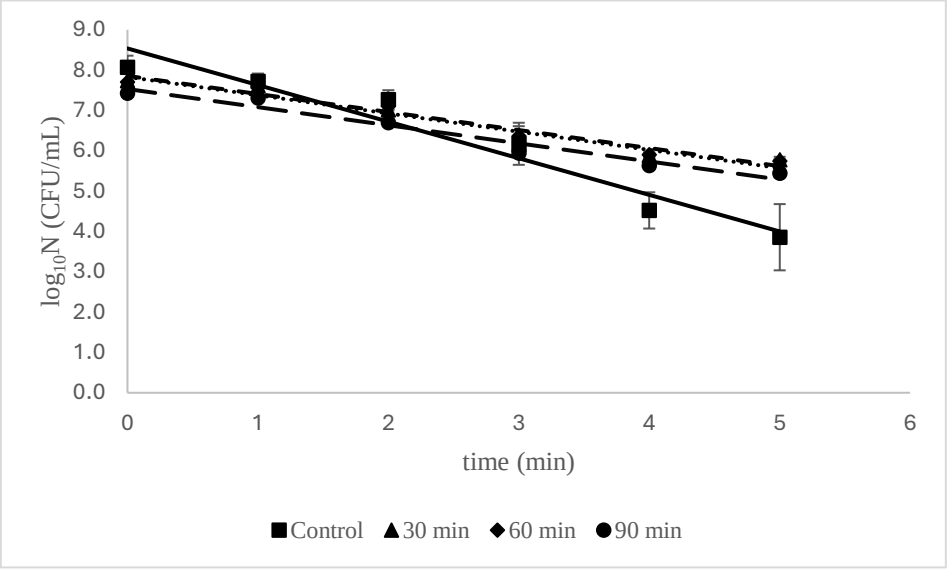


Figure 11. Isothermal heat inactivation of control and sublethal heat-treated L2 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

Control and sub-lethally treated L2 samples exhibited D-values of 1.12, 2.21, 2.24, and 2.23 minutes for 30, 60, and 90 minutes, respectively. The D-value of control samples was significantly

different from sublethal heat-treated samples ($P < 0.05$). Sublethal heat exposure caused up to a 2-fold increase in D-values of L2, compared to control (Table 8). Difference in D-value of sublethal heat-exposed L2 samples were not significant when the duration of the treatment was increased from 30 to 90 minutes ($P > 0.05$).

Table 8. D_{60}-values of control and sublethal heat-treated L2 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

Treatment	D_{60}-value (min)*	R^2
Control	1.12 (0.17)**a	0.95
30 min	2.21 (0.03)b	0.94
60 min	2.24 (0.11)b	0.96
90 min	2.23 (0.11)b	0.96

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each treatment based on the Student's t-test ($P < 0.05$).

Thermal inactivation and D-values of control and sublethal heat-treated L4 samples are shown in Figure 12 and Table 9.

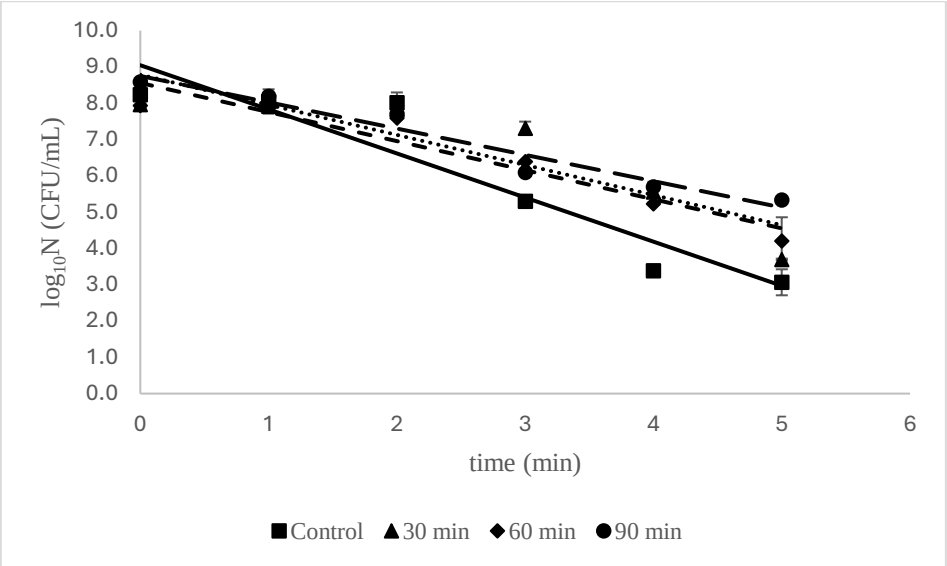


Figure 12. Isothermal heat inactivation of control and sublethal heat-treated L4 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

For both control and sub-lethally heat-treated L4 samples, the D-values were observed as 0.89, 1.21, 1.26, and 1.38 minutes for 30, 60, and 90 minutes, respectively. All sublethal treatments caused a significant increase in D-values compared to control samples ($P < 0.05$). Sublethal heat exposure caused up to a 1.55-fold increase in D-values of L4 compared to the control (Table 9). Between sublethal heat-treated samples, there was a trend of incline in D-values when the exposure time was increased. D-values for 30 and 60 minutes were not significantly different, just like 60 and 90 min. However, the increase in duration from 30 min to 90 min caused a significant increase in D-value (Table 9).

Table 9. D_{60}-values of control and sublethal heat-treated L4 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

Treatment	D_{60}-value (min)*	R^2
Control	0.89 (0.14)**[a]	0.89
30 min	1.21 (0.05)[b]	0.79
60 min	1.26 (0.16)[bc]	0.92
90 min	1.38 (0.03)[c]	0.95

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each treatment based on the Student's t-test ($P < 0.05$).

Thermal inactivation data and D-values for L7 were demonstrated in Figure 13 and Table 10.

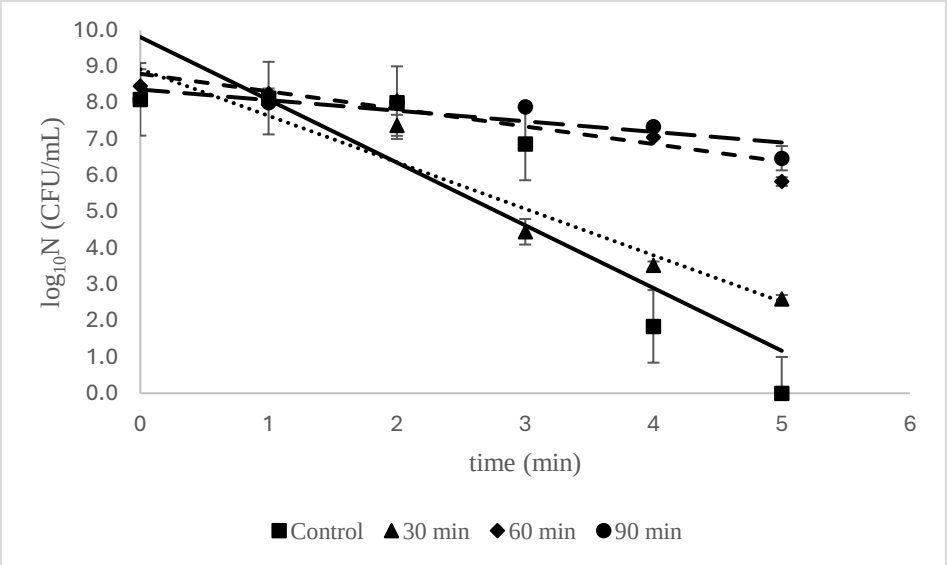


Figure 13. Isothermal heat inactivation of control and sublethal heat-treated L7 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

D values for control and sublethal treated samples for 30, 60, and 90 minutes were 0.58, 0.78, 2.10, and 3.50 minutes, respectively. D-values significantly differed in control and sublethal heat-

exposed samples of L7 ($P < 0.05$). D-values of L7 were increased up to 6-fold after sublethal heat-exposed samples compared to control samples.

Table 10. D_{60}-values of control and sublethal heat-treated L7 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using plate counting. All experiments were performed in triplicates on three separate days.

Treatment	D-value (min)*	R^2
Control	0.58 (0.00)**[a]	0.80
30 min	0.78 (0.01)[b]	0.93
60 min	2.10 (0.30)[c]	0.84
90 min	3.50 (0.59)[d]	0.75

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each treatment based on the Student's t-test ($P < 0.05$).

In summary, D-values increased in all strains when the sublethal heat treatments were applied, compared to the control. D-values between sublethal heat experiments also increased when the prior heat exposure treatment at 46 °C was prolonged, except for the 60-minute and 90-minute treatments in L2. The D-value at 60 °C for sublethal heat-treated samples for 60 minutes was 2.24 minutes, while the D-value for 90 minutes prior heat-treated samples was 2.23 minutes. However, there was no significant difference in the D-values of prior-heat-treated samples of L2 (Table 8).

When the D-values of all three strains were compared between each treatment, the results showed significant differences in all treatments except two cases. D-values for control samples of L2 and L4 and prior heat-treated samples at 46 °C for 60 min of L2 and L7 were not significantly different ($P > 0.05$). Except for these two cases, the D-values of all strains were statistically different from each other in all treatments ($P < 0.05$). The most significant increase in D-values occurred in L7 samples, which was more than a 6-fold increase obtained in prior treatment samples for 90 minutes compared to control samples. The lowest increase comprised in L4 samples, which caused a 1.55-fold increase in D-values in samples treated with 90 minutes of heat treatment compared to control

samples. The highest increase in D-values took place in L2 samples when the prior treatment for 60 minutes created a 2-fold increase compared to control samples.

5.3.2. Isothermal heat destruction of *L. monocytogenes*, determined by optical density measurement-based method

Isothermal heat treatments were conducted at 60 ℃ for control and sub-lethally treated strains previously exposed to 46 ℃ for 30, 60, and 90 minutes each. Samples were collected initially and every minute for 5 minutes. Microbial loads were determined using a standard curve, and D-values were calculated through linear regression analysis.

Figure 14 and Table 11 illustrate the thermal destruction curves and D-values of control and sub-lethally heat-treated samples of L2.

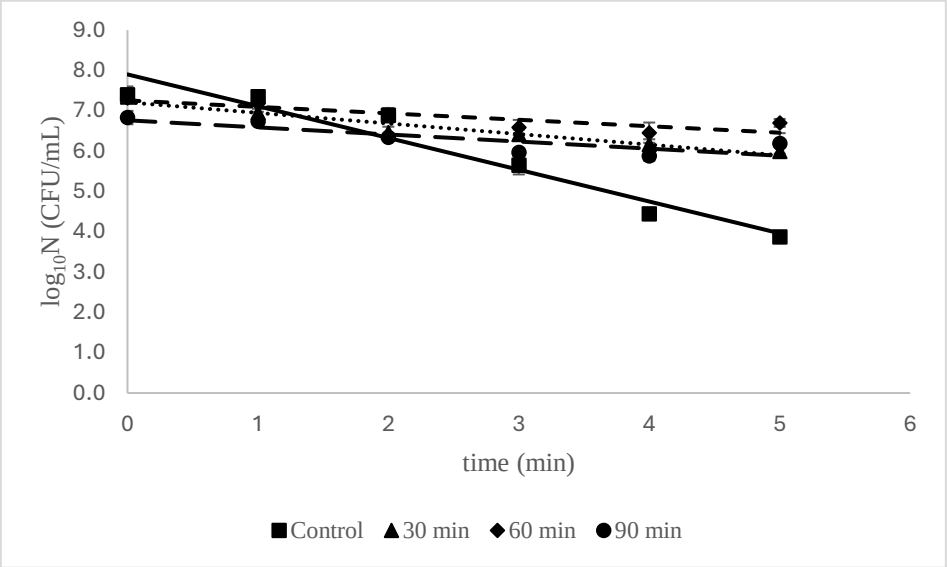


Figure 24. Isothermal heat inactivation of control and sublethal heat-treated L2 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using optical density measurement. All experiments were performed in triplicates on three separate days.

The D-values for control and sub-lethally treated samples of L2, spanning 30, 60, and 90 minutes, were 1.27, 3.84, 6.35, and 6.15 minutes, respectively. Control samples exhibited the lowest D-value, whereas the highest was observed in the 60-minute samples. Significant differences between

the D-values of control and sub-lethally treated samples were noted ($P < 0.05$). Sublethal heat exposure caused up to a 5-fold increase in D-values of L2, compared to control (Table 11). Among sub-lethally treated samples, the 30-minute and 60-minute samples showed significant differences ($P < 0.05$), while the 30-minute-90-minute and 60-minute-90-minute samples did not ($P > 0.05$). Sub-lethal treatment increased the D-value until the 60-minute treatment, after which a decrease was observed with the 90-minute treatment compared to the 60-minute treatment.

Table 11. D_{60}-values of control and sublethal heat-treated L2 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using optical density measurement. All experiments were performed in triplicates on three separate days.

Treatment	D_{60}-value (min)*	R^2
Control	1.27 (0.08)**[a]	0.94
30 min	3.84 (0.42)[b]	0.94
60 min	6.35 (0.93)[c]	0.77
90 min	6.15 (2.13)[bc]	0.70

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

Figure 15 and Table 12 display the thermal destruction curves and the D-values of control and sub-lethally treated L4 samples.

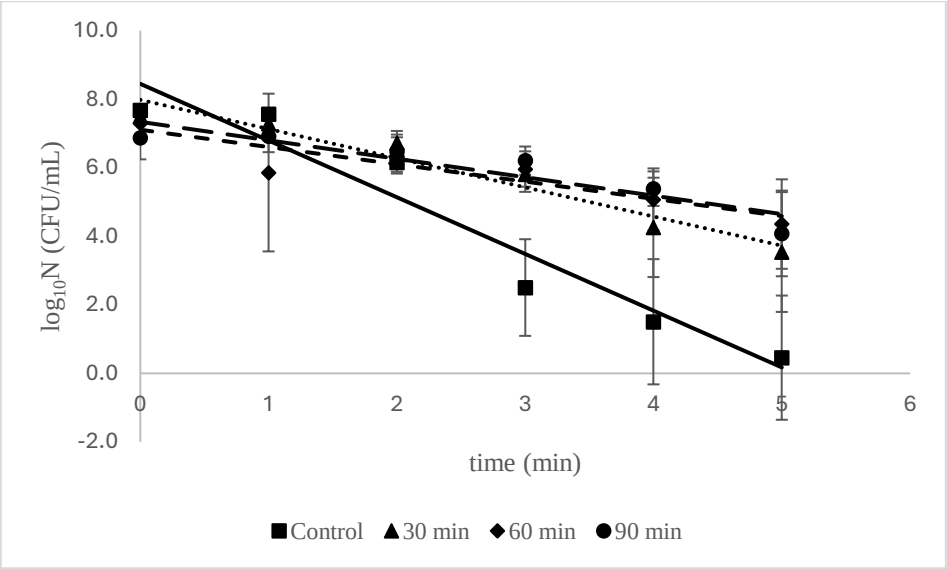


Figure 15. Isothermal heat inactivation of control and sublethal heat-treated L4 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using optical density measurement. All experiments were performed in triplicates on three separate days.

Control and sub-lethally treated L4 samples exhibited D-values of 0.63, 1.31, 2.04, and 1.90 minutes for 30, 60, and 90 minutes, respectively. The control samples demonstrated the lowest D-values, whereas the highest were observed in the 60-minute treatment. Sublethal heat exposure caused up to a 3.2-fold increase in D-values of L4, compared to the control (Table 12). No statistically significant difference was observed between the control and 30-minute samples. Among the sub-lethal treatments, there was no statistically significant difference in D-values ($P > 0.05$). Sub-lethal treatment led to an increase in D-values until the 60-minute treatment, followed by a decrease in the 90-minute treatment compared to the 60-minute treatment. A similar observation was made for L2. Extended exposure to mild stress may resulted in cellular damage and affect the capacity for adaptation to heat stress. However, it should be noted that there were no significant differences in D-values between the 60- and 90-minute treatments in both strains.

Table 12. D_{60}-values of control and sublethal heat-treated L4 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using optical density measurement. All experiments were performed in triplicates on three separate days.

Treatment	D_{60}-value (min)*	R^2
Control	0.63 (0.15)**[a]	0.93
30 min	1.31 (0.56)[ab]	0.94
60 min	2.04 (0.42)[b]	0.82
90 min	1.90 (0.33)[b]	0.85

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

Figure 16 and Table 13 present thermal destruction curves and D-values for both control and sub-lethally treated L7 samples across 30, 60, and 90-minute durations.

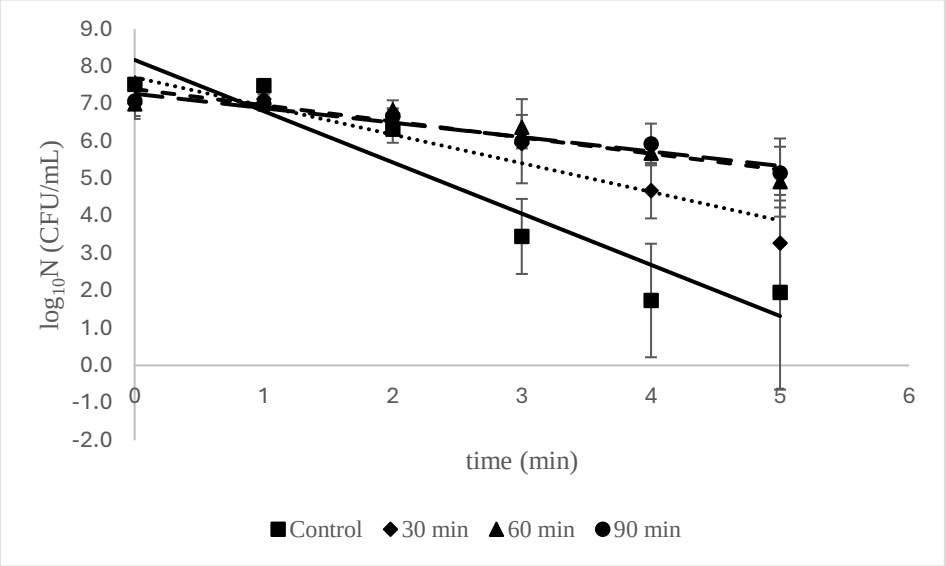


Figure 16. Isothermal heat inactivation of control and sublethal heat-treated L7 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using optical density measurement. All experiments were performed in triplicates on three separate days.

The D-values for control and sub-lethally treated L7 samples over 30, 60, and-90 minute durations were 0.83, 1.58, 2.54, and 3.01 minutes, respectively. Control samples exhibited the lowest D-

values, while the highest values were observed in the 90-minute treated samples. Sublethal heat exposure caused up to a 3.6-fold increase in D-values of L7, compared to the control. Prolonged sub-lethal treatment durations increased the D-values.

Table 13. D_{60}-values of control and sublethal heat-treated L7 samples. After sub-lethal heat treatment at 46 °C, including the control, samples were subjected to isothermal heat treatment at 60 °C. Microbial loads were enumerated using optical density measurement. All experiments were performed in triplicates on three separate days.

Treatment	D_{60}-value (min)*	R^2
Control	0.83 (0.38) **a	0.91
30 min	1.58 (0.55)ab	0.87
60 min	2.54 (0.85)b	0.88
90 min	3.01 (1.53)ab	0.94

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

Overall, D-values for strains L2, L4, and L7 varied across different treatment durations. For control samples, the D-values were recorded as 1.27, 0.63, and 0.83 minutes, respectively. While no significant differences were observed between L2-L7 and L4-L7, a statistically significant difference was noted between L2 and L4. In the 30-minute treatments, the D-values were 3.84, 1.31, and 1.58 minutes, respectively, with strain L2 demonstrating disparity from the other two strains. Similarly, for 60-minute treatments, the D-values were 6.35, 2.04, and 2.54 minutes, with strain L2 differing significantly from strains L4 and L7. Finally, for 90-minute treatments, the D-values were 6.15, 1.90, and 3.01 minutes, respectively. While no significant difference was observed between L2-L7 and L4-L7, a significant difference existed between strains L2 and L4.

In summary, L2 exhibited the highest D-values in each treatment among the three strains, while L4 consistently displayed the lowest D-values. Since the origins of L4 and L7 are both cheese, while the origin of L2 is dairy (Table 1), these differences can not be explained by the origins. Sub-lethal treatment induced an increase in D-values across all strains compared to control

conditions. This increase ranged between 3.10-5-fold for L2, 2.08-3.24-fold for L4, and 1.90-3.63-fold for L7 samples. Notably, in the L7 strain, prolonging the sub-lethal treatment duration resulted in a progressive rise in D-values, with the highest observed in the 90-minute treatment samples. Conversely, extended sub-lethal treatment durations in strains L2 and L4 led to an increase in D-values until the 60-minute treatment, after which a plateau was reached. Interestingly, the D-value for the 60-minute treatment exceeded that of the 90-minute treatment for both strains; however, this difference was not statistically significant ($P > 0.05$).

Table 14. Comparison of D_{60}-values between plate counting and optical density measurement methods across different strains and treatments

Treatment	Strain	D_{60}-value (min)*	
		Plate Count	Optical Density Measurement
Control	L2	1.12 (0.17)**[a]	1.27 (0.08)**[a]
	L4	0.89 (0.14)[a]	0.63 (0.15)[a]
	L7	0.58 (0.00)[a]	0.83 (0.38)[a]
30 min	L2	2.21 (0.03)[a]	3.84 (0.42)[b]
	L4	1.21 (0.05)[a]	1.31 (0.56)[a]
	L7	0.78 (0.01)[a]	1.58 (0.55)[a]
60 min	L2	2.24 (0.11)[a]	6.35 (0.93)[b]
	L4	1.26 (0.16)[a]	2.04 (0.42)[b]
	L7	2.10 (0.30)[a]	2.54 (0.85)[a]
90 min	L2	2.23 (0.11)[a]	6.15 (2.13)[b]
	L4	1.38 (0.03)[a]	1.90 (0.33)[a]
	L7	3.50 (0.59)[a]	3.01 (1.53)[a]

*Value within the bracket is the standard deviation.

**The means followed by different letters are significantly different within each row based on the Student's t-test ($P < 0.05$).

Table 14 presents the D-values of three *L. monocytogenes* strains, assessed through plate counting and optical density measurements. In the control group, the D-values did not significantly differ between the two methods for all strains. However, for sublethal heat treatment at 46 °C for 30 minutes, the D-values of L2 showed a significant difference between the two methods, whereas those of L4 and L7 did not. For sublethal heat treatment at 46 °C for 60 minutes, the D-values of L2 and L4 exhibited a significant difference between methods, while those of L7 did not. In contrast, for sublethal heat treatment at 46 °C for 90 minutes, the D-values of L2 were significantly different between the two methods, but those of L4 and L7 were not, similar to 30-minute treatment. Across all treatments, the D-values of L7 did not significantly differ between the two methods, while those of L2 and L4 exhibited different behaviour across various treatments.

Sergelidis and Abrahim (2009) reviewed the increase in D-values of *L. monocytogenes* after heat shock. It has been shown that the values of D for *L. monocytogenes* can increase by a factor of one to eight, depending on the duration of heat shock, temperature, and heating medium. Similarly, I found that the range of increase in D-values of *L. monocytogenes* was between 1.3-7.8-fold, depending on the enumeration method, strain, and sub-lethal heat treatment conditions. In my isothermal heat destruction of *L. innocua* study, I observed a 1.6-fold increase in D_{60} values after sublethal heat treatment at 46 °C for 60 min, whereas prior treatment at 46 °C for 30 min did not cause a significant difference compared to control samples. Ágoston et al. (2010) observed a 1.7 and 5.3-fold increase in D_{60} values of *L. monocytogenes* after prior exposure to 46 °C for 30 and 60 minutes, compared to control samples. Nevertheless, there was a decline in heat resistance after sublethal heat treatment at 50 °C for 60 minutes, compared to 30 minutes at the same temperature, demonstrating an upper limit for enhanced heat resistance after sublethal heat treatment. Shen et al. (2014) studied the impact of sublethal heating at 48 °C on survival following heat destruction at 60 °C across three *L. monocytogenes* strains, each representing distinct heat tolerance levels. Among these strains, the most effective heat stress adaptation occurred with sublethal heat stress at 48 °C for either 30 or 60 minutes. The D-values of these three groups of heat-tolerant strains

increased by a maximum of 2.5-folds. However, increasing the duration of preheating at 48 °C to 90 minutes led to a reduction in the ability of adaptation. They claimed that prolonged exposure to mild stress might cause cellular damage and hinder the ability to adapt to heat stress. My study and other studies indicate that heat stress adaptation of *Listeria* spp. depends on the strain and sublethal treatment conditions (Ágoston et al. 2010, Haykir et al. 2022).

Above literature regarding the increase in D-values after the sub-lethal heat treatment were based on conventional plate counting. There are research on investigation of stress conditions with optical density measurements (Guillier et al. 2005, Shen et al. 2014, Xuan et al. 2017). In such studies, growth parameters like lag time or growth rate were analyzed. Shen et al. (2014) observed increase in lag times when the *L. monocytogenes* cells subjected to sub-lethal heat treatment at 48 °C. Guillier et al. (2005) examined the impact of nine prevalent food-related stressors on the lag phases of *L. monocytogenes*. Out of all the stresses, they discovered that heat stress caused the biggest increase in TTD.

Even if most of the heat stress adaptation of *L. monocytogenes* studies were done in broth, there are also studies where food was used as the heating medium. In the study of Farber and Brown (1990) on different *L. monocytogenes* strains in sausage mix, prior treatment at 48 °C for 30 and 60 minutes did not cause a significant difference in D_{64} values compared to control samples. However, compared to control samples, there was a 2.4-fold increase in D_{64} values after the sublethal treatment at the same temperature for 120 min. Jorgensen et al. (1999) found that D_{60} was generally 2–6-fold higher in minced beef than in lactic acid added to tryptic phosphate broth, indicating that the presence of lactic acid in naturally occurring levels will have a specific effect on the response of *L. monocytogenes* to heat shock decreasing its thermotolerance. These results show the importance of the choice of media during heat resistance studies. In addition, it is also important to use foods as a matrix during those experiments.

From the food safety point of view, the increased heat resistance phenomenon is particularly significant in foods requiring long, low-temperature heating periods where bacterial pathogens may increase their thermotolerance in response to prior sublethal heat stress. For instance, meat products slowly heated to their final temperature, like sous-vide, can cause enhanced heat resistance. Another example is solid foods where slow heat penetration because of the conduction can create temperature gradients, inducing increased heat resistance in microbial cells. Similarly, gradual temperature increases are necessary for processing liquid foods like liquid whole eggs to prevent damage to sensitive food components (Sergelidis and Abrahim 2009). The introduction of milder thermal processing methods underscores the importance of precise safety predictions, particularly regarding the heating rate, especially when operating at lower processing temperatures. The slower the temperature increases, the greater the potential increase in heat resistance (Stephens et al. 1994). Foods left on warming trays or subject to interrupted cooking may also develop a more thermotolerant microbial population. *L. monocytogenes* needs to get special attention since it is a psychrotrophic species, meaning that it can proliferate in refrigerated conditions. Shen et al. (2014) observed that heat stress adaptation at 48 °C was reversed within 2 hours at 22 °C, but remained highly stable for up to 24 hours at 4 °C. While refrigeration typically delays the growth of *L. monocytogenes,* it can also preserve any gained heat stress adaptation. In such cases, higher reheating temperatures or longer reheating times may be necessary to effectively destroy heat stress-adapted *L. monocytogenes* cells and reduce any potential food safety risks following cold storage. To ensure food safety, heat processing procedures must target pathogenic microorganisms in their most heat-resistant state.

5.4. Predictive modelling of isothermal heat destruction of *L. monocytogenes* with GInaFit

Thermal inactivation data of three *L. monocytogenes*, obtained from plate counting were estimated through GInaFit. Nine different models in GInaFit cover commonly observed types of inactivation

curves. Data of four treatment types (control, prior sublethal heat treatment at 46 °C for 30, 60, and 90 minutes) on three *L. monocytogenes* strains (L2, L4, and L7) were estimated. For each experiment, three replicates on different days were applied.

For all data sets, microbial loads were estimated using nine different models. Three mathematical models, the classical log-linear curve, the log-linear curve with a shoulder, and the Weibull model were able to describe the inactivation data. The other six available models were unlikely to be suitable for the data. Models with tailing behaviour were inconvenient since the data did not show a tailing effect. The same dataset, expressed by three mathematical models was represented to demonstrate how the tool works (Figures 17, 18, and 19). GInaFit concluded that the tailing is unlikely for the data since the number of residues was less than the minimal measured value. Therefore, the tailing effect could be observed if the number of observations were increased. For the other models that were not appropriate, like the biphasic model, the number of data points was insufficient for the valid applications of these models.

Figure 17 shows the microbial inactivation of control samples of L2 described using the log-linear inactivation model, estimated with GInaFit.

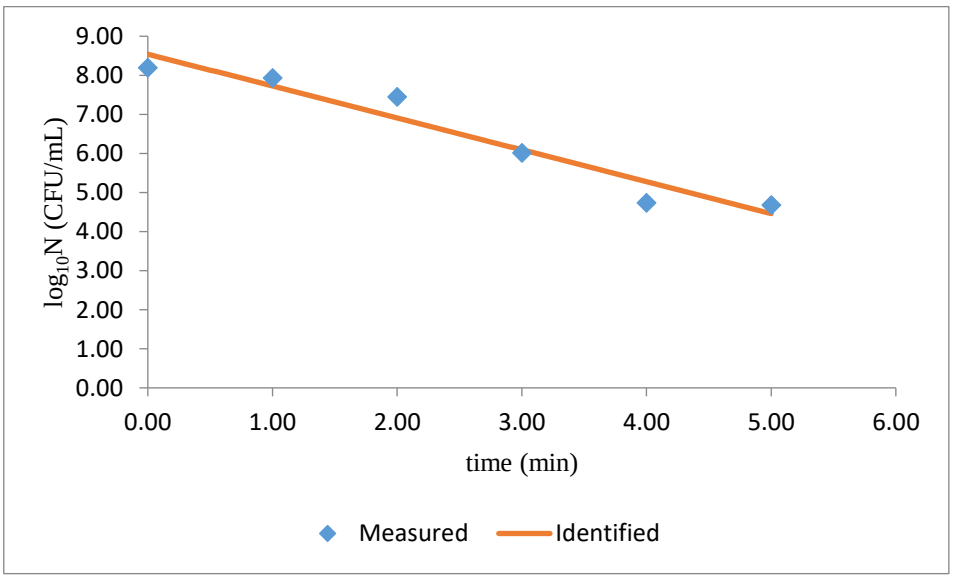


Figure 17. Comparison between the empirical observations (squares) of L2 control samples and the model predictions of the log-linear model (solid line) created from GInaFit

The log-linear model assumes that all cells within a population possess uniform heat sensitivity and that an individual's death is contingent upon random chance. The log-linear model (Bigelow and Esty, 1920) can be written as

$$\log_{10}(\mathrm{N}) = \log_{10}\big(\mathrm{N}(0)\big) - \frac{t}{D} = \log_{10}\big(N(0)\big) - \frac{k_{max}t}{\ln(10)}$$

In this equation, N denotes the microbial cell density, expressed as (CFU/mL), N(0) represents the initial microbial cell density (CFU/mL), k_{max} denotes the first-order inactivation constant in units of (1/time), and D stands for the decimal reduction time in time units.

The second model that estimated the microbial inactivation parameters was the log-linear model with a shoulder effect, described by Geeraerd et al. (2000). The model was defined by two equations,

$$\frac{\mathrm{dN}}{\mathrm{dt}} = -\mathrm{k_{max}} \times \mathrm{N} \times \frac{1}{1+\mathrm{C_c}} \times (1 - \frac{\mathrm{N_{res}}}{\mathrm{N}})$$

$$\frac{\mathrm{dC_c}}{\mathrm{dt}} = -\mathrm{k_{max}}\mathrm{C_c}$$

where C_c represents the physiological state of the cells, and N_{res} is the residual population density (CFU/mL). The dN/dt equation includes three factors: the first represents first-order inactivation kinetics, just like the previous equation; the second describes the shoulder effect due to the protective component around the cells; and the third indicates the presence of a more resistant subpopulation, i.e., the tail effect. In our experiments, there was no tailing effect. Figure 18 illustrates model fitting on empirical data of control samples of L2.

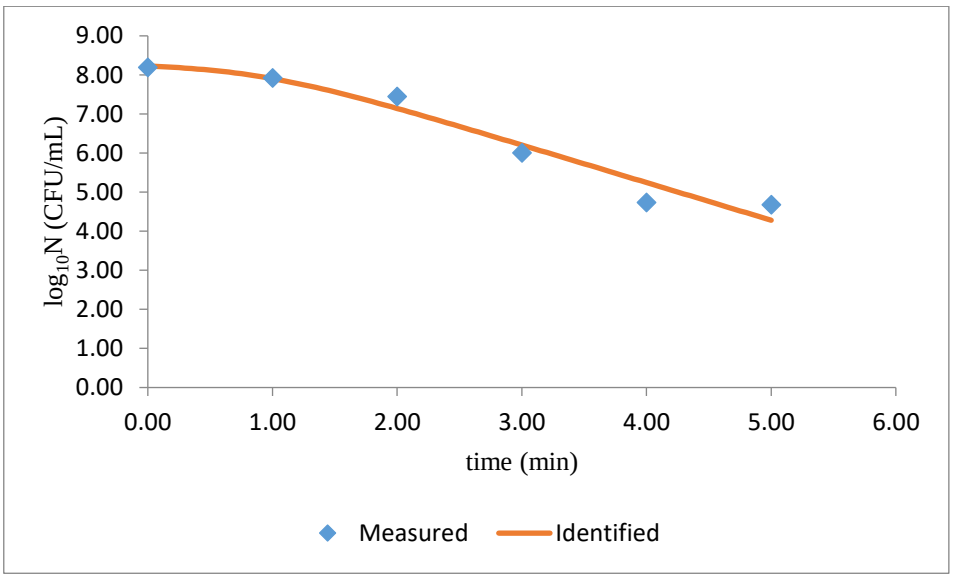


Figure 18. Comparison between the empirical observations (squares) of L2 control samples and the model predictions of the log-linear model with shoulder (solid line) created from GInaFit

The third model was applying the Weibull model to describe microbial inactivation, asserted by Mafart et al. (2002). The model is described as

$$\log_{10}(\mathrm{N}) = \log_{10}\big(N(0)\big) - (\frac{\mathrm{t}}{\delta})^{\mathrm{p}}$$

Here, δ represents the time required for the first decimal reduction when p equals 1. The shape parameter p indicates convex curves when greater than 1 and concave curves otherwise. Figure 19 displays the microbial inactivation curve of control samples of L2, estimated using the Weibull model in GInaFit.

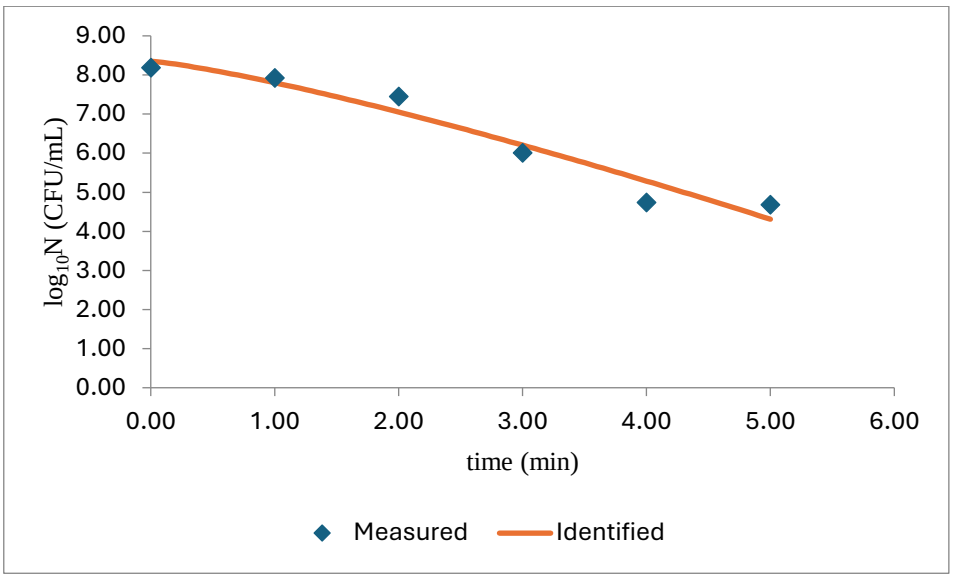


Figure 19. Comparison between the empirical observations (squares) of L2 control samples and the model predictions of the Weibull model (solid line) created from GInaFit

Table 15 shows model parameters for the thermal inactivation of L2, including log-linear k_{max} values. For control samples and those sub-lethally heat-treated at 46 °C for 30, 60, and 90 minutes, k_{max} values were 2.09, 1.04, 1.03, and 1.03, respectively. All sub-lethal heat treatments significantly altered k_{max} values compared to controls ($P < 0.05$), consistent with D-value comparisons with plate counting (Section 5.3.1.). This alignment is expected as both assessments rely on the same first-order inactivation model. The distinction lies in the parameter estimated from the model, with D-values in the prior section and the inactivation constant, k_{max}, in the current context. No significant difference was observed in k_{max} values between the sub-lethal heat treatments ($P > 0.05$).

Table 15. Model parameters for the three models estimated from the thermal inactivation data for L2

Treatment	log-linear	log-linear + shoulder		Weibull	
	k_{max} (min^{-1})*	k_{max} (min^{-1}) *	Sl (min)*	δ (min)*	p*
Control	2.09 (0.25) **a	2.74 (0.29)[a]	1.38 (0.36)[a]	1.88 (0.39)[a]	1.53 (0.30)[a]
30 min	1.04 (0.13)[b]	1.06 (0.28)[b]	0.10 (1.28)[b]	2.16 (0.74)[a]	0.98 (0.33)[a]
60 min	1.03 (0.11)[b]	1.15 (0.22)[c]	0.55 (0.86)[ab]	2.44 (0.57)[a]	1.11 (0.30)[a]
90 min	1.03 (0.11)[b]	1.07 (0.24)[b]	0.19 (1.04)[bc]	2.24 (0.62)[a]	1.01 (0.29)[a]

*Value within the bracket is the standard error.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

In the log-linear with a shoulder model, k_{max} values for control and sub-lethally heat-treated samples at 30, 60, and 90 minutes were 2.74, 1.06, 1.15, and 1.07, respectively. Control samples significantly differed from sub-lethally heat-treated samples. Among sub-lethally heat-treated samples, k_{max} values for 30 min and 90 min showed no significant difference ($P > 0.05$), while the k_{max} value for 60 min was different ($P < 0.05$). Control samples exhibited the most extended shoulder length (1.38 min), with the shortest in 30-minute samples (0.10 min). Interestingly, the control and 60-minute samples had 1.38 and 0.55 minutes, respectively, without a significant difference ($P > 0.05$). Similarly, sub-lethally heat-treated samples had 0.10, 0.55, and 0.19 min, respectively, without a significant difference ($P < 0.05$). Despite apparent differences, the high number of standard errors rendered those differences statistically non-significant.

In the Weibull model, the time required for the decimal reduction (δ) values for control and sub-lethally heat-treated samples at 30, 60, and 90 minutes were 1.88, 2.16, 2.44, and 2.24 minutes, respectively. Unlike the other two models, δ values did not significantly differ between the treatments. Similarly, p values did not exhibit a significant difference ($P > 0.05$).

Table 16 presents model parameters derived from thermal inactivation data for L4. In the log-linear model, the k_{max} values for the control and sub-lethally heat-treated samples for 30, 60, and 90

minutes were 2.80, 1.91, 1.84, and 1.67, respectively. Control samples exhibited a statistical difference from sub-lethally heat-treated samples. Among sub-lethally heat-treated samples, k_{max} values for 30 min and 60 min, and 60 min and 90 min showed no significant difference ($P > 0.05$), while the k_{max} values for 30 and 90 min were different ($P < 0.05$). There was a tendency for k_{max} values to decrease with increased duration of sub-lethal heat treatment.

Table 16. Model parameters for the three models estimated from the thermal inactivation data for L4

Treatment	log-linear	log-linear + shoulder		Weibull	
	k_{max} (min^{-1})*	k_{max} (min^{-1})*	Sl (min)*	δ (min)*	p*
Control	2.80 (0.50)**a	3.83 (0.80)a	1.52 (0.64)a	1.50 (0.78)a	1.50 (0.61)a
30 min	1.91 (0.48)b	4.30 (0.09)a	2.74 (0.04)b	3.07 (0.20)b	3.04 (0.39)b
60 min	1.84 (0.28)bc	2.70 (0.13)b	1.72 (0.14)a	2.33 (0.31)c	1.82 (0.30)a
90 min	1.67 (0.20)c	1.79 (0.35)c	0.41 (0.89)c	1.48 (0.63)a	1.05 (0.33)c

*Value within the bracket is the standard error.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

In the log-linear with a shoulder model, k_{max} values for control and sub-lethally heat-treated samples at 30, 60, and 90 minutes were 3.83, 4.30, 2.70, and 1.79, with shoulder lengths of 1.52, 2.74, 1.72, and 0.41, respectively. No significant difference in k_{max} values was observed between the control and 30 min samples ($P > 0.05$). However, increasing the sub-lethal treatment duration to 60 and 90 min resulted in a significant decrease in k_{max} values ($P < 0.05$). Shoulder lengths showed no significant difference between control and 60 min samples, while 30 and 90 min samples differed significantly.

In the Weibull model, δ values for control and sub-lethally heat-treated samples at 30, 60, and 90 minutes were 1.50, 3.07, 2.33, and 1.48, with corresponding p values of 1.50, 3.04, 1.82, and 1.05, respectively. δ values for control and 90 min did not show a significant difference ($P > 0.05$), but 30- and 60 min samples were significantly different ($P < 0.05$). Regarding p values, control and

60 min samples were not significantly different, while 30 and 90 min samples showed a significant difference.

Table 17 displays model parameters for L7 across three models. In the log-linear model, k_{max} values for control and sub-lethally heat-treated samples at 30, 60, and 90 minutes were 3.98, 2.95, 1.11, and 0.67, respectively. Notably, k_{max} values significantly varied among all treatments ($P < 0.05$), showing a tendency to decrease with longer sub-lethal heat treatment durations.

Table 17. Model parameters for the three models estimated from the thermal inactivation data for L7

Treatment	log-linear	log-linear + shoulder		Weibull	
	k_{max} (min^{-1})*	k_{max} (min^{-1})*	Sl (min)*	δ (min)*	p*
Control	3.98 (1.00)**a	7.90 (1.24)a	2.49 (0.30)a	2.04 (0.65)a	2.45 (0.84)a
30 min	2.95 (0.42)b	3.57 (0.62)b	1.06 (0.67)b	1.57 (0.75)b	1.54 (0.60)b
60 min	1.11 (0.24)c	2.59 (0.35)c	2.87 (0.26)ac	3.68 (0.17)c	3.00(0.40)ac
90 min	0.67 (0.19)d	2.20 (0.13)c	3.37 (0.09)c	4.43 (0.07)d	3.83 (0.38)c

*Value within the bracket is the standard error.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

In the log-linear with a shoulder model, k_{max} values for control and sub-lethally heat-treated samples at 30, 60, and 90 minutes were 7.90, 3.57, 2.59, and 2.20, with shoulder lengths of 2.49, 1.06, 2.87, and 3.37, respectively. While k_{max} values for 60 and 90 minutes did not significantly differ ($P > 0.05$), control and 30-minute samples showed a significant difference ($P < 0.05$). k_{max} values exhibited an inclination to decrease with longer durations of sub-lethal heat treatment. Shoulder lengths for control and 60 min samples were not significantly different ($P > 0.05$), similar to 60 min and 90 min samples ($P > 0.05$).

In the Weibull model, δ values for control and sub-lethally heat-treated samples at 30, 60, and 90 minutes showed a significant variation ($P < 0.05$). The δ values were 2.04, 1.57, 3.68, and 4.43,

respectively, with corresponding p values of 2.45, 1.54, 3.00, and 3.83. The δ value decreased after 30 minutes of sub-lethal heat treatment compared to control, while 60 min and 90 min treatments caused an increase in δ value. There was no significant difference in p values between the control and 60-minute samples, similar to the 60-minute and 90-minute samples, while the 30-minute samples significantly differed from the others.

Table 18 presents model parameters for three strains collectively. This table contains the same parameters as the Tables 15, 16, and 17. In those tables, model parameters of the same strain within different models were compared. Instead, in Table 18, the model parameters of three strains were compared within three different models to investigate strain differences. In the log-linear model, significant differences were observed in k_{max} values across all treatments except the 60-minute treatment. L2 and L7 exhibited no significant difference in k_{max} values after 60 minutes ($P > 0.05$). Significant differences were found in the log-linear with a shoulder model in k_{max} values for control and 30-minute samples across all strains. However, k_{max} values for L4 and L7 after 60 and 90 minutes of sub-lethal treatment did not significantly differ. Lastly, in the Weibull model, δ values for L2 showed no significant difference compared to L4 and L7 control samples. While in 60-minute treatment, L2 and L4 samples were not significantly different, L7 differed from the other two strains. Significant differences were observed in both 30 and 90-minute treatments across all strains.

Table 18. Model parameters of thermal inactivation of three *L. monocytogenes* strains. The model parameters of three strains were compared within three different models to investigate strain differences.

Treatment	Strain	log-linear	log-linear + shoulder	Weibull
		k_{max}*	k_{max}*	δ*
Control	L2	2.09 (0.25)**[a]	2.74 (0.29)[a]	1.88 (0.39)[ab]
	L4	2.80 (0.50)[b]	3.83 (0.80)[b]	1.50 (0.78)[a]
	L7	3.98 (1.00)[c]	7.90 (1.24)[c]	2.04 (0.65)[b]
30 min	L2	1.04 (0.13)[d]	1.06 (0.28)[d]	2.16 (0.74)[c]
	L4	1.91 (0.48)[e]	4.30 (0.09)[e]	3.07 (0.20)[d]
	L7	2.95 (0.42)[f]	3.57 (0.62)[f]	1.57 (0.75)[e]
60 min	L2	1.03 (0.11)[g]	1.15 (0.22)[g]	2.44 (0.57)[f]
	L4	1.84 (0.28)[h]	2.70 (0.13)[h]	2.33 (0.31)[f]
	L7	1.11 (0.24)[g]	2.59 (0.35)[h]	3.68 (0.17)[g]
90 min	L2	1.03 (0.11)[i]	1.07 (0.24)[i]	2.24 (0.62)[h]
	L4	1.67 (0.20)[j]	1.79 (0.35)[j]	1.48 (0.63)[i]
	L7	0.67 (0.19)[k]	2.20 (0.13)[j]	4.43 (0.07)[j]

*Value within the bracket is the standard error.

**The means followed by different letters are significantly different within each column based on the Student's t-test ($P < 0.05$).

Table 19 presents statistical measures for three models across three strains and four treatment types. GInaFit provided coefficient of determination (R^2) and root mean sum of squared error (RMSE) values. A lower RMSE score signifies a more accurate fit of the predictive models and thus, more reliable predictions. Similarly, R^2 close to 1 indicates a well-fitting model with minimal error in the predicted values (Lee et al. 2023).

For control samples in L2, the log-linear model with shoulder exhibited the lowest RMSE values across the three models. However, there was no significant difference between RMSE values across models ($P > 0.05$). For sublethal heat-treated samples for 30 minutes, the log-linear model showed the lowest RMSE values, significantly different from the other two models. For sublethal heat-treated samples for 60 and 90 minutes, there were no significant differences between three models ($P > 0.05$).

Similar results were observed for L4 control samples and sublethal heat-treated samples for 90 minutes, with no significant difference between models. Sublethal heat-treated samples for 30 minutes had the lowest value in the log-linear model with a shoulder, while sublethal heat-treated samples for 60 minutes had the lowest values in the log-linear model with shoulder and Weibull models, significantly different from the log-linear model ($P < 0.05$).

For L7 control samples, the log-linear model with shoulder exhibited the lowest RMSE value, significantly different from the other two models ($P < 0.05$). There was no significant difference between models in sublethal heat-treated samples for 30 minutes ($P > 0.05$). For sublethal heat-treated samples for 60 minutes, the lowest RMSE value was observed in the Weibull model, not significantly different from the log-linear model with a shoulder. Lastly, for sublethal heat-treated samples for 90 minutes, RMSE values of the log-linear model with shoulder and Weibull models had the same RMSE values, significantly lower than the log-linear model.

Table 19. Statistical indexes describing model fit/predictions

Strain	Treatment	log-linear		log-linear+shoulder		Weibull	
		R^2	RMSE*	R^2	RMSE*	R^2	RMSE*
L2	Control	0.95	0.50[a]	0.99	0.32[a]	0.98	0.36[a]
	30 min	0.94	0.24[a]	0.94	0.28[b]	0.94	0.28[b]
	60 min	0.96	0.21[a]	0.96	0.22[a]	0.96	0.23[a]
	90 min	0.96	0.20[a]	0.96	0.23[a]	0.96	0.23[a]
L4	Control	0.89	1.70[a]	0.95	1.72[a]	0.92	1.85[a]
	30 min	0.79	0.88[a]	1.00	0.07[b]	0.99	0.21[c]
	60 min	0.92	0.54[a]	1.00	0.16[b]	0.99	0.25[b]
	90 min	0.95	0.36[a]	0.95	0.40[a]	0.95	0.41[a]
L7	Control	0.80	1.82[a]	0.97	0.75[b]	0.94	1.10[c]
	30 min	0.93	0.78[a]	0.96	0.66[a]	0.93	0.80[a]
	60 min	0.84	0.47[a]	0.98	0.24[ab]	0.99	0.21[b]
	90 min	0.75	0.35[a]	1.00	0.06[b]	1.00	0.06[b]

R^2: coefficient of determination, RMSE: root mean sum of squared error,

*The means followed by different letters are significantly different in each strain within each column based on the Student's t-test ($P < 0.05$).

In summary, there was no consistent pattern observed in RMSE values, as no single model consistently outperformed others across different strains or treatments. The literature presents conflicting findings regarding the thermal destruction behaviour of *L. monocytogenes*. Hassani et al. (2005) reported that *L. monocytogenes* survival curves followed a first-order kinetic pattern in isothermal heat destruction. Liu et al. (2021) effectively incorporated the log-linear dynamic inactivation of *L. monocytogenes* in cooked beef under unsteady heating conditions. In contrast, Huang (2009) noted deviations from first-order kinetics in *L. monocytogenes* survival curves in ground beef, favoring Weibull-type and modified Gompertz models over the linear model.

Similarly, Velasco-Hernández et al. (2020) observed that modified Gompertz equation survival yielded a better fit compared to the log-linear pattern in the survival of *L. monocytogenes* in soursop pulp. In my study, there was no significant difference in RMSE values of the three models in L2 and L4 control samples. Instead, the log-linear model with shoulder yielded a lower RMSE value compared to the other two models in L7 control samples. Wang et al. (2024) found that the survival curves of *L. monocytogenes* in milk fit well with a log-linear model that includes a shoulder. In predictive microbiology, the shoulder length parameter is used to describe the accumulated damage to bacteria in the inactivation curve. Various factors such as experimental artefacts, heterogeneity in microbial resistance within populations, or the presence of mixed microbial communities have been postulated as potential sources for deviations observed in experimental outcomes. The appearance of the shoulder effect can happen due to the dimension of samples, meaning thick samples or samples in large containers, because it takes time for heat to spread through. Since come-up time was excluded from the heating time in my study, the shoulder effect in L7 control samples cannot be attributed to this reason. As also noted by Huang (2009), the shoulder observed in my study may be attributed to biological factors, wherein a thermal process must overcome an initial energy barrier before a lethal effect can be observed.

Previous research has shown that *L. monocytogenes* cells exhibited deviations from first-order inactivation kinetics when the cells experienced heat stress (Garre et al. 2019, Huang, 2009, Peleg and Cole 1998). Recent research indicates that log-linear kinetics are not consistently observed in heat processing, and the presence of shoulders in inactivation curves is quite common (Wang et al. 2015, Wason et al., 2022). The occurrence of a shoulder effect in survival curves is interpreted as suggestive of either the accumulation of sublethal damage or the overcoming of energy barriers (Fang et al. 2021). The deviations from the log-linear behaviour are commonly linked to damage caused by heat and subsequent repair mechanisms within the cells. Furthermore, in my results, neither the log-linear model with shoulder nor the Weibull models outperformed the standard log-linear model across different sub-lethal treatments.

5.5. Implementing Automated Cleaning Programs (ACPs) and Their Validation

A case study was conducted to deploy a CIP technique on a vending machine that makes smoothies. The project consisted of three distinct phases: establishing microbiological thresholds, developing cleaning protocols, and validating the effectiveness of the cleaning protocols by microbial analysis. The focus of my thesis on the enhanced heat resistance of *Listeria* spp. aligns closely with the case study on implementing ACPs. Firstly, defining microbial limits involved investigating the presence of *L. monocytogenes*, a key pathogen of interest in heat resistance studies. Secondly, the cleaning protocols developed and evaluated in the ACPs incorporated thermal processing as a critical component. Specifically, the cleaning cycles included a rinsing phase with cold water, sanitizing with hot water at 85-90 °C, and sterilizing with steam, directly addressing the thermal tolerance of microorganisms like *Listeria* spp. This integration highlights the practical application of understanding *Listeria*'s heat resistance in designing effective sanitation programs.

5.5.1. Defining microbial limits

Three pathogens, *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* were chosen as microorganisms to be evaluated during the validation process. *L. monocytogenes* was added to the case study since it is the main target of my research. The other two microorganisms were added because of their importance in fruit and vegetable juices. Limits for those pathogens were decided according to Commission Regulation (EC) No. 2073/2005 (European Commission 2005). In the regulation, ‘ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes category’ was chosen as a category for *L. monocytogenes*, whereas ‘unpasteurized fruit and vegetable juices (ready-to-eat)’ category was chosen for *Salmonella* and *E. coli*. Table 20 demonstrates microbial thresholds for three pathogens (Haykir et al. 2023).

Table 20. Microbial Limits of Three Pathogens for Smoothies

Microorganism	Sampling Plan		Limits		Analytical Reference Method
	n	c	m	M	
Listeria monocytogenes	5	0	Absence in 25g		ISO 11290-1, (2017)
Salmonella	5	0	Absence in 25g		ISO 6579-1, (2017)
E. coli	5	2	100 CFU/g	1000 CFU/g	ISO 16649-1, (2018; ISO 16649-2, (2001)

Note. n = the number of units comprising the sample; c = the number of sample units giving values over m or between m and M. Adapted from Regulation of the European Commission of 15 November 2005 on microbiological criteria for foodstuffs, 2073/2005/EC, by European Commission, 2005. Copyright 2005 by the European Commission.

5.5.2. Developing cleaning protocols

ACP procedure consisted of three cleaning cycles to reduce or eliminate microorganisms from the food contact materials in the vending machine. To make a smoothie in the vending machine, frozen fruits or vegetables are collected in the cup according to the recipe. Water is added to the cup, and ingredients are mixed with a blender for the smoothie production. The first cycle is called after smoothie cleaning and it occurs after every smoothie production. The primary goal of this step was to remove food residues from the blender and sanitize the food contact materials. The second cleaning cycle is called the 3-hour cleaning cycle and takes place every three hours automatically. The third cleaning cycle is called the full cleaning cycle and takes place at the end of each day. In every cleaning cycle, there are three phases: rinsing phase with cold water, sanitising with hot water around 85-90 ℃, and sterilizing with steam. All of these phases take place in each cleaning

cycle. The difference between cleaning cycles is the duration of the phases. For instance, since the main target is to remove residues after smoothie production, rinsing step-in after smoothie cleaning is the longest. Instead, sterilization duration is the longest in the full cleaning cycle since it is the last cleaning of the day. Rinsing duration was decided by visual inspections of the blender after cleaning, whereas sanitation and sterilization durations were chosen with respect to BRC Global Standard for Food Safety. According to the standard, minimum temperature and time combinations to destroy the target organism *L. monocytogenes* is 70 ℃ for two minutes or an equivalent process with using a z-value of 7.5 ℃ (BRC, 2018).

Even if the objective of this case study was to implement automated cleaning programs, manual cleaning of the vending machines is still unavoidable. Manual cleaning is done after the refilling of the machine. For manual cleaning, food-safe antibacterial wipes and disinfectants were chosen to clean dust and residues from the materials and disinfect the parts after the manual intervention. A cleaning manual was prepared and the operators were trained for effective cleaning.

5.5.3. Evaluation of Cleaning Programmes

As a final step, the validation of the cleaning procedures was carried out through the microbiological analysis of the ingredients and the blender. A sampling scheme has been established to replicate the typical usage of the vending machine. A plan for sampling over two days was developed to accomplish the evaluation. The first day began and the second day finished with manual cleaning, which is a routine task that involves reloading the machine and performing cleaning operations. During the two-day sampling period, all three cleaning cycles were performed and samples were collected by the laboratory in accordance with the standards.

This study aimed to employ a comprehensive sampling methodology, conducting sample collection on two separate occasions in August and October 2022, in order to assess the presence of pathogens in water, smoothies, and on the surface of blenders. The protocol for the two-day schedule and sampling intervals are presented in Table 21 (Haykir et al. 2023). Thirteen samples

were collected each month for a total of 26 samples analyzed. Samples were obtained aseptically, utilizing sterile swabs for the surfaces of the blender and sterile containers for the water and smoothie samples.

The samples were evaluated to determine the presence of three pathogens: *L. monocytogenes*, *Salmonella*, and *E. coli*. No pathogens were found in any of the 26 samples. In their study on the microbiological quality of smoothies from fresh bars, Krahulcová et al. (2021) detected the presence of *E. coli* in one out of the twenty samples. Raposo et al. (2015) revealed that the presence of *L. monocytogenes* in vending machine samples might be attributed to improper food handling and inadequate temperature control. In contrast, Hall et al. (2007) examined vending machines that dispense hot drinks with the result that regular on-site manual cleaning using detergent effectively maintained the microbial load at the desired level.

Table 21. Sampling plan during the cleaning validation

Day	Action	Time	Sample		
Day 1	Manual Cleaning	08:50			
	After Manual Cleaning	09:00	Swab 1	Water Sample 1	
	End of Day	16:00	Swab 2	Water Sample 2	Smoothie Sample 1
	24h Full Cleaning	16:20			
	After 24h Full Cleaning	16:30	Swab 3		
Day 2	24h Full Cleaning	08:50			
	After 24h Full Cleaning	09:00	Swab 4	Water Sample 3	
	End of Day	16:00	Swab 5	Water Sample 4	Smoothie Sample 2
	Manual Cleaning	16:20			
	After Manual Cleaning	16:30	Swab 6		Smoothie Sample 3

My study was conducted on a brand-new machine. Therefore, these results do not provide conclusive evidence about whether they are attributable to effective cleaning techniques. In addition to microbial analysis, a visual examination was carried out following each cleaning session to detect any traces of food on the blender. There was no visible residue on the blender.

According to my investigation, all the research in the literature employed the manual cleaning of the vending machines. There is a lack of research on the CIP system applied to vending machines. Further investigation is required to examine automated cleaning techniques. Karaman et al. (2012)

highlighted the difficulty of applying the HACCP system for small or medium-sized enterprises (SMEs) due to a deficiency in technical skills and food handling practices. According to Saltmarsh (2023), the majority of vending machines are run by SMEs. Therefore, enhancing the understanding of the cleaning protocols for vending machines can assist these organizations with establishing HACCP.

6. CONCLUSIONS AND RECOMMENDATIONS

L. monocytogenes is able to survive and proliferate in various conditions. These features render *L. monocytogenes* a major concern in the food industry regarding food safety. Heat stress adaptation of *L. monocytogenes* can enhance survival and resistance following heat treatments. Alternative enumeration techniques such as redox potential and optical density measurements can provide faster results than traditional methods. It is critical to evaluate the physiological responses of *L. monocytogenes* following sublethal heat treatment. Furthermore, it is essential to assess the effectiveness of alternate methods of enumeration when quantifying such replies.

The redox potential and optical density measurement-based methods were employed for rapid quantification of *L. monocytogenes*. Both methods offered the possibility of providing faster results compared to traditional methods. The repeatability of both methods was within the range of the repeatability of the standard conventional method. More research is needed to broaden the knowledge of their applicability in evaluating different stress conditions.

Isothermal heat destruction of *L. innocua* with and without sublethal heat treatment was employed. Sublethal heat treatment at 46 ℃ for 30 minutes did not change the D_{60} value, compared to control. Cluster analysis of peaks, obtained from MALDI-TOF also supported this result. Sublethal heat treatment at the same temperature for 60 minutes increased the D_{60} value compared to control.

Dynamic heat destruction of *L. innocua* with and without sublethal heat treatment at 46, 48, and 50 ℃ for 30, 60, and 90 minutes were evaluated. There were no significant differences in $\log_{10}D$ values of control and sublethal heat-treated samples at 46 ℃ for 30 and 60 minutes. All the other sublethal heat treatments altered the $\log_{10}D$ values significantly compared to the control. Since dynamic heat destruction occurs more in the industry than isothermal heat destruction, future research should focus on more dynamic heat destruction studies.

The effect of sublethal heat treatment at 46 ℃ for 30, 60, and 90 minutes was assessed on isothermal heat destruction of three *L. monocytogenes* strains. All the sublethal heat treatment

conditions caused a significant difference in D_{60} value compared to the control in all strains. Except in one instance, there was an increase in D_{60} values as sublethal treatment conditions were extended. No consistent pattern was observed among different strains when they were subjected to the same treatment conditions.

Subsequently, the same sublethal treatment conditions were applied to the same strains, but microbial loads were determined using the optical density measurement. Generally, there was no significant difference in D_{60} values seen between the plate counting method and the OD method in most cases. Nevertheless, there were instances where a variation in D_{60} values between the two approaches was observed.

Microbial inactivation data from the plate counting method was fitted by different mathematical models. The log-linear model, the log-linear models with a shoulder, and the Weibulls model successfully estimated the inactivation parameters for all situations. There was no particular model that surpassed others. However, different results were observed across different models when comparing sublethal treatment conditions. Further study can be done with the dynamic heat destruction. It is important to choose an appropriate model during the study of enhanced heat resistance.

In my research, experiments were done in broth. However, as mentioned in the literature overview section, different results were observed when the food was used as a matrix. Therefore, additional research is needed to better understand the effect of sublethal heat treatment on real foods. During my dissertation, I focused on the physiological responses of *Listeria* to sublethal temperature stress. Further research is needed to investigate genomic and proteomic responses with emerging methods.

Overall, the experimental results support the achievement of the initial objectives of my research. Two alternative enumeration methods that I utilized are promising methods for rapid quantification. *L. monocytogenes* elicits physiological responses to sublethal heat treatment as a

stress adaptation mechanism. This adaptation depends on different factors like strain variation, sub-lethal exposure conditions, and heat destruction methods.

7. NEW SCIENTIFIC RESULTS

1. It was proved that redox potential measurement can be applied to quantitatively assess the thermal inactivation of *Listeria* spp. for the first time (Haykir et al. 2025). In addition, optical density measurement was also suitable to evaluate the heat destruction of *L. monocytogenes*. Both methods provided rapid quantification within the repeatability range of the plate counting method.

2. The isothermal heat destruction of *L. innocua* T1 was examined following exposure to sub-lethal conditions at a temperature of 46 °C. The D_{60} value did not show a significant change when prior exposure for 30 minutes was followed by isothermal heat treatment at 60 °C, compared to the control group that had direct heat treatment at 60 °C. Instead, exposure to sub-lethal heat for 60 minutes at 46 °C altered the D_{60} value significantly from the control (Haykir et al. 2022).

3. The dynamic (non-isothermal) heat destruction with prior sub-lethal heat treatment of *L. innocua* T1 was evaluated. A paired t-test revealed that exposure at 46 °C for 30 and 60 minutes did not result in a statistically significant change in the $\log_{10}D$ values compared to the control samples. Nevertheless, all the other sub-lethal treatment conditions (46 °C for 90 minutes, 48 °C for 30, 60, and 90 minutes, and 50 °C for 30 and 60 minutes) resulted in significant alterations in the $\log_{10}D$ values (Haykir et al. 2025).

4. Enhanced heat resistance of three *L. monocytogenes* strains, *L. monocytogenes* L2, *L. monocytogenes* L4, and *L. monocytogenes* L7, were demonstrated when exposed to sub-lethal heat conditions at 46 °C for 30, 60 and 90 minutes. Enumeration was done with plate counting. Compared to control samples, all sub-lethal treatment conditions significantly increased the D_{60} values for all strains. With one exception in *L. monocytogenes* L2, all strains showed an increase in D_{60} values as the sub-lethal treatment durations were increased.

5. When the enumeration was done with an optical density measurement-based method, the same conditions also caused a significant increase in D_{60} values in all strains compared to control samples. Extension of sub-lethal treatment caused an increased trend in D_{60} values in *L.*

monocytogenes L7. However, extended sub-lethal treatment durations in strains *L. monocytogenes* L2 and *L. monocytogenes* L4 increased D_{60} values until the 60-minute treatment, after which a plateau was reached and the 90-minute treatment had a lower D_{60} value.

6. Log-linear model, log-linear model with a shoulder, and Weibull models were able to estimate isothermal heat destruction parameters for three *L. monocytogenes* strains with and without sub-lethal heat treatment. No tailing effect was observed for all conditions. Between the three models, none of the models surpassed the others in terms of statistical measures.

7. This study demonstrated that several parameters, such as strain variation, sub-lethal heat treatment temperature and duration, test settings, and the mathematical models used to estimate the data, all had an impact on the increased heat resistance of *Listeria* spp.

8. SUMMARY

Listeria monocytogenes is one of the pathogens that is a major concern in food safety. It has high case fatality rate and it can be found in various environments. Heat processing is still one of the most common food processing methods in the food industry. When microorganisms are subjected to sub-lethal heat treatment, they may gain enhanced heat resistance with their stress adaptation mechanism. The overall focus of my dissertation was to better understand the enhanced heat resistance of *L. monocytogenes* after sub-lethal heat exposure. Another goal of the research was to investigate the applicability of two alternative enumeration methods, the redox potential measurement-based method and the optical density (OD) measurement-based method, on the heat inactivation studies. *L. innocua* was utilized as a surrogate for *L. monocytogenes* in the first part of the study. Three *L. monocytogenes* strains (L2, L4, and L7) were used in the following part of the research.

Both the redox potential measurement and optical density measurement methods offered rapid quantification within the repeatability range of the plate counting method. Microbial loads were obtained from both methods and plate counting and analyzed to compare the enhanced heat resistance of *Listeria* spp.

Isothermal heat destruction of *L. innocua* was assessed with and without sub-lethal heat exposure at 46 °C for 30 and 60 minutes. Sub-lethal heat exposure at 46 °C for 30 minutes did not significantly increase the D_{60} value. This result was further supported by conducting a cluster analysis of the peaks obtained from MALDI-TOF. Instead, pre-exposure to a sub-lethal temperature of 46 °C for 60 minutes increased the D_{60} value compared to the control, from 3.66 minutes to 5.71 minutes.

During the dynamic heat destruction of *L. innocua*, sub-lethal heat exposure to 46 °C for 30 and 60 minutes did not significantly change the $\log_{10}D$ values compared to the control. The other sub-lethal treatment conditions (46 °C for 90 minutes, 48 °C for 30, 60, and 90 minutes, and 50 °C for

30 and 60 minutes) all altered the $log_{10}D$ values. The difference in results of isothermal and non-isothermal heat destruction experiments in this study shows the importance of choosing the appropriate method during thermal inactivation studies. Dynamic heat inactivation is much more common in the food industry compared to isothermal destruction. Therefore, more research on dynamic heat inactivation study is needed.

Sub-lethal heat treatments at 46 °C for 30, 60, and 90 minutes were all increased the D_{60} values of three *L. monocytogenes* strains compared to control when microbial loads were enumerated with the plate counting method. The highest increase in D_{60} values was observed in L7 with 6-fold increase compared to their control samples. The highest increase after sub-lethal heat treatment for L2 and L4 were 2- and 1.55-fold, respectively. Except for one case in L2, prolonging the sub-lethal exposure increased the D_{60} values.

Subsequently, the same conditions were applied to the same strains and microbial loads were enumerated using OD measurement. Doubling time, obtained from OD measurement using the GrowthCurver package, was used as TTD. Similar to plate counting, sub-lethal heat treatments at 46 °C for 30, 60, and 90 minutes all increased the D_{60} values of three *L. monocytogenes* strains compared to control. When the D_{60} values obtained from plate counting and OD measurement were compared, four values out of 12 were statistically different. Unlike the plate counting method, there was a limit for the increase in D_{60} values when the sub-lethal heat treatment duration was prolonged except in L7. For L2 and L4 samples, D_{60} values decreased after 90-minute treatment compared to 60-minute treatment. These results show that it is important to take into account experiment conditions when comparing results with existing literature.

Microbial inactivation data from plate counting were fitted to different mathematical models using the GInaFit tool. Out of nine models available in the tool, three models were able to estimate microbial inactivation parameters: the log-linear model, the log-linear model with a shoulder, and the Weibull model. When the statistical measures were compared for each model, no model

outperformed the other models. However, when the inactivation parameters were compared between different treatments within different models, different outcomes were obtained. Therefore, it shows that it is important to choose the right model during the study of heat resistance, but also compare the results with existing literature.

As a case study, the clean-in-place (CIP) technique was applied to clean a smoothie-making vending machine. The goal was to implement this technique to sanitize the machine effectively and reduce human intervention. Automated cleaning programmes were developed, microbial limits were decided, and microbial analysis were done for validation. For microbial analysis, blender, water, and smoothie samples were analyzed to find three pathogens, *L. monocytogenes*, *Salmonella* spp., and *E. coli*. After two months of sampling, no pathogen was detected in none of the samples.

Overall, in this research, it was demonstrated that enhanced heat resistance of *Listeria* spp. depends on various factors such as strain variability, sub-lethal heat exposure conditions, experiment settings and mathematical models. Further research is needed to evaluate those effects. The design of optimal thermal inactivation experiments is crucial when planning this kind of research. During my investigation, I focused on the physiological response, more specifically, the change in D-value after sub-lethal heat exposure. More research is needed for the genomic and proteomic responses of *L. monocytogenes* after sub-lethal heat exposure.

9. APPENDICES

9.1. References

Acuff, J.C., Dickson, J.S., Farber, J.M., Grasso-Kelley, E.M., Hedberg, C., Lee, A., Zhu, M.-J., (2023). Practice and Progress: Updates on Outbreaks, Advances in Research, and Processing Technologies for Low-moisture Food Safety. *J. Food Prot.* 86, 100018. https://doi.org/10.1016/j.jfp.2022.11.010

Ágoston, R., Soni, K., Jesudhasan, P.R., Russell, W.K., Mohácsi-Farkas, C., Pillai, S.D., (2009). Differential expression of proteins in *Listeria monocytogenes* under thermotolerance-inducing, heat shock, and prolonged heat shock conditions. *Foodborne Pathog.* Dis. 6, 1133–1140. https://doi.org/10.1089/fpd.2009.0286

Ágoston, R., Mohácsi-Farkas, C., Pillai, S., (2010). Exposure to sub-lethal temperatures induces enhanced heat resistance in *Listeria monocytogenes*. *Acta Aliment.* 39, 327–336. https://doi.org/10.1556/aalim.39.2010.3.9

Arioli, S., Montanari, C., Magnani, M., Tabanelli, G., Patrignani, F., Lanciotti, R., Mora, D., Gardini, F., (2019). Modelling of *Listeria monocytogenes* Scott A after a mild heat treatment in the presence of thymol and carvacrol: Effects on culturability and viability. *J. Food Eng.* 240, 73–82. https://doi.org/10.1016/j.jfoodeng.2018.07.014

Arvaniti, M., Tsakanikas, P., Papadopoulou, V., Giannakopoulou, A., Skandamis, P., (2021). *Listeria monocytogenes* Sublethal Injury and Viable-but-Nonculturable State Induced by Acidic Conditions and Disinfectants. *Microbiol. Spectr.* 9, e01377-21. https://doi.org/10.1128/Spectrum.01377-21

Aryani, D.C., den Besten, H.M.W., Hazeleger, W.C., Zwietering, M.H., (2015). Quantifying variability on thermal resistance of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 193, 130–138. https://doi.org/10.1016/j.ijfoodmicro.2014.10.021

Augustin, Carlier, Rozier, (1998). Mathematical modelling of the heat resistance of *Listeria monocytogenes*. *J. Appl. Microbiol.* 84, 185–191. https://doi.org/10.1046/j.1365-2672.1998.00317.x

Auvolat, A., Besse, N.G., (2016). The challenge of enumerating *Listeria monocytogenes* in food. *Food Microbiol.* 53, 135–149. https://doi.org/10.1016/j.fm.2015.09.003

Awuah, G.B., Ramaswamy, H.S., Economides, A., (2007). Thermal processing and quality: Principles and overview. *Chem. Eng. Process. Process Intensif.* 46, 584–602. https://doi.org/10.1016/j.cep.2006.08.004

Baka, M., Noriega, E., Stamati, I., Logist, F., Van Impe, J.F.M., (2015). Critical Assessment of the Time-to-Detection Method for Accurate Estimation of Microbial Growth Parameters. *J. Food Saf.* 35, 179–192. https://doi.org/10.1111/jfs.12170

Baranyi, J., Pin, C., (1999). Estimating Bacterial Growth Parameters by Means of Detection Times. *Appl. Environ. Microbiol.* 65, 732–736.

Baranyi, J., Buss da Silva, N., (2017). The use of predictive models to optimize risk of decisions. *Int. J. Food Microbiol.*, Special Issue: 9th International Conference on Predictive Modelling in Food (Rio de Janeiro, Brazil) 240, 19–23. https://doi.org/10.1016/j.ijfoodmicro.2016.10.016

Barbuddhe, S.B., Maier, T., Schwarz, G., Kostrzewa, M., Hof, H., Domann, E., Chakraborty, T., Hain, T., (2008). Rapid identification and typing of *Listeria* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl. Environ. Microbiol.* 74, 5402–5407. https://doi.org/10.1128/AEM.02689-07

Beal, J., Farny, N.G., Haddock-Angelli, T., Selvarajah, V., Baldwin, G.S., Buckley-Taylor, R., Gershater, M., Kiga, D., Marken, J., Sanchania, V., Sison, A., Workman, C.T., (2020). Robust estimation of bacterial cell count from optical density. *Commun. Biol.* 3, 1–29. https://doi.org/10.1038/s42003-020-01127-5

Berthold, M.R., Cebron, N., Dill, F., Gabriel, T.R., Kötter, T., Meinl, T., Ohl, P., Sieb, C., Thiel, K., Wiswedel, B., (2008). KNIME: The Konstanz Information Miner, in: Preisach, C., Burkhardt, H., Schmidt-Thieme, L., Decker, R. (Eds.), Data Analysis, Machine Learning and Applications. Springer, Berlin, Heidelberg, pp. 319–326. https://doi.org/10.1007/978-3-540-78246-9_38

Besse, N.G., Colin, P., (2004). Enumerating *Listeria Monocytogenes* in Food: A Problem of Low Numbers. *J. Rapid Methods Autom. Microbiol.* 12, 83–105. https://doi.org/10.1111/j.1745-4581.2004.tb00052.x

Bigelow, W.D., Esty, J.R., (1920). The Thermal Death Point in Relation to Time of Typical Thermophilic Organisms. *J. Infect. Dis.* 27, 602–617. https://doi.org/10.1093/infdis/27.6.602

Bolívar, A., Costa, J.C.C.P., Posada-Izquierdo, G.D., Valero, A., Zurera, G., Pérez-Rodríguez, F., (2018). Modelling the growth of *Listeria monocytogenes* in Mediterranean fish species from aquaculture production. *Int. J. Food Microbiol.* 270, 14–21. https://doi.org/10.1016/j.ijfoodmicro.2018.02.005

Bonsaglia, E.C.R., Silva, N.C.C., Fernades Júnior, A., Araújo Júnior, J.P., Tsunemi, M.H., Rall, V.L.M., (2014). Production of biofilm by *Listeria monocytogenes* in different materials and temperatures. *Food Control* 35, 386–391. https://doi.org/10.1016/j.foodcont.2013.07.023

Boyer, R.R., Matak, K., Sumner, S.S., Meadows, B., Williams, R.C., Eifert, J.D., Birbari, W., (2009). Survival of *Listeria monocytogenes*, *Listeria innocua*, and lactic acid bacteria in chill brines. *J. Food Sci.* 74, M219-223. https://doi.org/10.1111/j.1750-3841.2009.01182.x

Brasca, M., Morandi, S., Lodi, R., Tamburini, A., (2007). Redox potential to discriminate among species of lactic acid bacteria. *J. Appl. Microbiol.* 103, 1516–1524. https://doi.org/10.1111/j.1365-2672.2007.03392.x

BRC, (2018). BRC Issue 8: Cooking instruction validation at Campden BRI [WWW Document]. URL https://www.campdenbri.co.uk/white-papers/cooking-instruction-validation.php (accessed 10.12.23).

Buchanan, R.L., Gorris, L.G.M., Hayman, M.M., Jackson, T.C., Whiting, R.C., (2017). A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control* 75, 1–13. https://doi.org/10.1016/j.foodcont.2016.12.016

Bucur, F.I., Grigore-Gurgu, L., Crauwels, P., Riedel, C.U., Nicolau, A.I., (2018). Resistance of *Listeria monocytogenes* to Stress Conditions Encountered in Food and Food Processing Environments. *Front. Microbiol.* 9, 2700. https://doi.org/10.3389/fmicb.2018.02700

Caggiano, G., Marcotrigiano, V., D'Ambrosio, M., Marzocca, P., Spagnuolo, V., Fasano, F., Diella, G., Leone, A., Lopuzzo, M., Sorrenti, D., Sorrenti, G., Montagna, M., (2023). Preliminary

Investigation on Hygienic-Sanitary Quality of Food Vending Machines. *Int. J. Environ. Res. Public. Health* 20, 5557. https://doi.org/10.3390/ijerph20085557

Cardaci, R., Burgassi, S., Golinelli, D., Nante, N., Battaglia, M., Bezzini, D., Messina, G., (2016). Automatic Vending-Machines Contamination: A Pilot Study. Glob. *J. Health Sci.* 9, p63. https://doi.org/10.5539/gjhs.v9n2p63

Carpentier, B., Cerf, O., (2011). Review — Persistence of *Listeria monocytogenes* in food industry equipment and premises. *Int. J. Food Microbiol.* 145, 1–8. https://doi.org/10.1016/j.ijfoodmicro.2011.01.005

Cassini, A., Colzani, E., Pini, A., Mangen, M.-J.J., Plass, D., McDonald, S.A., Maringhini, G., Lier, A. van, Haagsma, J.A., Havelaar, A.H., Kramarz, P., Kretzschmar, M.E., Consortium, on behalf of the Bc., (2018). Impact of infectious diseases on population health using incidence-based disability-adjusted life years (DALYs): results from the Burden of Communicable Diseases in Europe study, European Union and European Economic Area countries, 2009 to 2013. *Eurosurveillance* 23, 17. https://doi.org/10.2807/1560-7917.ES.2018.23.16.17-00454

CDC, (2023). CDC: *Listeria* Outbreak Linked to Deli Meat and Cheese [WWW Document]. *Cent. Dis. Control Prev.* URL https://www.cdc.gov/listeria/outbreaks/deli-11-22/index.html (accessed 5.18.24).

Chan, Y.C., Wiedmann, M., (2008). Physiology and Genetics of *Listeria Monocytogenes* Survival and Growth at Cold Temperatures. *Crit. Rev. Food Sci. Nutr.* 49, 237–253. https://doi.org/10.1080/10408390701856272

Chaturongakul, S., Raengpradub, S., Wiedmann, M., Boor, K.J., (2008). Modulation of stress and virulence in *Listeria monocytogenes*. *Trends Microbiol.* 16, 388–396. https://doi.org/10.1016/j.tim.2008.05.006

Chikhi, L., Mancier, M., Brugère, H., Lombard, B., Faouzi, L., Guillier, L., Gnanou Besse, N., (2024). Comparison of *Listeria monocytogenes* alternative detection methods for food microbiology official controls in Europe. I*nt. J. Food Microbiol.* 408, 110448. https://doi.org/10.1016/j.ijfoodmicro.2023.110448

Clemente-Carazo, M., Cebrián, G., Garre, A., Palop, A., (2020). Variability in the heat resistance of Listeria monocytogenes under dynamic conditions can be more relevant than that evidenced by isothermal treatments. *Food Res. Int.* 137, 109538. https://doi.org/10.1016/j.foodres.2020.109538

Cossu, F., Spanu, C., Deidda, S., Mura, E., Casti, D., Pala, C., Lamon, S., Spanu, V., Ibba, M., Marrocu, E., Scarano, C., Piana, A., De Santis, E.P.L., (2016). *Listeria* Spp. and *Listeria Monocytogenes* Contamination in Ready-To-Eat Sandwiches Collected from Vending Machines. *Ital. J. Food Saf.* 5, 5500. https://doi.org/10.4081/ijfs.2016.5500

Dalgaard, P., Koutsoumanis, K., (2001). Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models. *J. Microbiol. Methods* 43, 183–196. https://doi.org/10.1016/s0167-7012(00)00219-0

Dawan, J., Ahn, J., (2022). Bacterial Stress Responses as Potential Targets in Overcoming Antibiotic Resistance. *Microorganisms* 10, 1385. https://doi.org/10.3390/microorganisms10071385

Dawoud, T.M., Davis, M.L., Park, S.H., Kim, S.A., Kwon, Y.M., Jarvis, N., O'Bryan, C.A., Shi, Z., Crandall, P.G., Ricke, S.C., (2017). The Potential Link between Thermal Resistance and

Virulence in *Salmonella*: A Review. *Front. Vet. Sci.* 4, 93. https://doi.org/10.3389/fvets.2017.00093

De Angelis, M., Gobbetti, M., (2004). Environmental stress responses in *Lactobacillus*: A review. *PROTEOMICS* 4, 106–122. https://doi.org/10.1002/pmic.200300497

De Noordhout, C.M., Devleesschauwer, B., Angulo, F.J., Verbeke, G., Haagsma, J., Kirk, M., Havelaar, A., Speybroeck, N., (2014). The global burden of listeriosis: a systematic review and meta-analysis. *Lancet Infect. Dis.* 14, 1073–1082. https://doi.org/10.1016/S1473-3099(14)70870-9

de Oliveira, C.A.F., da Cruz, A.G., Tavolaro, P., Corassin, C.H., (2016). Chapter 10 - Food Safety: Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), Hazard Analysis and Critical Control Point (HACCP), in: Barros-Velázquez, J. (Ed.), Antimicrobial Food Packaging. Academic Press, San Diego, pp. 129–139. https://doi.org/10.1016/B978-0-12-800723-5.00010-3

Disanto, C., Celano, G., Dambrosio, A., Quaglia, N.C., Bozzo, G., Tritto, A., Celano, G.V., (2021). Food safety in collective catering: knowledge, attitudes and correct application of GHP/GMP knowledge among foodservice workers. *Ital. J. Food Saf.* 9, 8453. https://doi.org/10.4081/ijfs.2020.8453

Dlusskaya, E.A., McMullen, L.M., Gänzle, M.G., (2011). Characterization of an extremely heat-resistant *Escherichia coli* obtained from a beef processing facility. *J. Appl. Microbiol.* 110, 840–849. https://doi.org/10.1111/j.1365-2672.2011.04943.x

Doyle, M.E., Mazzotta, A.S., Wang, T., Wiseman, D.W., Scott, V.N., (2001). Heat resistance of *Listeria monocytogenes*. *J. Food Prot.* 64, 410–429. https://doi.org/10.4315/0362-028x-64.3.410

Duze, S.T., Marimani, M., Patel, M., (2021). Tolerance of *Listeria monocytogenes* to biocides used in food processing environments. *Food Microbiol.* 97, 103758. https://doi.org/10.1016/j.fm.2021.103758

ECDC and EFSA, (2024). Rapid Outbreak Assessment: Prolonged multi-country outbreak of *Listeria monocytogenes* ST1607 linked to smoked salmon products [WWW Document]. URL https://www.ecdc.europa.eu/en/publications-data/rapid-outbreak-assessment-prolonged-multi-country-outbreak-listeria-monocytogenes (accessed 5.18.24).

ECDC and EFSA (European Centre for Disease Prevention and Control and European Food Safety Authority)., (2023). The European Union One Health 2022 Zoonoses Report. *EFSA J.* 21, e8442. https://doi.org/10.2903/j.efsa.2023.8442

EFSA and ECDC, (2018). Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update. *EFSA Support. Publ.* 15, 1448E. https://doi.org/10.2903/sp.efsa.2018.EN-1448

EFSA, (2024). The European Union One Health 2023 Zoonoses report. EFSA Journal. 2024;22:e9106. https://doi.org/10.2903/j.efsa.2024.9106

EFSA, L. updated, (2022). *Listeria monocytogenes* story map [WWW Document]. Story Map List. Monocytogenes. URL https://storymaps.arcgis.com/stories/629e6627e6c64111bfd5b9257473c74a (accessed 5.18.24).

Egan, M.B., Raats, M.M., Grubb, S.M., Eves, A., Lumbers, M.L., Dean, M.S., Adams, M.R., (2007). A review of food safety and food hygiene training studies in the commercial sector. *Food Control* 18, 1180–1190. https://doi.org/10.1016/j.foodcont.2006.08.001

Engelhardt, T., Szakmár, K., Kiskó, G., Mohácsi-Farkas, C., Reichart, O., (2018). Combined effect of NaCl and low temperature on antilisterial bacteriocin production of *Lactobacillus plantarum* ST202Ch. *LWT* 89, 104–109. https://doi.org/10.1016/j.lwt.2017.10.027

Engstrom, S.K., Cheng, C., Seman, D., Glass, K.A., (2020). Growth of *Listeria monocytogenes* in a Model High-Moisture Cheese on the Basis of pH, Moisture, and Acid Type. *J. Food Prot.* 83, 1335–1344. https://doi.org/10.4315/JFP-20-069

Erdősi, O., Szakmar, K., Oliver, R., Szekely Kormoczy, P., Laczay, P., (2012). Application of the redox potential measurement based rapid method in the microbial hygienic control. *Acta Aliment.* 41, 45–55. https://doi.org/10.1556/AAlim.2011.0005

Erdősi, O., Szakmár, K., Reichart, O., Szili, Z., László, N., Székely Körmöczy, P., Laczay, P., 2014a). Rapid detection of *Listeria monocytogenes* in raw milk and soft cheese by a redox potential measurement based method combined with real-time PCR. *Acta Vet. Hung.* 62, 304–316. https://doi.org/10.1556/AVet.2014.013

Erdősi, O., Szakmár, K., Reichart, O., Szili, Zs., László, N., Balogh, Z., Székely Körmöczy, P., Laczay, P., (2014b). Rapid detection of *Salmonella* in Food by redox-potential measurement based method combined with real-time PCR. *Acta Aliment.* 43, 660–667. https://doi.org/10.1556/AAlim.43.2014.4.16

Espinosa, M.F., Sancho, A.N., Mendoza, L.M., Mota, C.R., Verbyla, M.E., (2020). Systematic review and meta-analysis of time-temperature pathogen inactivation. *Int. J. Hyg. Environ. Health* 230, 113595. https://doi.org/10.1016/j.ijheh.2020.113595

European Commission, (2007). Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs [WWW Document]. URL https://eur-lex.europa.eu/eli/reg/2007/1441/oj/eng (accessed 1.22.25).

European Commission, (2005). Regulation of the European Commission of 15 November 2005 on microbiological criteria for foodstuffs, 2073/2005/EC.

Fagerlund, A., Langsrud, S., Møretrø, T., (2021). Microbial diversity and ecology of biofilms in food industry environments associated with *Listeria monocytogenes* persistence. *Curr. Opin. Food Sci.* 37, 171–178. https://doi.org/10.1016/j.cofs.2020.10.015

Fang, T., Wu, Y., Xie, Y., Sun, L., Qin, X., Liu, Y., Li, H., Dong, Q., Wang, X., (2021). Inactivation and Subsequent Growth Kinetics of *Listeria monocytogenes* After Various Mild Bactericidal Treatments. *Front. Microbiol*. 12. https://doi.org/10.3389/fmicb.2021.646735

Farber, J.M., Brown, B.E., (1990). Effect of prior heat shock on heat resistance of *Listeria monocytogenes* in meat. *Appl. Environ. Microbiol.* 56, 1584–1587. https://doi.org/10.1128/aem.56.6.1584-1587.1990

FDA and CDC, (2024). Outbreak Investigation of *Listeria monocytogenes*: Queso Fresco and Cotija Cheese (February 2024). FDA.

Fernández-Segovia, I., Pérez-Llácer, A., Peidro, B., Fuentes, A., (2014). Implementation of a food safety management system according to ISO 22000 in the food supplement industry: A case study. *Food Control* 43, 28–34. https://doi.org/10.1016/j.foodcont.2014.02.042

Ferreira, V., Wiedmann, M., Teixeira, P., Stasiewicz, M.J., (2014). *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *J. Food Prot.* 77, 150–170. https://doi.org/10.4315/0362-028X.JFP-13-150

Gandhi, M., Chikindas, M.L., (2007). *Listeria*: A foodborne pathogen that knows how to survive. *Int. J. Food Microbiol.* 113, 1–15. https://doi.org/10.1016/j.ijfoodmicro.2006.07.008

Garre, A., Fernández, P.S., Lindqvist, R., Egea, J.A., (2017). Bioinactivation: Software for modelling dynamic microbial inactivation. *Food Res. Int. Ott. Ont* 93, 66–74. https://doi.org/10.1016/j.foodres.2017.01.012

Garre, A., González-Tejedor, G.A., Aznar, A., Fernández, P.S., Egea, J.A., (2019). Mathematical modelling of the stress resistance induced in *Listeria monocytogenes* during dynamic, mild heat treatments. *Food Microbiol. 84*, 103238. https://doi.org/10.1016/j.fm.2019.06.002

Geeraerd, A.H., Herremans, C.H., Van Impe, J.F., 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. Int. J. Food Microbiol. 59, 185–209. https://doi.org/10.1016/S0168-1605(00)00362-7

Geeraerd, A.H., Valdramidis, V.P., Van Impe, J.F., (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *Int. J. Food Microbiol.* 102, 95–105. https://doi.org/10.1016/j.ijfoodmicro.2004.11.038

Gil, M.M., Miller, F.A., Brandão, T.R.S., Silva, C.L.M., (2017). Mathematical Models for Prediction of Temperature Effects on Kinetic Parameters of Microorganisms' Inactivation: Tools for Model Comparison and Adequacy in Data Fitting. *Food Bioprocess Technol.* 10, 2208–2225. https://doi.org/10.1007/s11947-017-1989-x

Godic Torkar, K., Kirbiš, A., Vadnjal, S., Biasizzo, M., Galicic, A., Jevšnik, M., (2017). The microbiological quality of Slovenian raw milk from vending machines and their hygienic-technical conditions. *Br. Food J.* 119, 377–389. https://doi.org/10.1108/BFJ-06-2016-0291

Goncharuk, V.V., Bagrii, V.A., Mel'nik, L.A., Chebotareva, R.D., Bashtan, S.Yu., (2010). The use of redox potential in water treatment processes. *J. Water Chem.* Technol. 32, 1–9. https://doi.org/10.3103/S1063455X10010017

Guillier, L., Pardon, P., Augustin, J.-C., (2005). Influence of stress on individual lag time distributions of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 71, 2940–2948. https://doi.org/10.1128/AEM.71.6.2940-2948.2005

Gurtler, J.B., Fan, X., Jin, T., Niemira, B.A., (2019). Influence of Antimicrobial Agents on the Thermal Sensitivity of Foodborne Pathogens: A Review. *J. Food Prot.* 82, 628–644. https://doi.org/10.4315/0362-028X.JFP-18-441

Haaber, J., Cohn, M.T., Petersen, A., Ingmer, H., (2016). Simple method for correct enumeration of *Staphylococcus aureus*. *J. Microbiol. Methods* 125, 58–63. https://doi.org/10.1016/j.mimet.2016.04.004

Hall, A., Short, K., Saltmarsh, M., Fielding, L., Peters, A., (2007). Development of a microbial population within a hot-drinks vending machine and the microbial load of vended hot chocolate drink. *J. Food Sci.* 72, M263-266. https://doi.org/10.1111/j.1750-3841.2007.00473.x

Hassani, M., Álvarez, I., Raso, J., Condón, S., Pagán, R., (2005). Comparing predicting models for heat inactivation of *Listeria monocytogenes* and *Pseudomonas aeruginosa* at different pH. *Int. J. Food Microbiol.*, The Fourth International Conference on Predictive Modelling in Foods 100, 213–222. https://doi.org/10.1016/j.ijfoodmicro.2004.10.017

Haykir, O., Mohácsi-Farkas, C., Engelhardt, T., (2022). Enhanced heat resistance of *Listeria innocua* as a surrogate of *Listeria monocytogenes* after sublethal heat treatment. *Acta Aliment.* 51, 241–248. https://doi.org/10.1556/066.2022.00013

Haykir, O., Forrez, R., Mathijssen, G., Vanderborght, B., (2023). Food safety and quality assessment of an automated vending machine for smoothies – A case study. https://doi.org/10.1556/446.2023.00077

Haykir, O., Tőzsér, D., Mohácsi-Farkas, C., Engelhardt, T., (2025). Studying heat stress adaptation of Listeria spp. under dynamic conditions by redox potential measurement-based method. *LWT*. 117543. https://doi.org/10.1016/j.lwt.2025.117543

Hazan, R., Que, Y.-A., Maura, D., Rahme, L.G., (2012). A method for high throughput determination of viable bacteria cell counts in 96-well plates. *BMC Microbiol.* 12, 259. https://doi.org/10.1186/1471-2180-12-259

Huang, L., (2009). Thermal inactivation of *Listeria monocytogenes* in ground beef under isothermal and dynamic temperature conditions. *J. Food Eng.* 90, 380–387. https://doi.org/10.1016/j.jfoodeng.2008.07.011

Hunter, P.R., (1992). Bacteriological, hygienic, and public health aspects of food and drink from vending machines. *Crit. Rev. Environ. Control* 22, 151–167. https://doi.org/10.1080/10643389209388434

ISO 6579-1, 2017. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of Salmonella spp. [WWW Document]. ISO. URL https://www.iso.org/standard/56712.html (accessed 7.22.24).

ISO 11290-1, 2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method [WWW Document]. ISO. URL https://www.iso.org/standard/60313.html (accessed 4.28.23).

ISO 11290-2, 2017. ISO 11290-2:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 2: Enumeration method [WWW Document]. ISO. URL https://www.iso.org/standard/60314.html (accessed 5.31.24).

ISO 11290-2:1998, 2004. ISO 11290-2:1998/Amd 1:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of *Listeria monocytogenes* — Part 2: Enumeration method — Amendment 1: Modification of the enumeration medium [WWW Document]. ISO. URL https://www.iso.org/standard/36146.html (accessed 1.22.25).

ISO 16649-1, 2018. ISO 16649-1:2018 Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* — Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide [WWW Document]. ISO. URL https://www.iso.org/standard/64951.html (accessed 7.22.24).

ISO 16649-2, 2001. ISO 16649-2:2001 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide [WWW Document]. ISO. URL https://www.iso.org/standard/29824.html (accessed 7.22.24).

Jadhav, S., Gulati, V., Fox, E.M., Karpe, A., Beale, D.J., Sevior, D., Bhave, M., Palombo, E.A., (2015). Rapid identification and source-tracking of *Listeria monocytogenes* using MALDI-TOF mass spectrometry. *Int. J. Food Microbiol.* 202, 1–9. https://doi.org/10.1016/j.ijfoodmicro.2015.01.023

Jamshidi, A., Zeinali, T., (2019). Significance and Characteristics of *Listeria monocytogenes* in Poultry Products. *Int. J. Food Sci.* 2019, 7835253. https://doi.org/10.1155/2019/7835253

Jánosity, A., Vajna, B., Kiskó, G., Baranyi, J., (2022). Distribution of bacterial single cell parameters and their estimation from turbidity detection times. *Food Microbiol.* 104, 103972. https://doi.org/10.1016/j.fm.2021.103972

Jasson, V., Jacxsens, L., Luning, P., Rajkovic, A., Uyttendaele, M., (2010). Alternative microbial methods: An overview and selection criteria. *Food Microbiol.* 27, 710–730. https://doi.org/10.1016/j.fm.2010.04.008

Jombart, T., (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinforma. Oxf. Engl.* 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jorgensen, F., Hansen, T.B., Knochel, S., (1999). Heat shock-induced thermotolerance in *Listeria monocytogenes* 13-249 is dependent on growth phase, pH and lactic acid. *Food Microbiol.*

Juneja, V.K., Huang, L., Yan, X., (2011). Thermal inactivation of foodborne pathogens and the USDA pathogen modeling program. *J. Therm. Anal. Calorim.* 106, 191–198. https://doi.org/10.1007/s10973-011-1453-5

Karaman, A.D., Cobanoglu, F., Tunalioglu, R., Ova, G., (2012). Barriers and benefits of the implementation of food safety management systems among the Turkish dairy industry: A case study. *Food Control 25*, 732–739. https://doi.org/10.1016/j.foodcont.2011.11.041

Kesisoglou, I., Tam, V.H., Tomaras, A.P., Nikolaou, M., (2022). Discerning in vitro pharmacodynamics from OD measurements: A model-based approach. *Comput. Chem. Eng.* 158, 107617. https://doi.org/10.1016/j.compchemeng.2021.107617

Krahulcová, M., Barbora Micajová, Petra Olejníková, Klára Cverenkárová, Lucia Bírošová, (2021). *Foods* | Free Full-Text | Microbial Safety of Smoothie Drinks from Fresh Bars Collected in Slovakia [WWW Document]. URL https://www.mdpi.com/2304-8158/10/3/551 (accessed 4.28.23).

Law, J.W.-F., Ab Mutalib, N.-S., Chan, K.-G., Lee, L.-H., (2015). An insight into the isolation, enumeration, and molecular detection of *Listeria monocytogenes* in food. *Front. Microbiol.* 6. https://doi.org/10.3389/fmicb.2015.01227

Lee, Y.-J., Pan, Y.-C., Chang, C.-W., Lu, K.-H., (2023). Thermal inactivation kinetics of uropathogenic *Escherichia coli* in *sous-vide* processed chicken breast. *Food Res. Int.* 164, 112316. https://doi.org/10.1016/j.foodres.2022.112316

Leistner, L., (2000). Basic aspects of food preservation by hurdle technology. *Int. J. Food Microbiol.* 55, 181–186. https://doi.org/10.1016/S0168-1605(00)00161-6

Li, H., Gänzle, M., (2016). Some Like It Hot: Heat Resistance of *Escherichia coli* in Food. *Front. Microbiol.* 7. https://doi.org/10.3389/fmicb.2016.01763

Lianou, A., Koutsoumanis, K.P., (2013). Strain variability of the behavior of foodborne bacterial pathogens: a review. *Int. J. Food Microbiol.* 167, 310–321. https://doi.org/10.1016/j.ijfoodmicro.2013.09.016

Lianou, A., Sofos, J.N., (2007). A review of the incidence and transmission of *Listeria monocytogenes* in ready-to-eat products in retail and food service environments. *J. Food Prot.* 70, 2172–2198. https://doi.org/10.4315/0362-028x-70.9.2172

Lin, Y.-H., Chien, W.-S., Duan, K.-J., Chang, P.R., (2011). Effect of aeration timing and interval during very-high-gravity ethanol fermentation. *Process Biochem.* 46, 1025–1028. https://doi.org/10.1016/j.procbio.2011.01.003

Liu, C.-G., Wang, N., Lin, Y.-H., Bai, F.-W., (2012). Very high gravity ethanol fermentation by flocculating yeast under redox potential-controlled conditions. *Biotechnol. Biofuels* 5, 61. https://doi.org/10.1186/1754-6834-5-61

Liu, Y., Dong, Q., Wang, X., Liu, B., Yuan, S., (2021). Analysis and probabilistic simulation of *Listeria monocytogenes* inactivation in cooked beef during unsteady heating. *Int. J. Food Sci.* Technol. 56, 2282–2290. https://doi.org/10.1111/ijfs.14849

Lou, Y., Yousef, A.E., (1997). Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. *Appl. Environ. Microbiol.* 63, 1252–1255. https://doi.org/10.1128/aem.63.4.1252-1255.1997

Mafart, P., Couvert, O., Gaillard, S., Leguerinel, I., (2002). On calculating sterility in thermal preservation methods: Application of the Weibull frequency distribution model. *Int. J. Food Microbiol.* 72, 107–113. https://doi.org/10.1016/S0168-1605(01)00624-9

Matthews, M.A., Horacek, T.M., (2015). Vending machine assessment methodology. A systematic review. *Appetite* 90, 176–186. https://doi.org/10.1016/j.appet.2015.03.007

Mazaheri, T., Cervantes-Huamán, B.R.H., Bermúdez-Capdevila, M., Ripolles-Avila, C., Rodríguez-Jerez, J.J., (2021). *Listeria monocytogenes* Biofilms in the Food Industry: Is the Current Hygiene Program Sufficient to Combat the Persistence of the Pathogen? *Microorganisms* 9, 181. https://doi.org/10.3390/microorganisms9010181

McCann, M.S., McDowell, D.A., Sheridan, J.J., (2009). Effects of reduction in beef surface water activity on the survival of *Salmonella Typhimurium* DT104 during heating. *J. Appl. Microbiol.* 106, 1901–1907. https://doi.org/10.1111/j.1365-2672.2009.04154.x

McGoverin, C., Steed, C., Esan, A., Robertson, J., Swift, S., Vanholsbeeck, F., (2021). Optical methods for bacterial detection and characterization. *APL Photonics* 6, 080903. https://doi.org/10.1063/5.0057787

Meloni, D., (2019). High-Hydrostatic-Pressure (HHP) Processing Technology as a Novel Control Method for *Listeria monocytogenes* Occurrence in Mediterranean-Style Dry-Fermented Sausages. *Foods Basel Switz.* 8, 672. https://doi.org/10.3390/foods8120672

Métris, A., George, S.M., Peck, M.W., Baranyi, J., (2003). Distribution of turbidity detection times produced by single cell-generated bacterial populations. *J. Microbiol. Methods* 55, 821–827. https://doi.org/10.1016/j.mimet.2003.08.006

Miller, F.A., Ramos, B.F., Gil, M.M., Brandão, T.R.S., Teixeira, P., Silva, C.L.M., (2011). Heat inactivation of *Listeria innocua* in broth and food products under non-isothermal conditions. *Food Control* 22, 20–26. https://doi.org/10.1016/j.foodcont.2010.06.004

Mira, P., Yeh, P., Hall, B.G., (2022). Estimating microbial population data from optical density. *PLoS ONE* 17, e0276040. https://doi.org/10.1371/journal.pone.0276040

Moerman, F., Rizoulières, P., Majoor, F.A., (2013). Cleaning in place (CIP) in food processing. *Hyg. Food Process. Princ. Pract.* Second Ed. 305–383. https://doi.org/10.1533/9780857098634.3.305

Moorhead, S.M., Dykes, G.A., (2003). The Role of the sigB Gene in the General Stress Response of *Listeria monocytogenes* Varies between a Strain of Serotype 1/2a and a Strain of Serotype 4c. *Curr. Microbiol.* 46, 0461–0466. https://doi.org/10.1007/s00284-002-3867-6

Myers, J.A., Curtis, B.S., Curtis, W.R., (2013). Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC Biophys.* 6, 4. https://doi.org/10.1186/2046-1682-6-4

Mytilinaios, I., Bernigaud, I., Belot, V., Lambert, R.J.W., (2015). Microbial growth parameters obtained from the analysis of time to detection data using a novel rearrangement of the Baranyi-Roberts model. *J. Appl. Microbiol.* 118, 161–174. https://doi.org/10.1111/jam.12695

Mytilinaios, I., Salih, M., Schofield, H.K., Lambert, R.J.W., (2012). Growth curve prediction from optical density data. *Int. J. Food Microbiol.* 154, 169–176. https://doi.org/10.1016/j.ijfoodmicro.2011.12.035

Nelms, P.K., Larson, O., Barnes-Josiah, D., (1997). Time to *B. cereus* about hot chocolate. *Public Health Rep.* 112, 240–244.

Nelson, J., Coleman, R., Chacón-Rodríguez, L., Runnebaum, R., Boulton, R., Knoesen, A., (2023). Advanced Monitoring and Control of Redox Potential in Wine Fermentation across Scales. *Fermentation* 9, 7. https://doi.org/10.3390/fermentation9010007

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., der Giessen, J.V., Kruse, H., (2010). Food-borne diseases - The challenges of 20years ago still persist while new ones continue to emerge. *Int. J. Food Microbiol.* 139, S3–S15. https://doi.org/10.1016/j.ijfoodmicro.2010.01.021

Nyhan, L., Johnson, N., Begley, M., O'Leary, P., Callanan, M., (2020). Comparison of predicted and impedance determined growth of *Listeria innocua* in complex food matrices. *Food Microbiol.* 87, 103381. https://doi.org/10.1016/j.fm.2019.103381

O'Bryan, C.A., Crandall, P.G., Martin, E.M., Griffis, C.L., Johnson, M.G., (2006). Heat Resistance of *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* 0157:H7, and *Listeria innocua* M1,

a Potential Surrogate for *Listeria monocytogenes*, in Meat and Poultry: A Review. *J. Food Sci.* 71, R23–R30. https://doi.org/10.1111/j.1365-2621.2006.tb15639.x

O'Driscoll, B., Gahan, C.G., Hill, C., (1996). Adaptive acid tolerance response in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. *Appl. Environ. Microbiol.* 62, 1693–1698. https://doi.org/10.1128/aem.62.5.1693-1698.1996

Orsi, R.H., Wiedmann, M., (2016). Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. *Appl. Microbiol. Biotechnol.* 100, 5273–5287. https://doi.org/10.1007/s00253-016-7552-2

Osek, J., Lachtara, B., Wieczorek, K., (2022). *Listeria monocytogenes* – How This Pathogen Survives in Food-Production Environments? *Front. Microbiol.* 13, 866462. https://doi.org/10.3389/fmicb.2022.866462

Pang, X., Wong, C., Chung, H.-J., Yuk, H.-G., (2019). Biofilm formation of *Listeria monocytogenes* and its resistance to quaternary ammonium compounds in a simulated salmon processing environment. *Food Control* 98, 200–208. https://doi.org/10.1016/j.foodcont.2018.11.029

Patterson, M.F., Mackle, A., Linton, M., (2011). Effect of high pressure, in combination with antilisterial agents, on the growth of *Listeria monocytogenes* during extended storage of cooked chicken. *Food Microbiol.* 28, 1505–1508. https://doi.org/10.1016/j.fm.2011.08.006

Peleg, M., (2021). The Thermal Death Time Concept and Its Implications Revisited. *Food Eng. Rev.* 13, 291–303. https://doi.org/10.1007/s12393-021-09279-8

Peleg, M., Cole, M.B., (1998). Reinterpretation of microbial survival curves. *Crit. Rev. Food Sci. Nutr.* 38, 353–380. https://doi.org/10.1080/10408699891274246

Peñalver-Soto, J.L., Garre, A., Esnoz, A., Fernández, P.S., Egea, J.A., (2019). Guidelines for the design of (optimal) isothermal inactivation experiments. *Food Res. Int.* 126, 108714. https://doi.org/10.1016/j.foodres.2019.108714

Pérez-Baltar, A., Alía, A., Rodríguez, A., Córdoba, J.J., Medina, M., Montiel, R., (2020). Impact of Water Activity on the Inactivation and Gene Expression of *Listeria monocytogenes* during Refrigerated Storage of Pressurized Dry-Cured Ham. *Foods* 9, 1092. https://doi.org/10.3390/foods9081092

Pla, M.-L., Oltra, S., Esteban, M.-D., Andreu, S., Palop, A., (2015). Comparison of Primary Models to Predict Microbial Growth by the Plate Count and Absorbance Methods. *BioMed Res. Int.* 2015, 365025. https://doi.org/10.1155/2015/365025

Pratap Singh, A., Yen, P.P.-L., Ramaswamy, H.S., Singh, A., (2018). Recent advances in agitation thermal processing. Curr. Opin. Food Sci., Food Engineering & Processing part 1 * Food Engineering & Processing part 2 * *Food Mycology* 23, 90–96. https://doi.org/10.1016/j.cofs.2018.07.001

R Core Team, (2024). R: The R Project for Statistical Computing [WWW Document]. URL https://www.r-project.org/ (accessed 3.20.24).

Raposo, A., Carrascosa, C., Pérez, E., Saavedra, P., Sanjuán, E., Millán, R., (2015). Vending machines: Food safety and quality assessment focused on food handlers and the variables involved in the industry. *Food Control* 56, 177–185. https://doi.org/10.1016/j.foodcont.2015.01.052

Reichart, O., Szakmár, K., Jozwiak, A., Felföldi, J., Baranyai, L., (2007). Redox potential measurement as a rapid method for microbiological testing and its validation for coliform determination. *Int. J. Food Microbiol.* 114, 143–148. https://doi.org/10.1016/j.ijfoodmicro.2006.08.016

Ricci, A., Alinovi, M., Martelli, F., Bernini, V., Garofalo, A., Perna, G., Neviani, E., Mucchetti, G., (2021). Heat Resistance of *Listeria monocytogenes* in Dairy Matrices Involved in Mozzarella di Bufala Campana PDO Cheese. *Front. Microbiol.* 11. https://doi.org/10.3389/fmicb.2020.581934

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P.S., Girones, R., Herman, L., Koutsoumanis, K., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Takkinen, J., Wagner, M., Arcella, D., Da Silva Felicio, M.T., Georgiadis, M., Messens, W., Lindqvist, R., (2018). *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. *EFSA J.* 16, e05134. https://doi.org/10.2903/j.efsa.2018.5134

Ripolles-Avila, C., Martínez-Garcia, M., Capellas, M., Yuste, J., Fung, D.Y.C., Rodríguez-Jerez, J.-J., (2020). From hazard analysis to risk control using rapid methods in microbiology: A practical approach for the food industry. *Compr. Rev. Food Sci. Food Saf.* 19, 1877–1907. https://doi.org/10.1111/1541-4337.12592

Rohde, A., Hammerl, J.A., Boone, I., Jansen, W., Fohler, S., Klein, G., Dieckmann, R., Al Dahouk, S., (2017). Overview of validated alternative methods for the detection of foodborne bacterial pathogens. *Trends Food Sci. Technol.* 62, 113–118. https://doi.org/10.1016/j.tifs.2017.02.006

Ruiz, L., Aertsen, A., Nguyen-The, C., Gänzle, M.G., Alvarez-Ordóñez, A., (2017). Editorial: Industrial and Host Associated Stress Responses in Food Microbes. Implications for Food Technology and Food Safety. *Front. Microbiol.* 8. https://doi.org/10.3389/fmicb.2017.01522

Saltmarsh, M.E., (2023). Safety of Food in Vending Machines, in: *Reference Module in Food Science.* Elsevier. https://doi.org/10.1016/B978-0-12-822521-9.00101-5

Saunders, L.P., Sen, S., Wilkinson, B.J., Gatto, C., (2016). Insights into the Mechanism of Homeoviscous Adaptation to Low Temperature in Branched-Chain Fatty Acid-Containing Bacteria through Modeling FabH Kinetics from the Foodborne Pathogen *Listeria monocytogenes*. *Front. Microbiol.* 7. https://doi.org/10.3389/fmicb.2016.01386

Schimel, J., Balser, T.C., Wallenstein, M., (2007). Microbial Stress-Response Physiology and Its Implications for Ecosystem Function. *Ecology* 88, 1386–1394. https://doi.org/10.1890/06-0219

Schirmer, B.C.T., Heir, E., Lindstedt, B.-A., Møretrø, T., Langsrud, S., (2014). Use of used vs. fresh cheese brines and the effect of pH and salt concentration on the survival of *Listeria monocytogenes*. *J. Dairy Res.* 81, 113–119. https://doi.org/10.1017/S0022029913000666

Schlech, W.F., (2019). Epidemiology and Clinical Manifestations of *Listeria monocytogenes* Infection. *Microbiol. Spectr.* 7, 10.1128/microbiolspec.gpp3-0014–2018. https://doi.org/10.1128/microbiolspec.gpp3-0014-2018

Schmid, B., Klumpp, J., Raimann, E., Loessner, M.J., Stephan, R., Tasara, T., (2009). Role of Cold Shock Proteins in Growth of *Listeria monocytogenes* under Cold and Osmotic Stress Conditions. *Appl. Environ. Microbiol.* 75, 1621–1627. https://doi.org/10.1128/AEM.02154-08

Schott, A.-S., Behr, J., Quinn, J., Vogel, R.F., (2016). MALDI-TOF Mass Spectrometry Enables a Comprehensive and Fast Analysis of Dynamics and Qualities of Stress Responses of *Lactobacillus*

paracasei subsp. paracasei F19. *PloS One* 11, e0165504. https://doi.org/10.1371/journal.pone.0165504

Sergelidis, D., Abrahim, A., (2009). Adaptive response of *Listeria monocytogenes* to heat and its impact on food safety. *Food Control* 20, 1–10. https://doi.org/10.1016/j.foodcont.2008.01.006

Shamloo, E., Hosseini, H., Moghadam, A.Z., Larsen, H.M., Haslberger, A., Alebouyeh, M., (2019). Importance of *Listeria monocytogenes* in food safety: A review of its prevalence, detection, and antibiotic resistance. *Iran. J. Vet. Res.* 20, 241–254.

Shen, Q., Jangam, P.M., Soni, K.A., Nannapaneni, R., Schilling, W., Silva, J.L., (2014). Low, medium, and high heat tolerant strains of *Listeria monocytogenes* and increased heat stress resistance after exposure to sublethal heat. *J. Food Prot.* 77, 1298–1307. https://doi.org/10.4315/0362-028X.JFP-13-423

Sibanda, T., Buys, E.M., (2022). *Listeria monocytogenes* Pathogenesis: The Role of Stress Adaptation. *Microorganisms* 10, 1522. https://doi.org/10.3390/microorganisms10081522

Skandamis, P.N., Yoon, Y., Stopforth, J.D., Kendall, P.A., Sofos, J.N., (2008). Heat and acid tolerance of *Listeria monocytogenes* after exposure to single and multiple sublethal stresses. *Food Microbiol.* 25, 294–303. https://doi.org/10.1016/j.fm.2007.10.008

Soni, A., Bremer, P., Brightwell, G., (2022). A Comprehensive Review of Variability in the Thermal Resistance (D-Values) of Food-Borne Pathogens—A Challenge for Thermal Validation Trials. *Foods* 11, 4117. https://doi.org/10.3390/foods11244117

Sprouffske, K., Wagner, A., (2016). Growthcurver: An R package for obtaining interpretable metrics from microbial growth curves. *BMC Bioinformatics* 17. https://doi.org/10.1186/s12859-016-1016-7

Stephens, P.J., Cole, M.B., Jones, M.V., (1994). Effect of heating rate on the thermal inactivation of *Listeria monocytogenes*. *J. Appl. Bacteriol.* 77, 702–708. https://doi.org/10.1111/j.1365-2672.1994.tb02822.x

Stevenson, K., McVey, A.F., Clark, I.B.N., Swain, P.S., Pilizota, T., (2016). General calibration of microbial growth in microplate readers. *Sci. Rep.* 6, 38828. https://doi.org/10.1038/srep38828

Szakmár, K., Reichart, O., Szatmári, I., Erdősi, O., Szili, Z., László, N., Székely Körmöczy, P., Laczay, P., (2014). In vitro study on the effect of doxycycline on the microbial activity of soil determined by redox-potential measuring system. *Acta Microbiol. Immunol. Hung.* 61, 317–328. https://doi.org/10.1556/AMicr.61.2014.3.6

Tarlak, F., (2023). The Use of Predictive Microbiology for the Prediction of the Shelf Life of Food Products. *Foods* 12, 4461. https://doi.org/10.3390/foods12244461

Tasara, T., Stephan, R., (2006). Cold stress tolerance of *Listeria monocytogenes*: A review of molecular adaptive mechanisms and food safety implications. *J. Food Prot.* 69, 1473–1484. https://doi.org/10.4315/0362-028x-69.6.1473

Taylor, B.J., Quinn, A.R., Kataoka, A., (2019). *Listeria monocytogenes* in low-moisture foods and ingredients. *Food Control* 103, 153–160. https://doi.org/10.1016/j.foodcont.2019.04.011

Török, T., Reichart, O., (1983). Thermal inactivation kinetics of sugar-tolerant yeasts. *Eur. J. Appl. Microbiol. Biotechnol.* 17, 191–196. https://doi.org/10.1007/BF00505888

Tsai, H.-C., Taylor, M.H., Song, X., Sheng, L., Tang, J., Zhu, M.-J., (2019). Thermal resistance of *Listeria monocytogenes* in natural unsweetened cocoa powder under different water activity. *Food Control* 102, 22–28. https://doi.org/10.1016/j.foodcont.2019.03.006

Välimaa, A.-L., Tilsala-Timisjärvi, A., Virtanen, E., (2015). Rapid detection and identification methods for *Listeria monocytogenes* in the food chain – A review. *Food Control* 55, 103–114. https://doi.org/10.1016/j.foodcont.2015.02.037

van der Veen, S., Abee, T., (2011). Mixed species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid. *Int. J. Food Microbiol.* 144, 421–431. https://doi.org/10.1016/j.ijfoodmicro.2010.10.029

van der Veen, S., Hain, T., Wouters, J.A., Hossain, H., de Vos, W.M., Abee, T., Chakraborty, T., Wells-Bennik, M.H.J., (2007). The heat-shock response of *Listeria monocytogenes* comprises genes involved in heat shock, cell division, cell wall synthesis, and the SOS response. *Microbiol. Read. Engl.* 153, 3593–3607. https://doi.org/10.1099/mic.0.2007/006361-0

Vasavada, P.C., Lee, A., Betts, R., (2020). Conventional and Novel Rapid Methods for Detection and Enumeration of Microorganisms, in: Demirci, A., Feng, H., Krishnamurthy, K. (Eds.), Food Safety Engineering. Springer International Publishing, Cham, pp. 85–128. https://doi.org/10.1007/978-3-030-42660-6_4

Velasco-Hernández, A., Saucedo-Veloz, C., Ramírez-Guzmán, M.E., Chávez-Franco, S.H., Valle-Guadarrama, S., Saucedo-Reyes, D., (2020). Effect of moderate thermal treatments on the inactivation of a strain of *Listeria monocytogenes* and physicochemical properties of soursop pulp. *Food Sci. Technol. Int.* 26, 535–548. https://doi.org/10.1177/1082013220913357

Verheyen, D., Govaert, M., Seow, T.K., Ruvina, J., Mukherjee, V., Baka, M., Skåra, T., Van Impe, J.F.M., (2020). The Complex Effect of Food Matrix Fat Content on Thermal Inactivation of *Listeria monocytogenes*: Case Study in Emulsion and Gelled Emulsion Model Systems. *Front. Microbiol.* 10. https://doi.org/10.3389/fmicb.2019.03149

Walker, S.J., Archer, P., Banks, J.G., (1990). Growth of *Listeria monocytogenes* at refrigeration temperatures. *J. Appl. Bacteriol.* 68, 157–162. https://doi.org/10.1111/j.1365-2672.1990.tb02561.x

Wang, X., Lahou, E., De Boeck, E., Devlieghere, F., Geeraerd, A., Uyttendaele, M., (2015). Growth and inactivation of *Salmonella enterica* and *Listeria monocytogenes* in broth and validation in ground pork meat during simulated home storage abusive temperature and home pan-frying. *Front. Microbiol.* 6. https://doi.org/10.3389/fmicb.2015.01161

Wang, X., Zheng, J., Luo, L., Hong, Y., Li, X., Zhu, Y., Wu, Y., Bai, L., (2024). Thermal inactivation kinetics of *Listeria monocytogenes* in milk under isothermal and dynamic conditions. *Food Res. Int.* 179, 114010. https://doi.org/10.1016/j.foodres.2024.114010

Wason, S., Verma, T., Wei, X., Mauromoustakos, A., Subbiah, J., (2022). Thermal inactivation kinetics of *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 as a function of temperature and water activity in fine ground black pepper. *Food Res. Int.* 157, 111393. https://doi.org/10.1016/j.foodres.2022.111393

Wawerla, M., Stolle, A., Schalch, B., Eisgruber, H., (1999). Impedance microbiology: applications in food hygiene. *J. Food Prot.* 62, 1488–1496. https://doi.org/10.4315/0362-028x-62.12.1488

Wesche, A.M., Gurtler, J.B., Marks, B.P., Ryser, E.T., (2009). Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. *J. Food Prot.* 72, 1121–1138. https://doi.org/10.4315/0362-028x-72.5.1121

Wiktorczyk-Kapischke, N., Skowron, K., Grudlewska-Buda, K., Wałecka-Zacharska, E., Korkus, J., Gospodarek-Komkowska, E., (2021). Adaptive Response of *Listeria monocytogenes* to the Stress Factors in the Food Processing Environment. *Front. Microbiol.* 12, 710085. https://doi.org/10.3389/fmicb.2021.710085

Wiktorczyk-Kapischke, N., Skowron, K., Wałecka-Zacharska, E., Grudlewska-Buda, K., Wnuk, K., Buszko, K., Gospodarek-Komkowska, E., (2023). Assessment of the influence of selected stress factors on the growth and survival of *Listeria monocytogenes*. *BMC Microbiol.* 23, 27. https://doi.org/10.1186/s12866-023-02766-4

World Health Organization, (2015). WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization, Geneva.

Xuan, X.-T., Ding, T., Li, J., Ahn, J.-H., Zhao, Y., Chen, S.-G., Ye, X.-Q., Liu, D.-H., (2017). Estimation of growth parameters of *Listeria monocytogenes* after sublethal heat and slightly acidic electrolyzed water (SAEW) treatment. *Food Control* 71, 17–25. https://doi.org/10.1016/j.foodcont.2016.06.018

Yakdhane, E., Tőzsér, D., Haykir, O., Yakdhane, A., Labidi, S., Kiskó, G., Baranyai, L., (2024). Recognition of environmental contaminant and pathogenic bacteria by means of redox potential methodology. *MethodsX* 13, 102811. https://doi.org/10.1016/j.mex.2024.102811

Yesil, M., Kasler, D.R., Huang, E., Yousef, A.E., (2024). Thermal Inactivation of Escherichia Phage OSYSP and Host Strain *Escherichia coli* O157:H7 EDL933: A Comparative Kinetic Analysis. *J. Food Prot.* 87, 100215. https://doi.org/10.1016/j.jfp.2023.100215

Zhang, Y., Pérez-Reyes, M.E., Qin, W., Hu, B., Wu, Q., Liu, S., (2022). Modeling the effect of protein and fat on the thermal resistance of *Salmonella enterica* Enteritidis PT 30 in egg powders. *Food Res. Int.* 155, 111098. https://doi.org/10.1016/j.foodres.2022.111098

Ziegler, M., Kent, D., Stephan, R., Guldimann, C., (2019). Growth potential of *Listeria monocytogenes* in twelve different types of RTE salads: Impact of food matrix, storage temperature and storage time. *Int. J. Food Microbiol.* 296, 83–92. https://doi.org/10.1016/j.ijfoodmicro.2019.01.016

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