The Thesis of the PhD Dissertation

Oktay Haykir

Budapest

2025

Hungarian University of Agriculture and Life Sciences

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

DOI: 10.54598/006490

Oktay Haykir

Budapest

2025

Doctoral School

Name: Doctoral School of Food Science

Field: Food Science

Head: Livia Simon Sarkadi, DSc

Institute of Food Science and Technology

Hungarian University of Agriculture and Life Sciences

Supervisors:

Csilla Mohácsi-Farkas, PhD

Department of Food Microbiology, Hygiene and Safety

Institute of Food Science and Technology

Hungarian University of Agriculture and Life Sciences

Tekla Engelhardt, PhD

Institute of Food Chain Science

University of Veterinary Medicine Budapest

The applicant met the requirements of the Ph.D. regulations of the Hungarian University of Agriculture and Life Sciences and the thesis is accepted for the defense process.

.....

Head of the Doctoral School

....

Supervisor Supervisor

INTRODUCTION AND OBJECTIVES

L. monocytogenes is ubiquitous in the environment, meaning it can be found in various sources like soil, water, and animals. 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.

Stress adaptation is a response of microorganisms to stresses caused by different factors, 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 like GInaFiT that allow users to apply their data to various models without requiring a thorough comprehension of the mathematical principles underlying such models.

The primary objective of this 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.

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 *Listeria* spp. and compare with plate counting method;
- to compare different mathematical models of heat inactivation of *L. monocytogenes*.

MATERIALS AND METHODS

Bacterial Strains

Four *Listeria* strains from the collection of the Department of Food Microbiology, Hygiene and Safety, Hungarian University of Agriculture and Life Sciences were used in this study: *L. innocua* T1 (origin not known), *L. monocytogenes* L2 (isolated from a dairy product), *L. monocytogenes* L4 (isolated from cheese), *L. monocytogenes* L7 (isolated from cheese).

Indirect enumeration methods

Redox potential measurement-based method

To establish a standard curve for *Listeria* strains, test tubes containing 9 mL of ½ concentration Tryptic Soy Broth (TSB) 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 Tryptic Soy Agar (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. All parameters were subjected to arithmetic mean computations when working with replicates.

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.

Optical density measurement method

The optical density (OD) measurements were conducted in TSA at 37 °C. 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.

Heat Stress Adaptation of *L. innocua*

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

For sub-lethal heat treatment, 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 °C 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 °C. 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 were serially diluted and 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 were determined 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.

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*. 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.

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

Sub-lethal heat treatment experiments were conducted as previously explained. 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. 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.

Heat stress adaptation of *L. monocytogenes*

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

All three *L. monocytogenes* strains were utilized for heat destruction studies. 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 previously explained. Each experiment was performed in triplicate on three different days.

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

The same sublethal treatment conditions in the previous section were applied to the same strains, but microbial loads were determined using the optical density measurement. Enumeration was determined with OD measurement using the standard curve. D_{60} values were determined and analyzed using one-way ANOVA and Student's t-test for post-hoc analysis, utilizing Microsoft Excel 2021.

Predictive modelling of thermal inactivation parameters with GInaFiT

The isothermal heat destruction data of three *L. monocytogenes* strains, obtained from the plate counting, 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.

Inactivation parameters 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.

RESULTS AND DISCUSSION

Evaluation of Indirect Enumeration Methods

For the redox potential measurement-based method, detection limits were determined from intercepts of standard curves, yielding from 17.18 to 26.85 hours for TTD values corresponding to $\log_{10}N=0$, indicating a single cell. In traditional plate counting, the enumeration process generally takes 48-72 hours (Reichart et al. 2007). Therefore, the method provided a faster quantification of *Listeria* spp. 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 errors for the determinations ranged 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).

For the optical density measurement method, detection limits for a single cell fell from 18.78 to 22.41 hours. The repeatability of the method, evaluated similarly to the redox potential measurement method, showed standard errors of regression analysis ranged from 0.22 to 0.33.

Heat Stress Adaptation of *L. innocua*

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

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 ($P > 0.05$). The outcome of different clusters from the MALDI-TOF MS analysis did not show a comprehensive result, indicating three clusters did not show a meaningful pattern. This verifies our heat destruction findings, showing no significant difference ($P > 0.05$) in D_{60}-values between control and pre-treated samples at 46 °C for 30 minutes. However, a 60-minute sublethal treatment at 46 °C significantly improved the survival at 60 °C ($P < 0.05$).

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

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 ($P < 0.05$).

Z-values were computed for nine treatments, spanning a range of 11.5 to 28.4 °C. 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. These results underscore the significance of enhanced heat resistance in modelling heat destruction parameters, with increased D and z values following prior treatment at milder temperatures.

Heat stress adaptation of *L. monocytogenes*

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

D_{60}-values increased in all three strains when the sublethal heat treatments were applied, compared to the control. The most significant increase in D-values occurred in *L. monocytogenes* L7 samples, which was more than a 6-fold increase obtained in sub-lethally treated samples for 90 minutes compared to control samples. The lowest increase comprised in *L. monocytogenes* L4 samples, which caused a 1.55-fold increase in D-values in samples treated with 90 minutes of sub-lethal heat treatment compared to control samples. The highest increase in D-values took place in *L. monocytogenes* L2 samples when the prior treatment for 60 minutes created a 2-fold increase compared to control samples.

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

When the same experimental conditions were applied as in the previous section, but the enumeration method was changed to OD measurement from the plate counting, some different

results were obtained compared to the previous section. Sub-lethal treatment induced an increase in D_{60}-values across all strains compared to control conditions. *L. monocytogenes* L2 exhibited the highest D-values in each treatment among the three strains, while *L. monocytogenes* L4 consistently displayed the lowest D-values. When I compared the D_{60}-values between the two methods (plate counting and OD measurement) across all treatments, the D_{60}-values of *L. monocytogenes* L7 did not significantly differ between the two methods, while those of *L. monocytogenes* L2 and *L. monocytogenes* 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.

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. To ensure food safety, heat processing procedures must target pathogenic microorganisms in their most heat-resistant state.

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. Three mathematical models, the classical log-linear curve (Bigelow and Esty 1920), the log-linear curve with a shoulder (Geeraerd et al. 2000), and the Weibull model (Mafart et al. 2002) were able to describe the inactivation data. Statistical analysis of Root Mean Square Errors (RMSE values) revealed that no single model consistently outperformed other models across different strains or treatments. Previous research has shown that *L. monocytogenes* cells

exhibited deviations from first-order inactivation kinetics when the cells experienced heat stress (Huang 2009, Garre et al. 2019). 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.

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, different results could be 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.

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.

LIST OF PUBLICATIONS IN THE FIELD OF STUDIES

IF articles or Q1-Q4 articles in the foreign language:

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

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, Paper: 102811 , 6 p. https://doi.org/10.1016/j.mex.2024.102811

Haykir, O., Forrez, R., Mathijssen, G., Vanderborght, B. (2023). Food safety and quality assessment of an automated vending machine for smoothies – A case study. *Progress in Agricultural Engineering Sciences*, 19(S1), 9-15. https://doi.org/10.1556/446.2023.00077

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 Alimentaria*, 51(2), 241-248. https://doi.org/10.1556/066.2022.00013

Presentations at international conferences:

Oktay, Haykir ; Csilla, Mohácsi-Farkas ; Tekla, Engelhardt: Mathematical modelling of the stress adaptation of *Listeria monocytogenes* under isothermal conditions, Oral Presentation, 28th International ICFMH Conference, 8-11 July 2024, Burgos, Spain.

Oktay, Haykir ; Csilla, Mohácsi-Farkas ; Tekla, Engelhardt: Applying redox potential measurement based method in the study of the enhanced heat resistance, Oral Presentation, 4th FoodConf - International Conference on Food Science and Technology, 10-11 June 2022, Budapest, Hungary.

Oktay Haykir; Csilla Mohácsi-Farkas; Tekla Engelhardt: Study of *Listeria Innocua* Heat Resistance after Sublethal Heat Treatment with MALDI-TOF, Poster presentation, IAFP's European Symposium, 4-6 May 2022, Munich, Germany.

REFERENCES

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

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

Berthold, M.R. et al. (2008): KNIME: The Konstanz Information Miner, in C. Preisach et al. (eds) *Data Analysis, Machine Learning and Applications*. Berlin, Heidelberg: Springer, pp. 319–326. Available at: https://doi.org/10.1007/978-3-540-78246-9_38.

Bigelow, W.D. and Esty, J.R. (1920): The Thermal Death Point in Relation to Time of Typical Thermophilic Organisms, *The Journal of Infectious Diseases*, 27(6), pp. 602–617. Available at: https://doi.org/10.1093/infdis/27.6.602.

Garre, A. et al. (2019): Mathematical modelling of the stress resistance induced in *Listeria monocytogenes* during dynamic, mild heat treatments, *Food Microbiology*, 84, p. 103238. Available at: https://doi.org/10.1016/j.fm.2019.06.002.

Geeraerd, A.H., Herremans, C.H. and Van Impe, J.F. (2000): Structural model requirements to describe microbial inactivation during a mild heat treatment, *International Journal of Food Microbiology*, 59(3), pp. 185–209. Available at: https://doi.org/10.1016/S0168-1605(00)00362-7.

Geeraerd, A.H., Valdramidis, V.P. and Van Impe, J.F. (2005): GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves, *International Journal of Food Microbiology*, 102(1), pp. 95–105. Available at: https://doi.org/10.1016/j.ijfoodmicro.2004.11.038.

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. *Progress in Agricultural Engineering Sciences*, 19(S1), 9-15. 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.

Huang, L. (2009): Thermal inactivation of *Listeria monocytogenes* in ground beef under isothermal and dynamic temperature conditions, *Journal of Food Engineering*, 90(3), pp. 380–387. Available at: https://doi.org/10.1016/j.jfoodeng.2008.07.011.

Jombart, T. (2008): adegenet: a R package for the multivariate analysis of genetic markers, *Bioinformatics*, 24(11), pp. 1403–1405. Available at: https://doi.org/10.1093/bioinformatics/btn129.

Mafart, P. et al. (2002): On calculating sterility in thermal preservation methods: Application of the Weibull frequency distribution model, *International Journal of Food Microbiology*, 72(1–2), pp. 107–113. Available at: https://doi.org/10.1016/S0168-1605(01)00624-9.

R Core Team (2024): *R: The R Project for Statistical Computing*. Available at: https://www.r-project.org/ (Accessed: 20 March 2024).

Reichart, O. et al. (2007): Redox potential measurement as a rapid method for microbiological testing and its validation for coliform determination, *International Journal of Food Microbiology*, 114(2), pp. 143–148. Available at: https://doi.org/10.1016/j.ijfoodmicro.2006.08.016.

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

Sprouffske, K. and Wagner, A. (2016): Growthcurver: An R package for obtaining interpretable metrics from microbial growth curves, *BMC Bioinformatics*, 17(1). Available at: https://doi.org/10.1186/s12859-016-1016-7.

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.