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Mariana Pimentel-González , Arícia Possas , Antonio Valero * , Eduardo Sánchez-García , José Rodríguez-Rodríguez , Sandra Castillo *

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Inactivation Kinetics of *Listeria monocytogenes* Applying Mild Temperatures and Fractionated Mexican Oregano Essential Oil (*Poliomintha longiflora* Gray)

Mariana Pimentel-González [1,2], Antonio Valero [2,*], Arícia Possas [2], Eduardo Sánchez-García [1], José Rodríguez-Rodríguez [3] and Sandra Castillo [1,*]

[1] Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Avenida Universidad s/n, Ciudad Universitaria, 66455, San Nicolás de los Garza, Nuevo León, México

[2] Department of Food Science and Technology, UIC Zoonosis y Enfermedades Emergentes (ENZOEM), CeiA3, Universidad de Córdoba, Campus Rabanales, 14014 Córdoba, Spain

[3] Tecnológico de Monterrey. Escuela de Ingeniería y Ciencias. Av. Eugenio Garza Sada 2501 Sur, Tecnológico C.P. 64849, Monterrey, Nuevo León, México

* Correspondence: AV bt2vadia@uco.es; Tel.: +34 665820947; SC sandra.castillohrn@uanl.edu.mx; Tel.: +52 8182877056

Abstract: The susceptibility of meat products to contamination with *Listeria monocytogenes*, foodborne pathogen associated with high mortality among consumers, requires the exploration of novel strategies with the use of hurdle technology. Essential oils have been shown to reduce exposure times to heat treatments, while the concentration of their main antimicrobial compounds impacts food safety by reducing their quantities applied in foods. The aim of this study was to evaluate the effect of the sublethal concentrations of *Poliomintha longiflora* pure oregano essential oil (PEO) and fractionated at 140 °C (FIV) on *L. monocytogenes* inactivation at 52, 54, 57.5 and 63 °C. The Weibull-Mafart model was suitable to describe the observed inactivation data. The FIV group, characterized by a chemical profile of 60.23 % carvacrol and 21.17 % thymol, resulted in δ-values of $\delta_{52\,°C}$ = 0.40, $\delta_{54\,°C}$ = 0.44, $\delta_{57.5°C}$= 0.35 and $\delta_{63°C}$=0.33 min, representing a significant reduction ($P < 0.05$) in comparison to the control group. The z-values were estimated as 5.75, 5.20 and 5.00 °C for control, PEO and FIV, respectively. Therefore, the application of fractionated oregano essential oil in meat products presents a promising alternative to reducing exposure times to heat treatments, effectively lowering the incidence of *L. monocytogenes* contamination while preserving product quality.

Keywords: thermal inactivation; predictive microbiology; foodborne pathogens; food safety; hurdle technology; natural antimicrobials

1. Introduction

Meat products have the necessary nutritional composition for the survival and growth of a wide diversity of foodborne pathogens, making them potentially hazardous [1]. *Listeria monocytogenes*, a gram-positive bacterium found in soil, water, and mammals can cause listeriosis when contaminated foods are consumed [2]. It has been highlighted as one of the food-associated bacteria with the highest mortality [3]. Gastrointestinal symptoms are common in listeriosis, but the infection can spread to the nervous system, leading to severe complications in immunocompromised individuals. In pregnant women, it can result in infections that may cause miscarriage or neonatal sepsis [4].

In the latest report from the European Food Safety Authority (EFSA), it was stated that there was a 5.80 % increase in listeriosis cases in 2023 compared to 2022, with nearly 3,000 reported cases

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in the European Union. Of these, 96.50 % resulted in hospitalizations with a total of 335 deaths [4]. In North America, the Centers for Disease Control and Prevention (CDC) monitor and control foodborne outbreaks, with *L. monocytogenes* being one of the major concerns in the United States, being associated with a wide variety of products, including vegetables [5], ice cream [6,7], cheeses [8], and meat products [9]. The CDC reports that *L. monocytogenes* is responsible for more than 1,500 illnesses and 200 deaths annually in the U.S., with a significant portion of these cases requiring hospitalization [10]. The continued presence of *L. monocytogenes* in food products not only poses a risk to public health in the U.S., but also has potential implications for neighboring countries, such as Mexico, where similar risks are likely associated with cross-border food trade and consumption patterns [11].

It has been reported that *L. monocytogenes* has the ability to persist in food environments and survive against preservation treatments generally applied to inhibit or stop the growth of other foodborne pathogens [12]. These preservation techniques in food processing include the use of salt amounts lower than 10 % [13], acidic pH levels within 4 and 6 [14] and low (< 5 °C) or high temperatures (> 60°C) [1,15]. Long-term exposure to these stress conditions has been shown to enhance the adaptive response of *L. monocytogenes*, leading to alter its lag phase and its growth rate [16,17]. As a result, researchers are increasingly exploring combined treatments to reduce the incidence of this pathogen and its application in food matrices [18]. When implementing thermal treatments, avoiding the misuse of time-temperature parameters has prompted the application of novel sustainable strategies for microbial inactivation in foods. These include the use of natural additives like bacteriocins, organic acids, plant extracts and essential oils (EO) [19–21].

Essential oils are natural substances usually classified as “Generally Recognized as Safe'' (GRAS) that interact with the bacterial membrane changing its permeability, decreasing proton force, and causing coagulation of cytoplasm [22]. Oregano and thyme EOs contain high concentrations of monoterpenes, which have been reported to exhibit antilisterial activity in foods, with significant logarithmic reductions of *L. monocytogenes* observed in pork meat stored at 4 °C [23]. *Origanum vulgare*, a European species of oregano, has been widely explored for its antimicrobial properties against Gram-positive and Gram-negative bacteria with Minimum Inhibitory Concentrations (MIC) ranging from 0.49 to 1.90 mg/mL, and Minimum Bactericidal Concentrations (MBC) ranging from 0.99 to 7.90 mg/mL, where Gram-positive bacteria has been more resistant than negative [24,25]. Sublethal concentrations (SLC) of *O. vulgare* (1.25 µL/mL) at 30 °C can alter the growth parameters of *L. monocytogenes* after 1 h of exposure. This effect may improve the efficacy of antibiotics in medical applications or be combined with other inactivation treatments in the food sector [26]. Thus, the inactivation of *L. monocytogenes* can be achieved through novel strategies using mild concentrations, aligning with the hurdle technology concept [22].

In developing countries, including Mexico, regulations for the surveillance of *L. monocytogenes* in foods and infected consumers are often limited [27]. However, its presence in Mexican vegetables [28], dairy products [29], and meat products [11,30] indicate a possible health risk to the population. The EO and extracts of Mexican oregano, *Lippia graveolens*, are very popular in America due to its antimicrobial activity against *Salmonella* Typhimurium [31,32]. *Poliomintha longiflora* is another Mexican species of oregano but it has been scarcely studied in comparison with other oregano species. The chemical composition of a wild species from northern Mexico revealed an increased concentration of carvacrol and thymol [33]. This composition influences its antimicrobial activity as in *O. vulgare* and *L. graveolens*. By fractionating the pure EO, the concentration of key terpenes such as carvacrol and thymol can be enhanced, leading to increased antilisterial activity [34]. The investigation of natural sources plays a crucial role in understanding their potential in combating pathogens, fostering innovation in the region. This Mexican species represents a valuable and locally sourced resource, offering a promising alternative for food safety interventions [35]. Moreover, it provides an opportunity to develop sustainable, natural antimicrobial solutions that leverage Mexico’s rich biodiversity [36]. However, when applying these natural compounds, it is essential to

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assess SLC to optimize processing treatments. Despite being considered safe, EOs can be toxic to health when used under inappropriate conditions or at excessive concentrations [37].

Previous studies have reported that combining physical treatments such as High-Pressure Processing (HPP), freezing, irradiation, or thermal treatments, with sublethal concentrations of EOs can successfully reduce foodborne pathogens such as *Salmonella* spp., *L. monocytogenes*, *Escherichia coli, Bacillus cereus* and *Campylobacter jejuni* in food matrices [1,38,39]. The use of temperatures below 70 °C with oregano OE (OEO) in low concentrations improves thermal inactivation in food processing [40–42]. The microbial reductions yielded from combining EOs with other technologies can be estimated using predictive models [41]. Predictive models are mathematical equations that help elucidate the effects of intrinsic and extrinsic factors (e.g., temperature, pH, water activity) on the microbial responses in foods and are valuable tools for enabling the assessment of interventions to ensure food safety [43,44]. Among these interventions, the use of *P. longiflora* EO fractions with high concentrations of carvacrol and thymol has garnered attention due to its strong antimicrobial properties [34]. This approach reduces the need for high concentrations of EOs, making it a sustainable and effective option for food safety interventions. Besides, by focusing on local resources like *P. longiflora*, the potential toxic effects and bacterial resistance can be reduced, while promoting sustainable practices.

The aim of this work was to assess the impact of pure and fractionated *P. longiflora* EO (PEO and FIV) on the thermal inactivation of *L. monocytogenes* at mild temperatures (52, 54, 57.5 and 63 °C). This was achieved by determining the pathogen inactivation parameters in an In vitro assay using a simulated meat medium.

2. Materials and Methods

2.1. Bacterial Culture and Growth Conditions

L. monocytogenes PM1, a clinical isolate from the listeriosis outbreak in Andalusia linked to the consumption of ready-to-eat meats in 2019, was provided by the Food Science and Technology Department from the University of Córdoba, Spain. The strain was stored at -80 °C in 20 % v/v glycerol/Brain Heart Infusion broth (BHI; Difco Laboratories, Sparks MD., USA) until use. To obtain working cultures, two successive transfers were performed in BHI broth. Initially, an aliquot of 100 µL of the frozen culture was transferred to a tube containing 10 mL of BHI and incubated at 37 °C for 24 h. After incubation, 0.1 mL of the grown culture was transferred to another 10 mL BHI tube and incubated at the same conditions described above. The inoculum level was then adjusted to an OD 600 = 0.5 (ONDA V-10 Plus spectrophotometer CE) to obtain an initial inoculum of ~10^8 CFU/mL.

2.2. Essential Oil of P. longiflora

P. longiflora EO and its fraction was provided by the School of Engineering and Sciences from Tecnológico de Monterrey (México) and obtained as follows [34]: the plant was collected during the spring-summer period flowering period in northern Mexico and identified as *P. longiflora* by the herbarium of the Faculty of Biological Sciences of the Autonomous University of Nuevo Leon (UANL). *P. longiflora* EO was obtained from the leaves, flowers, and stem (ratio of 90:9:1, respectively) via steam distillation. This oil was referred to as PEO. PEO was treated by fractional distillation at 140 °C to obtain a new concentrated oil identified as FIV. PEO and FIV were characterized by Gas Chromatography (GC) (Perkin Elmer-Clarus 690) coupled to Mass Spectroscopy (MS) analysis (Perkin Elmer-Clarus SQ8T) and characterization results were reported in a previous study performed by the authors [45]. The relative area of each compound was considered as an equivalent of the composition in percentage. The major component was carvacrol with the highest concentration identified in FIV with 60.23 % followed by thymol with 21.17 %. The concentration of carvacrol in PEO was 34.09 % followed by o-Cymene with 21.51 %. However, PEO, showed a greater diversity of compounds in lower concentrations, including eucalyptol, myrcene, terpinene, humulene, and caryophyllene [45].

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2.2.1. Preparation of Oil Working Solutions

Due to the hydrophobicity of the OEO, oil working solutions were prepared according to our previous data [45] for the antimicrobial screening tests. Briefly, a 1 % (v/v) of Polysorbate 80 (Tween 80) solution was prepared and sterilized. Subsequently, 400 µL of PEO and the FIV were separately mixed with 600 µL of the 1 % Tween 80 solution and stirred for 2 min to ensure dilution [46]. The resulting oil working solution was stored in the dark at refrigeration until use.

2.3. Antimicrobial Activity: Minimum Bactericidal Concentration and Sublethal Concentrations.

The MBC was determined using the microdilution method from CLSI [47] with slight modifications. A 96-well microplate (Corning, Costar; Cambridge, MA; USA) was used, with each well filled with 190 µL of BHI broth previously inoculated with L. monocytogenes (1 % v/v; $1x10^6$ CFU/mL). Subsequently, 10 µL of PEO and FIV working solution was added to the first column of wells, followed by 10 µL dilutions across the rows. Each experiment was performed in duplicate, with two replicates per treatment (PEO, FIV, and a control without EO). After incubation at 37 °C for 24 h, 10 µL from each well was transferred to TSA plates using the drip method. The plates were incubated under the same conditions, and the MBC was identified as the lowest concentration of PEO or FIV at which no bacterial growth was observed. To estimate the Sublethal Concentrations (SLC), the same microdilution method used for MBC was used but, in this case, the OEO was diluted to concentrations between the MBC and the Minimum Inhibitory Concentration (MIC, results not shown) to achieve a reduction in L. monocytogenes without reaching a lethal effect, ensuring that the concentration remains compatible with minimal processing conditions such as the use of mild temperatures [48]. SLC was considered the maximum concentration where the OEO did not cause a decrease in the bacterial population.

2.4. Thermal Inactivation Treatments

The effect of PEO or FIV in its SLC on the thermal inactivation of *L. monocytogenes* was evaluated at 52, 54, 57.5 and 63 °C [1,40,49,50] in a modified Simulated Meat Medium (SMM), based on BHI broth [51]. The SMM was prepared in assay tubes containing 4 mL of broth and supplemented with glucose (18 g/L) and yeast extract (3 g/L) without modifying water activity. After sterilization of the SMM, 0.5 mL of OEO (PEO and FIV) solutions were added to reach the SLC and a control with only Tween 80 was considered (each tube represented a sample). Tubes containing SMM and OEO were placed into a hot water bath, with temperature monitored using a mercury thermometer in a control tube. When the internal temperature approached the experimental temperature (± 1.0 °C), 0.5 mL of the adjusted inoculum (10^8 CFU/mL) of *L. monocytogenes* was added to obtain a final volume of 5 mL. When the internal temperature reached the experimental temperature, samples from the three treatments (PEO, FIV and control) were taken from the tubes at appropriate intervals, ranging from 0 to 30 min, depending on the temperature evaluated. Samples at time zero did not undergo heat treatment. Samples were treated and analyzed in triplicate and the entire experiment was performed twice.

Serial dilutions of the samples were carried out in saline solution (0.85 %v/v) and aliquots of 100 µL were plated in Oxford agar petri dishes (90 x 14 mm), using a Spiral Plater (Eddy Jet 2W, IUL Instruments SA, Spain) and the plates were incubated at 37 °C for 48 h. The enumeration of the colonies (CFU/mL) per sampling time at different temperatures was performed using an automatic colony counter Flash & Go (Interscience®, IUL Instruments, Spain).

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2.4.1. Estimation of the *L. monocytogenes* Inactivation Parameters

The Weibull-Mafart and Bigelow primary inactivation models, available in the user-friendly software Bioinactivation4 [52], were fitted to the experimental data obtained (log CFU/mL) over time (sec) for each treatment (PEO, FIV and control without EO) and temperature condition (52, 54, 57.5 and 63 °C). The Mafart model describes the time required to reduce a microbial population by one Log10 unit at a constant temperature, represented by the δ-value. This model is often combined with the Weibull model to interpret non-linear regression [53]. In contrast, the Bigelow model represents microbial inactivation at a constant temperature but follows a first-order, linear relationship [54,55]. To assess the goodness-of-fit of the primary models, several statistical indices were calculated to select the best model to fit the obtained results from the thermal inactivation of *L. monocytogenes* (RMSE, AIC, B*f*, and A*f*, as described below). These indicators were particularly useful for estimating the D-values (δ value for the Mafart model), which represent the time required to achieve 1-log reduction in the *L. monocytogenes* population. The Bigelow primary model is described by Equation 1 [54].

$$Log_{10}N(t) = Log_{10}N_0 - \frac{1}{D_T}t \quad (1)$$

where the D-value at a constant temperature T is identified as D_T; N(t) is the population at time t (CFU/g); and N_0 is the initial population (CFU/g).

The Weibull-Mafart model is described by the Equation 2 [53]:

$$Log_{10}N(t) = log_{10}N_0 - (\frac{t}{\delta_T})^p \quad (2)$$

where the time to reduce 90 % of the microbial population at temperature T is represented by δ_T (scale parameter); and p is the shape parameter (dimensionless). When p > 1, the curve is convex, when p < 1 the curve is concave and, when p = 1 the curve follows a linear trend [56].

For the secondary models, both one-step and two-step approaches were employed to estimate the z-values, which describe the temperature dependence of the inactivation process. These secondary models were fitted to the complete experimental dataset, incorporating all temperature and treatment conditions. The time-temperature data were adjusted using 57.5 °C as the reference temperature (T_R), as recommended for similar studies on *L. monocytogenes* [40,57,58]. The z-values were calculated by fitting the secondary models to the entire dataset, ensuring that temperature variation was adequately accounted for the inactivation curves. For the Mafart model, Equation 3 describes the secondary behavior and evaluates the sensitivity of microorganisms at a certain temperature [55]:

$$Log_{10}\delta_T = log_{10}\delta_R + \frac{T_R - T}{z} \quad (3)$$

where δ_R is the δ-value at the T_R.

In evaluating the goodness-of-fit of each model, the Root Mean Square Error (RMSE, Equation 4) was calculated. Values lower than 0.50, indicate an acceptable fit between the model and the experimental data [56,59].

$$\mathrm{RMSE} = \sqrt{\sum_{i=1}^{n} \frac{(X_{obs} - X_{fit})^2}{n-s}} \quad (4)$$

where X_{obs} and X_{fit} represent the observed and fitted values, respectively; n is the total number of observations; and s is the number of model parameters.

In the two-step inactivation model, the Residual Standard Error (RSE) is used to measure how well the residuals fit in the regression model, accounting for the degrees of freedom (df). It is calculated using the Equation 5 [60], which is closely related to RMSE, in the one-step, but is not identical:

$$\mathrm{RSE} = \sqrt{\sum_{i=1}^{n} \frac{(X_{obs} - X_{fit})^2}{df}} \quad (5)$$

To facilitate a direct comparison on a common scale between the RMSE and RSE in the two-step inactivation model, the RMSE is standardized ($RMSE_{std}$) by adjusting for the degrees of freedom (Equation 6). This standardization accounts for the difference in the number of parameters between the one- and two-step models, allowing for a more meaningful comparison of their goodness-of-fit [60].

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$$\mathrm{RMSE_{std}} = \mathrm{RMSE}\sqrt{\frac{df}{\mathrm{n-s}}} \quad (6)$$

The Akaike Information Criteria (AIC) related the maximum likelihood (loglik) to the estimated parameters was calculated according to Equation 7 [61].

$$\mathrm{AIC} = \mathrm{n}\ln\left(\frac{\mathrm{SSE}}{\mathrm{n}}\right) + 2\mathrm{s} \quad (7)$$

where n is the total number of experiments, SSE is the sum of squares of errors, and s (or k) is the number of parameters in the model [61,62].

Additionally, the Bias Factor (B*f*, Equation 8) and Accuracy Factor (A*f*, Equation 9) were assessed, with values closer to 1.0 indicating good predictive performance and minimal discrepancy between predicted and observed data [63].

$$B_f = 10^{\frac{\sum_{i=1}^{n}\log\left(\frac{x_{pred}}{x_{obs}}\right)}{n}} \quad (8)$$

$$A_f = 10^{\frac{\sum_{i=1}^{n}\left|\log\left(\frac{x_{pred}}{x_{obs}}\right)\right|}{n}} \quad (9)$$

where x_{pred} represent the predicted values; X_{obs} represents the observed values; and n is the total number of experimental data points [62,64].

2.5. Statistical Analysis

The estimated D- and/or δ-values were analyzed using the statistical program OpenStat, comparing means across different temperatures for the same treatment and across different treatments at the same temperature, with $P < 0.05$ set as the level of significance.

3. Results and Discussion

Fractionation has been shown to enhance the activity of EOs by exerting bactericidal effects and interfering with microbial resistance and virulence mechanisms, providing a multifaceted approach to microbial control [65]. The MBC was considered as the lowest concentration at which no microbial growth was observed across all replicates and repetitions. The MBC against *L. monocytogenes* obtained when using PEO was 3.10 ± 0.00 µg/mL, while the MBC with FIV was significantly lower (0.02 ± 0.00 µg/mL, $P < 0.05$). To allow a more viable result and use of the OEO in the thermal treatments, a SLC test was performed and considered as the lowest concentration of EO where the bacterial growth was not affected. In this way, the SLC of PEO was 0.13 ± 0.00 % and 0.09 ± 0.05 % for FIV.

For setting the concentration for thermal treatments, 0.06 % of PEO and FIV were used because of the rapid inactivation that made colony counting difficult when using the SLC value (data not shown). OEO has promising antibacterial properties, but its effectiveness can be influenced by factors such as bacterial strain and oil composition. Guo et al. [66] determined the Minimum Inhibitory Concentration (MIC) of OEO (does not specify the oregano species used) against *L. monocytogenes* to be 0.03 % (v/v), then treated *L. monocytogenes* with approximately 0.05 % OEO for 30 minutes, resulting in around 6-log reductions of the bacterial count. In other investigation, Bakkali et al. [67] assessed the MBC of *Origanum compactum* (68.99 % of carvacrol) against *E. coli*, reporting an MBC of 0.63 % (v/v) and a MIC of 0.31 %.

Figure 1 presents the inactivation curves of *L. monocytogenes* (log N/N_0 versus time) at 52, 54, 57.5 and 63 °C in the SMM. A more pronounced initial reduction can be observed in the treatments with OEO compared to the control group. The Weibull-Mafart and Bigelow inactivation models were compared for their suitability for describing the observed data (Table 1). The models were fitted to the inactivation data for the selection of the best model in estimating the D-value. Both models showed low RMSE; however, all the goodness-of-fit indices evaluated indicated better performance for the Weibull-Mafart model. Specifically, RMSE and SER showed lower values while A_f and B_f were closer to one. Therefore, the Weibull-Mafart model was chosen in this study as the most suitable for describing the observed data.

The Weibull-Mafart model allows to estimate the delta (δ) value which represents the time to achieve one logarithm reduction of the microbial population under a specific temperature, which is

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equivalent to the D value (min) [53]. Table 2 shows the estimations of δ (min) of *L. monocytogenes* for each group at the four temperatures evaluated. While the temperature increases, it is observed that in the three groups, δ decreases. In all cases, δ-values using FIV were significantly lower ($P < 0.05$) than the control. Only at 52 and 63 °C there was a significant difference ($P < 0.05$) between PEO and control, while at 54 and 57.5 °C significant differences ($P < 0.05$) were observed between PEO and FIV.

Table 1. Goodness-of-fit indices estimated by fitting the primary models Weibull-Mafart and Bigelow to the inactivation data: RMSE (Root Mean Square Error), Loglik (Log likelihood), AIC (Akaike Information Criteria), A_f (Accuracy factor) and B_f (Bias factor).

		Weibull-Mafart				Bigelow			
Group	**T (°C)**	**RMSE**	**Loglik**	**AIC**	**A_f/B_f**	**RMSE**	**Loglik**	**AIC**	**A_f/B_f**
Control	52	0.10	42.70	-81.40	1.01/0.99	0.20	22.36	-42.72	1.02/1.00
	54	0.16	24.40	-44.80	1.02/1.00	0.29	13.30	-24.60	1.04/1.00
	57.5	0.08	47.44	-90.88	1.01/1.00	0.37	11.92	-21.84	1.04/1.00
	63	0.06	46.21	-88.42	1.01/0.99	0.48	5.35	-8.70	1.07/1.00
PEO	52	0.42	10.36	-16.72	1.07/1.00	0.55	7.57	-13.14	1.09/1.00
	54	0.18	22.73	-41.46	1.02/1.00	0.30	14.33	-26.66	1.05/1.01
	57.5	0.33	10.96	-17.92	1.07/1.00	0.62	6.47	-10.94	1.45/1.42
	63	0.34	11.06	-18.12	1.06/1.00	1.00	2.52	-3.04	1.26/0.99
FIV	52	0.20	19.14	-34.28	1.06/1.03	0.61	6.22	-10.44	1.12/1.02
	54	0.33	11.55	-19.10	1.05/1.01	0.51	8.35	-14.70	1.06/1.00
	57.5	0.20	19.50	-35.00	1.10/0.98	1.04	4.14	-6.28	1.30/0.94
	63	0.24	11.94	-19.88	0.99/1.07	1.11	2.28	-2.56	1.39/0.95

The estimated Weibull-Mafart model p parameter values were lower than 1 (Table 2) for all the treatments, indicating a concave shape of the curves (Figure 1). As inactivation time was extended, a tail appeared in the curve, suggesting an adaptation of the microorganism to the environment (EO and medium) and/or the presence of a more susceptible subpopulation, within the *L. monocytogenes* population (Figure 1) [68].

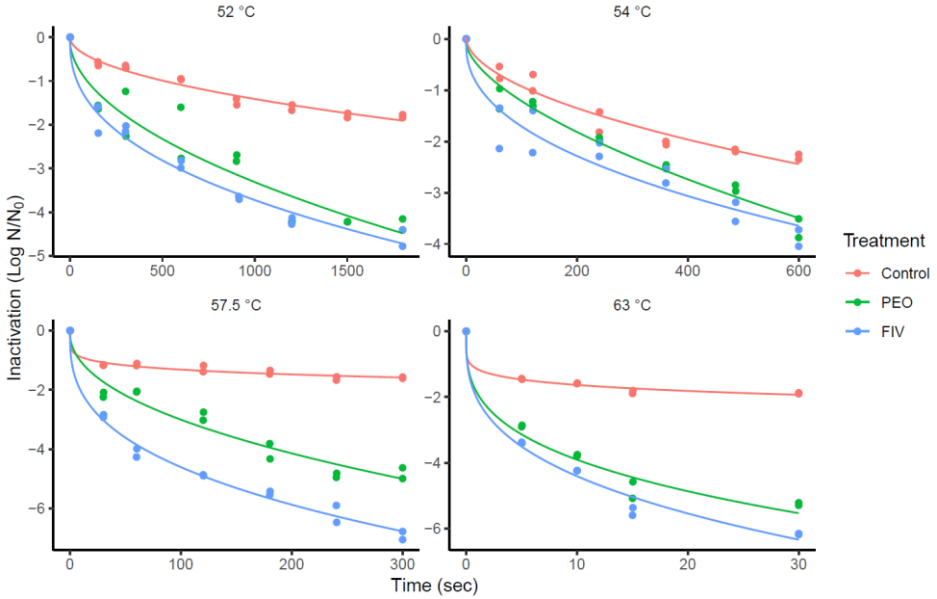

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Figure 1. Thermal inactivation curves In vitro of *L. monocytogenes* at 52, 54, 57.5 and 63 °C exposed to the PEO (Pure Oregano Essential Oil), FIV (Fractionated Oregano Essential Oil) and Control groups. The data points represent two independent replicates.

Table 2. Weibull-Mafart model parameters estimated by fitting the inactivation data obtained at 52, 54, 57.5, and 63 °C for the PEO (Pure Oregano Essential Oil), FIV (Fractionated Oregano Essential Oil), and Control groups.

	Control		PEO		FIV	
Temp (°C)	**δ (min)**	p	**δ (min)**	p	**δ (min)**	p
52	8.470 ± 1.510	0.51	1.750 ± 1.050	0.52	0.640 ± 0.240	0.40
54	1.800 ± 0.500	0.53	1.350 ± 0.350	0.61	0.540 ± 0.350	0.44
57.5	0.330 ± 0.170	0.17	0.170 ± 0.090	0.47	0.020 ± 0.010	0.35
63	0.007 ± 0.004	0.15	0.002 ± 0.001	0.32	0.002 ± 0.002	0.33

Considering the goodness-of-fit indices for both primary and secondary models, the One-Step procedure was selected to fit the model to the experimental data to estimate the z-values (Table 3). The resulting secondary models are shown in Figure 2. This One-Step model fitting were carried out using the bionactivation4 shiny and verified using the negative inverse of the slope of each line in MS Excel. The z-values estimated were 5.75, 5.20 and 5.00 °C for Control, PEO and FIV, respectively, where no significant differences were observed between the groups ($P > 0.05$). Similar z-values around 5 °C were obtained in previous studies of the use of OEO against *L. monocytogenes* species in seafood [40,69,70]. These previous results, along with those obtained in this study for the z- and δ-values are consistent with the thermal resistance profile and susceptibility to the applied antimicrobial agents. These results indicate that while *L. monocytogenes* maintains a consistent sensitivity to temperature, its susceptibility differs depending on the antimicrobial agent applied [39].

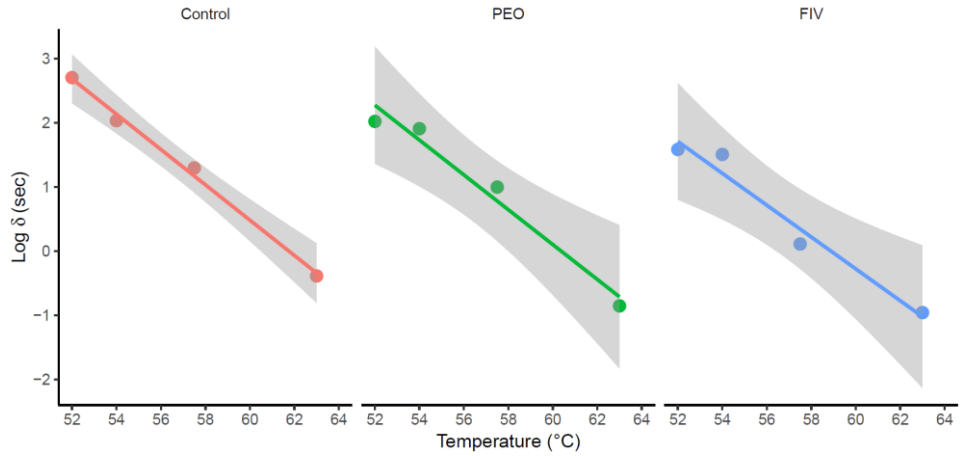

Figure 2. Relationship between the δ parameter estimated for *L. monocytogenes* with the mild heat treatment temperature applied for the Control group, PEO (Pure Oregano Essential Oil) and FIV (Fractionated Oregano Essential Oil) groups. Grey zones represent the confidence intervals of parameter estimates.

Table 3. Z-values (°C) estimated by the One-Step and Two-Step procedures and the Root Mean Square Error (RMSE), Residual Standard Error (RSE) and Standardized RMSE ($RMSE_{std}$) indices, estimated from the thermal inactivation of *L. monocytogenes* using PEO (Pure Oregano Essential Oil), FIV (Fractionated Oregano Essential Oil) and the control group.

	One-Step			Two-Step			
Group	**RMSE**	**$RMSE_{std}$**	**z-value**	**z-value**	**RSE**	**t value**	**Pr (>|t|)**
Control	0.31	0.06	5.75 ± 0.28	3.63 ± 0.19	0.12	19.29	0.003
PEO	0.43	0.08	5.20 ± 0.14	3.69 ± 0.46	0.28	8.00	0.02
FIV	0.46	0.09	5.00 ± 0.13	4.03 ± 0.55	0.28	7.27	0.02

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Table 4 summarizes the findings from previous studies on thermal inactivation of *L. monocytogenes* at temperatures ranging from 52 to 65 °C, using EOs, phenolic compounds and plant extracts as natural antimicrobial agents. Notably, at 55 °C, the D value varied depending on the food matrix, highlighting the influence of the matrix on the effectiveness of thermal treatments. The lowest D values were observed with treatments containing isolated EO compounds, such as carvacrol, thymol, and cinnamaldehyde, indicating their strong antimicrobial properties. These results aligned with those obtained in our study using FIV, where the higher concentration of active compounds and reduced interference from EO components with lower antimicrobial activity contributed to more effective bacterial inactivation.

Table 4. D or δ- and z-values of *L. monocytogenes* using mild temperatures and essential oil reported in previous studies.

Matrix	Treatment	D- or δ-values (min)							z Values (°C)	Reference
		D_{52}	D_{54}	D_{55}	$D_{57.5}$	D_{60}	D_{63}	D_{65}		
BHI broth supplemented with glucose and yeast extract	*P. longiflora* PEO 0.06%:	1.75	1.35	-	0.17	-	$2.00x10^{-3}$	-	5.20	Present study
	P. longiflora FIV 0.06%:	0.64	0.54	-	0.02	-	$2.00x10^{-3}$	-	5.00	
Sous-vide salmon	*Origanum vulgare* EO 1%	-	-	10.03	4.88	1.81	-	-	5.62	[40]
Sous-vide beef	*Salvia officinalis* EO 0.6%	-	-	21.17	-	-	-	-	-	[71]
Ground pork	Cinnamaldehyde 0.5%	-	-	3.61	-	0.63	-	0.52	-	[72]
BHI broth	Carvacrol 30 μg/mL	-	-	8.17	-	0.67	0.17	0.07	-	[73]
	2-Hexenal 65 μg/mL	-	-	8.03	-	0.60	0.12	0.08	-	
	Citral 50 μg/mL	-	-	8.42	-	0.66	0.16	0.08	-	
TSBYE	Thymol	-	-	0.25	-	-	-	-	-	[74]
	Carvacrol	-	-	0.25	-	-	-	-	-	
	Thymol + Carvacrol	-	-	0.18	-	-	-	-	-	
PBS	Thymol	-	-	1.47	-	-	-	-	-	[75]
	Carvacrol	-	-	1.48	-	-	-	-	-	
	Thymol + Carvacrol	-	-	0.38	-	-	-	-	-	
Pineapple juice	Mexican coriander 15 μg/mL			5.61		0.53		0.28		[76]
	Mexican coriander 60 μg/mL			5.47		0.44		0.16		
Beef marinated	Grapefruit seed extract 200 ppm	-	-	22.17	6.11	3.69	-	-	7.98	[59]
Ground Turkey	Sodium chloride (1%) and green tea polyphenol extract (1.5%)	-	-	30.40	-	5.50	-	0.90	-	[77]

Incorporating FIV and PEO into food processing could provide an effective strategy to control *L. monocytogenes*, especially in Ready-to-Eat (RTE) foods where the pathogen's ability to grow at refrigeration temperatures poses a challenge [78]. Furthermore, FIV offers the potential to enhance antimicrobial efficacy while reducing the sensory impact on food products, a critical consideration for maintaining consumer acceptability [37]. By leveraging predictive microbiology models, the thermal and antimicrobial effects of FIV can be optimized, allowing for evidence-based decisions that balance food safety and quality. This approach represents an opportunity to minimize heat treatments while ensuring safe food products, particularly in regions with limited regulatory oversight for listeriosis [79]. Moreover, integrating these predictive models into user-friendly tools could facilitate widespread adoption, enabling food processors and regulators to make informed decisions with greater ease and efficiency.

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4. Conclusions

P. longiflora fractionated oregano essential oil significantly reduced the time for achieving *L. monocytogenes* logarithmic reductions under heat treatments at all the mild temperatures applied in this study. This effect is related to the high concentration of carvacrol, and thymol reached by the fractional distillation at 140 °C. The inactivation parameters estimated in our study through model fitting can be used to assess suitable oregano essential oil and mild heat treatments to achieve desired levels of pathogen inactivation. The thermal inactivation experiments of *L. monocytogenes* in a food matrix using ground meat showed results consistent with those obtained in the meat simulation medium, suggesting that Mexican *P. longiflora* fractionated essential oil has strong potential for application in this food matrix. Further investigations on the sensory effects of this application are underway.

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Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism
°C	Degree Celsius
µL	microliter
A*f*	Accuracy factor
AIC	Akaike Information Criterion
B*f*	Bias factor
BHI	Brain Heart Infusion
CFU/mL	Colony Forming Units per milliliter
EO	Essential Oils
FIV	Fractionated Oregano Essential Oil
g/L	grams per liter
GRAS	Generally Recognized As Safe
h	hours
HPP	High Pressure Processing
MBC	Minimum Bactericidal Concentration
min	minutes
mL	milliliter
OD	Optical Density
OEO	Oregano Essential Oil

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PEO	Pure Oregano Essential Oil
RMSE	Root Mean Square Error
sec	seconds
SER	Standard Error of Regression
v/v	volume/volume

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