PATHOGENIC BACTERIA INACTIVATION BY COMBINED HURDLE TREATMENTS: PERACETIC ACID AND PULSED LIGHT.

Karen B. Battani[1], Laila Schramm[1], Ana G. Massia[1], Bruno A. M. Carciofi[2]

[1] *Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina - Florianópolis, Brazil*
[2] *Department of Biological and Agricultural Engineering, University of California Davis - Davis, USA*
*karenb.battani@gmail.com
lailaschramm@gmail.com
anagmassia@gmail.com
bacarciofi@davis.edu

ABSTRACT

Listeriosis is a disease caused by food contaminated with *L. monocytogenes*, and it is a public health problem due to the occurrence of outbreaks, characterized by a high mortality rate in vulnerable populations. Pulsed light (PL) uses high-intensity pulses with UV light, capable of leading to cell death mainly by DNA changes. Peracetic acid (PAA) is a hurdle treatment that act oxidizing cellular components. This study aimed to investigate the combination of PL and PAA on the inactivation of *L. monocytogenes* on agar, evaluating the benefits of the combination compared to individual treatments. PAA concentrations of 50-200 ppm do not inactivate *L. monocytogenes* cells until 240 minutes of acid exposure, and 400-600 ppm concentrations resulted in reductions up to 5 Log/cm^2 at 180, and 210 minutes, respectively. The inactivation kinetics by PAA data fit well ($R^2 \geqslant 0.879$) to the Baranyi & Roberts mode for acid concentrations of 400, and 600 ppm tested. The μ values for the acid concentration of 400 ppm was -0.045, 2 times lower compared to 600 ppm concentration (μ=0.095). The results for the combined treatments shows a cooperative response between the treatments (acid+PL) resulting in inactivation values greater than the method alone. The organic acid (PAA) shows a higher inactivation levels compared to the inorganic acid (HCl). The obtained results proved that PAA+PL combination is efficient to inactivate *L. monocytogenes* cells in agar and could be potentially used for food safety benefits.

Keywords: 1. *L. monocytogenes* 2. Peracetic Acid 3. Pulsed Light 4. Inactivation

1 INTRODUCTION

Listeriosis is a serious disease caused by food contaminated with *L. monocytogenes*, posing a significant public health issue, especially for vulnerable groups such as immunosuppressed individuals, the elderly, pregnant women, and children.[1] This bacterium can form biofilms, enhancing its resistance to disinfectants and antimicrobial treatments, thereby complicating control efforts in food processing environments [2,3].

Effective methods to eliminate *L. monocytogenes* are crucial in industrial food production settings. Pulsed light (PL) technology utilizes intense light pulses to deactivate microorganisms by targeting cellular DNA, while peracetic acid (PAA) serves as a broad-spectrum antimicrobial alternative with no by-products when in contact with organic matter [4,5] .This study explored the combination of PL and PAA for *L. monocytogenes* inactivation in agar, varying PAA concentrations and acid exposure times to assess potential synergistic effects in combating this pathogenic bacterium

2 MATERIAL & METHODS

The inoculum of *L. monocytogenes* (CCT 7474 isolated from sausage) was prepared from a colony-forming unit (previously made) incubated in Tryptone Soy Broth (TSB) broth (37 °C, 24 h). Afterward, an aliquot (50 µL) of the previous inoculum was subcultured in 5 mL of fresh TSB (37 °C, 7 h). When the initial stationary phase was reached, the inoculum was centrifuged and resuspended in phosphate buffer solution; this process was repeated three times to remove the residual TSB.

To obtain the acidified agar, Tryptone Soy Agar (TSA) was prepared according to the manufacturer's guidelines, sterilized, and cooled (45 °C). PAA was added to obtain 100, 200, 400, and 600 ppm concentrations. The agar was poured into Petri dishes and the pH was measured. At the same time, samples of acidified TSA with added HCl were prepared until they reached the pH of the corresponding PAA agars to compare organic (PAA) and inorganic (HCl) acids. Control samples were made with TSA without acid addition.

For the inactivation kinetics, 50 µL of the inoculum (1010 CFU/mL) were inoculated into the acidified agar (PAA concentrations of 100-600 ppm) and control. The agars were incubated at 37 °C immediately after inoculation. Samples were taken at predetermined time intervals for cell recovery and the inactivation curves were carried out in duplicate.

The inactivation by the combined treatments (acid+PL) was made into 3 different agars after inoculation with *L. monocytogenes* (1010 CFU/mL): acidified agar with PAA (organic acid), with HCl (inorganic acid), and control (no acid). The combined treatments followed the experimental design shown in Fig. 1, 5 combinations were made varying the time of incubation/acid exposure (at 37 °C), and the moment of PL application. The PL parameters used were 2000 V and 1 single pulse of 75 µs.

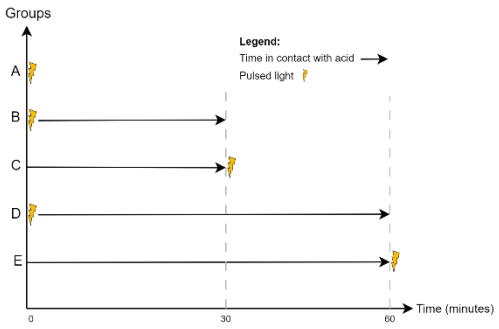

Figure 1 - Experimental design for *L. monocytogenes* inactivation by the combined treatments of PL and acid to different combinations varying the time of incubation/acid exposure (0, 30, or 60 min), and the moment of PL application (after 0, 30, or 60 min of acid exposure).

The *L. monocytogenes* cells count followed the surface count method on TSA using phosphate buffer solution as a diluent. The microbial concentration (N) was calculated by multiplying the CFU count by the inverse of the dilutions and dividing by the area of the plate, expressed as Log CFU/cm². The Baranyi & Roberts model (Equation 1) was used to model the microbial inactivation data over time.

$$\ln(N) = \ln(N0) + \mu_{máx} \cdot F(t) - \ln\left(1 + \frac{\exp(\mu máx \cdot F(t)) - 1}{\exp(\ln(Nmáx) - \ln(N0))}\right) \quad (1)$$

3 RESULTS & DISCUSSION

This study sought to evaluate the effectiveness of acid concentration on cell inactivation under different conditions. The concentrations of 100, and 200 ppm did not significantly reduce the cell population over a 240 min period. These concentrations are insufficient to cause any significant effect on cell inactivation, suggesting that higher concentrations are needed to obtain substantial results.

Model parameters	PAA concentration (ppm)	
	400	600
λ	30 ± 17	28 ± 7
μ	-0.045 ± 0.016	-0.095 ± 0.023
R^2	0.79	0.879

Table 1 Primary parameters of Baranyi & Roberts model for *L. monocytogenes* inactivation data by different PAA concentrations (400-600 ppm).

The results in Figure 2 show that higher acid concentrations significantly impact the inactivation of *L. monocytogenes* cells. Using the Baranyi and Roberts model to describe the inactivation kinetics by PAA at 400 and 600 ppm, the curves indicate an initial plateau, suggesting a delayed cellular response. At 400 ppm, the delay time is approximately 29.94 minutes, with a maximum inactivation rate of -0.0449 and a total reduction of -3.585 after 210 minutes (R^2 = 0.79). At 600 ppm, the delay time is shorter at 28.379 minutes, with a maximum inactivation rate of -0.0945 and a total reduction of -4.298 after 210 minutes (R^2 = 0.879). The cell inactivation at 600 ppm is 110.47% higher than at 400 ppm, indicating that the effectiveness of the treatment more than doubles with the increase in concentration.

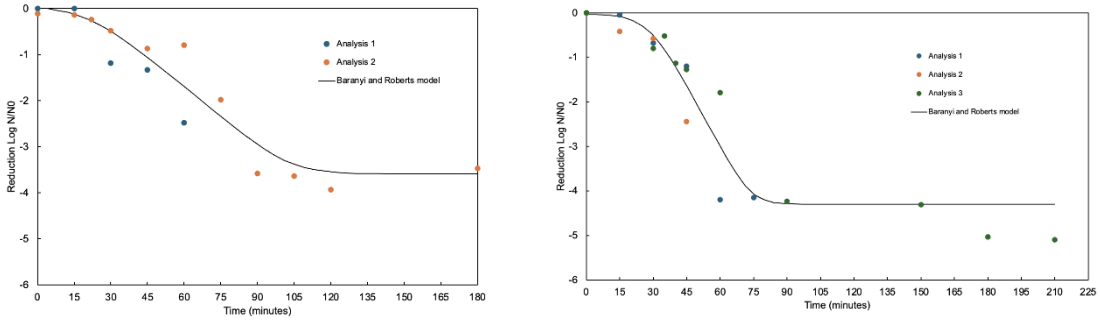

Figure 2 - Reduction of *L. monocytogenes* over time by different concentrations of PAA 400 (A) and 600 (B) ppm. Data points represent experimental data, and continuous line represent Baranyi and Roberts model.

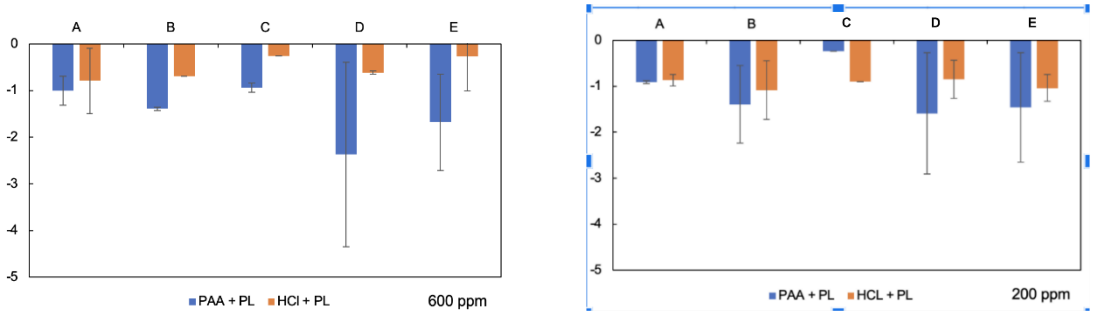

Figure 3 - Reduction of *L. monocytogenes* for different combination treatments combining acid and PL (A, B, C, D, and E). PAA concentrations of 200 (left side), and 600 (right side) ppm, and correspondingly acidified HCL agar.

The study evaluated the effectiveness of different acid concentrations in cell inactivation, comparing peracetic acid (PAA) and hydrochloric acid (HCl) in combination with pulsed light (PL) at 200 ppm and 600 ppm (Figure 3). At 200 ppm, both acids showed limited efficacy, with an average reduction of approximately -1 log and noticeable variations under specific conditions. PAA+PL and HCl+PL resulted in similar reductions, except in Group C where HCl+PL was more effective. At 600 ppm, effectiveness increased significantly, with PAA+PL achieving greater reductions in cell population than HCl+PL, indicating that PAA is more effective at higher concentrations. This highlights that higher acid concentrations favor cell inactivation and have a greater synergistic impact.

4 CONCLUSION

The concentration of peracetic acid has a significant impact on cell inactivation, with more effective results observed at higher concentrations (400 and 600 ppm). The comparison between 400 ppm and 600 ppm revealed significantly greater cell inactivation at the latter, with a maximum inactivation rate of approximately 110.47% higher. Analysis of the final population also corroborated the superior efficacy of 600 ppm, showing a smaller cell population. These results reinforce the importance of acid concentration in cell inactivation, highlighting that the increase from 400 ppm to 600 ppm more than doubles the effectiveness of the treatment. Additionally, the combination of peracetic acid and light (PL) was more effective in inactivating *Listeria monocytogenes* than using the method alone. The organic acid (PAA) proved to be more efficient than the inorganic acid (HCl). In conclusion, peracetic acid, especially at high concentrations, in combination with light, appears to be a promising approach for effective cell inactivation.

5 REFERENCES

[1]BUCHANAN, R. L. et al. **A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments**. **Food Control**Elsevier Ltd, , 1 maio 2017.

[2] COLAGIORGI, A. et al. ***Listeria monocytogenes* Biofilms in the wonderland of food industry**. **Pathogens**MDPI AG, , 1 set. 2017.

[3] COUGHLAN, L. M. et al. **New weapons to fight old enemies: Novel strategies for the (bio)control of bacterial biofilms in the food industry**. **Frontiers in Microbiology**Frontiers Media S.A., , 18 out. 2016.

[4] GÓMEZ-LÓPEZ, V. M. et al. Pulsed light for food decontamination: a review. **Trends in Food Science and Technology**, v. 18, n. 9, p. 464–473, set. 2007.

[5] KITIS, M. Disinfection of wastewater with peracetic acid: A review. **Environment International**, v. 30, n. 1, p. 47–55, 2004.

[6]LONGHI, D. A. et al. Microbial growth models: A general mathematical approach to obtain μ max and λ parameters from sigmoidal empirical primary models. **Brazilian Journal of Chemical Engineering**, v. 34, n. 2, p. 369–375, 1 abr. 2017

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