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EFFECT OF HYPOCHLOROUS ACID ON *LISTERIA MONOCYTOGENES*: INFLUENCE OF TIME AND SURFACE MATERIAL.

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I. INTRODUCTION

Listeria monocytogenes has recently been identified as one of the main bacterial hazards associated with persistence in food and feed processing environments by the European Food Safety Authority [1]. In food processing plants, sanitation procedures are routinely carried out using various chemical detergents and biocides to mitigate the presence of *L. monocytogenes* in processing environments. However, some of these substances may negatively impact the environment and, if not properly employed, can pose health risks to personnel and may contribute to the development of tolerance or resistance in microorganisms, leading to diminished effectiveness over time [2]. In this sense, hypochlorous acid (HOCl) has been considered a potential alternative method for controlling bacteria in the food industry due to its strong disinfection activity and environmental friendliness [3]. The aim of this study was to assess the effect of hypochlorous acid on *L. monocytogenes* strains isolated from meat products, on two main surface materials commonly used in the meat sector (polystyrene and stainless steel), in order to explore its potential as a strategy for reducing contamination by this pathogen in meat processing plants.

II. MATERIALS AND METHODS

To achieve this objective, the ISO 13697:2015+A1:2019 guidelines were followed. To simulate practical application conditions, the chosen conditions were: a contact time of 5 to 15 minutes at 5-minute intervals (5, 10, and 15 minutes); room temperature (18-25 °C); 0.3 g/l of bovine albumin as an interfering substance (clean conditions); and polystyrene and stainless steel as porous and non-porous surfaces. Regarding test organisms, a total of four strains of *L. monocytogenes*, previously isolated from environments in a meat processing industry, were used in the study. These strains included the four main serotypes (1/2a, 1/2b, 1/2c, and 4b). Finally, the hypochlorous acid employed in this study (Klorxitol) was provided by a local company (ICB Ibérica, Salamanca, Spain) as a product ready to use.

In summary, the procedure followed involved preparing a test suspension of a mix of four *L. monocytogenes* strains in a solution of interfering substances. The suspension was inoculated onto a stainless steel or polystyrene surface and dried. An aliquot of the product under test was applied in a manner that covered the dried film. The surface under evaluation was maintained at room temperature for 5, 10, or 15 minutes and then transferred to a neutralization medium to immediately neutralize the disinfectant's action. The number of surviving *L. monocytogenes* that could be recovered from the surface was determined quantitatively using TSA agar (Scharlau, Barcelona, Spain). The number of *L. monocytogenes* on each surface treated with water instead of the disinfectant was also determined. Also, in parallel, tests to verify the absence of toxicity of the neutralizer and to validate the dilution neutralization method were carried out.

For each surface and experimental condition, the reduction (R) in viable counts attributed to the disinfectant was calculated by difference between the log number of cfu recovered from the test surface treated with water and the log number of cfu recovered from the test surface treated with the disinfectant. The reduction (R) was expressed as log cfu.

All experiments were performed at least six times. Graphical representation was made using R software (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.).

III. RESULTS AND DISCUSSION

Data on the logarithmic reduction of *L. monocytogenes* counts after hypochlorous acid treatments are reported in Fig. 1. As can be observed, no significant differences were found among treatments. It is known that, in comparison with hydrophilic surfaces such as stainless steel, hydrophobic and non-polar surfaces such as polystyrene favor quicker bacterial adhesion and promote biofilm formation, thereby increasing the resistance of cell communities. In this study, no significant differences were found between surfaces, and the effect of hypochlorous acid was comparable between polystyrene and stainless steel.

Similarly, no significant differences were observed among the different treatment times. (5, 10, or 15 min). Greater variability was observed in the logarithmic reductions obtained after 15 minutes of treatment compared with those obtained after 5 or 10 minutes.

Considering all the experiments conducted, an average reduction of 5.1 ± 1.3 log cfu was achieved. Taking into account that European Standard EN 13697:2015 +A1:2019 establishes a 4-log reduction as the benchmark for verifying the bactericidal effectiveness of a disinfectant under different surface soiling conditions, we can consider the results obtained in this study—under all tested conditions—as meeting the 4-log reduction threshold.

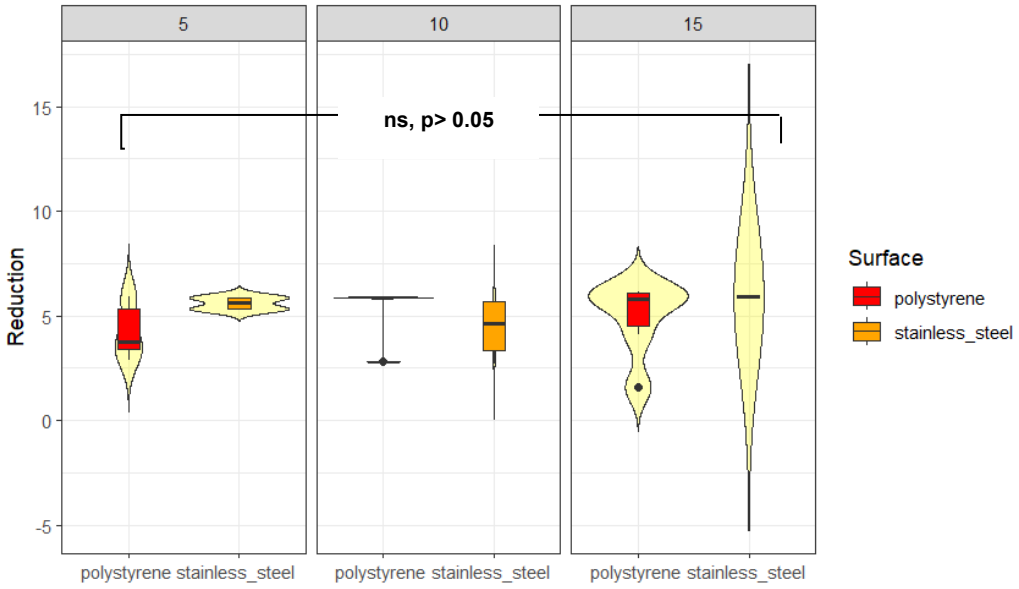


Figure 1. Violin plots combining box plots representing the distribution of *L. monocytogenes* reductions (log cfu) obtained after hypochlorous acid treatment applied for 5, 10, or 15 minutes on polystyrene or stainless steel surfaces. Each box/violin represents 6 data points (n = 6).
* ns: no significant differences among treatments, as determined by ANOVA

IV. CONCLUSION

Based on the results obtained, hypochlorous acid, as applied in this study, can be used as a tool to reduce and control *L. monocytogenes*, which remains one of the main foodborne pathogens posing a public health risk. Hypochlorous acid could represent an interesting approach given its antimicrobial activity, ease of use, and low environmental impact.

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