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QUANTITATIVE MICROBIAL RISK ASSESSMENT REVEALS PRIORITY STRATEGIES TO COMBAT LISTERIOSIS ASSOCIATED WITH COOKED HAM

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I. INTRODUCTION

Listeria monocytogenes is a significant public health concern due to the high hospitalization and fatality rates of listeriosis cases, which has been increasing in incidence in the last years [1]. Ready-to-eat (RTE) foods, especially cooked meat products, are common vehicles for *L. monocytogenes* transmission [2].
This study aims to understand, from a quantitative risk assessment perspective, the effectiveness of various intervention strategies on reducing the risk of listeriosis in cooked ham. Technological strategies were focused on the growth inhibition by antimicrobial agents (organic acids and bioprotective cultures) and/or postlethality treatements based on in-package high-pressure processing (HPP) aiming at pathogen inactivation.

II. MATERIALS AND METHODS

A quantitative microbial risk assessment (QMRA) model was developed, with particular focus on expossure assessment linked to food processing and preservation strategies. The strategies assessed included the use of potassium lactate as an antimicrobial agent in the product formulation, the addition of bioprotective microbial cultures, and the application of HPP. Eleven scenarios were considered, ranging from no intervention (baseline) to combinations of strategies (i.e., lactate and HPP). The inactivation and growth kinetics of *L. monocytogenes* were modeled, considering the protective effects of lactate and the piezo-stimulation effect of HPP based on previous works [3,4,5]. The initial prevalence and concentration of *L. monocytogenes* in RTE cooked meat products were estimated using data from a baseline survey conducted at retail in the EU. The concentration was described using a beta-general distribution, and the initial contamination per package was calculated. The growth rate of *L. monocytogenes* was modeled using the gamma concept approach, considering factors such as temperature, water activity, pH, and lactic acid concentration [6]. The maximum population density was set at 8.5 log CFU/g (without antimicrobial agents), with adjustments for the presence of lactate and bioprotective cultures. The model included detailed temperature and time distributions for the industry, retail, and home stages. These distributions were based on published studies and surveys of domestic refrigerator temperatures. The ingested dose of *L. monocytogenes* was calculated considering a standard serving size (35.2 g), and an exponential dose-response model [2] was used to estimate the probability of illness in elderly population incorporating variability. One million Monte Carlo simulations were performed to determine the distribution of the probability of illness per serving and the number of cases per million servings using the *mc2d* package in R software. Sensitivity analysis based on Spearman rank correlation was conducted to identify the key factors influencing the variability in the estimates of listeriosis risk per serving.

III. RESULTS AND DISCUSSION

The arithmetic mean probability of illness per serving in the baseline scenario with no intervention strategy was $3.7 \cdot 10^{-5}$ (i.e. 37 predicted mean number of cases per million servings of cooked ham). The risk of listeriosis was driven by the impact of storage conditions (time and temperature) on the growth of *L. monocytogenes* from the industry stage and until consumption. Reducing the product's shelf-life from 45 days to 21 days resulted in a 99% reduction in listeriosis risk. This significant reduction was attributed to the decreased growth potential of *L. monocytogenes* during the shorter storage period. Further reduction to 5 days nearly eliminated the risk, highlighting the importance of controlling storage time to mitigate pathogen growth.
Regarding the impact of interventions based on the use of antimicrobial agent, formulating cooked ham with 2% lactate reduced the mean number of listeriosis cases per million servings by 99.8%, while 2.8% lactate achieved an additional order of magnitude reduction. The effectiveness of lactate was due to its ability to lower the growth rate and the maximum population density of *L. monocytogenes*, particularly at lower storage temperatures. The addition of bioprotective cultures, such as the bacteriocin-producing *L. sakei* CTC494, demonstrated substantial risk

reduction potential by lowering its maximum population density. At high ratios of bioprotective strain to pathogen, the mean number of cases was reduced by five orders of magnitude. This indicated that the bioprotective strategy would contribute to mitigating the relative impact of the long storage time (including the lack of adherence to date marking) by the consumer. This finding highlights the primary importance of the maximum population density of the pathogen on the risk estimation, being in agreement with the QMRA model of EFSA BIOHAZ Panel [2].
Mild HPP treatments (400 MPa for 5 min) provided a modest risk reduction of 30.7% in products without lactate. The effectiveness of HPP was primarily due to the inactivation of *L. monocytogenes*, with minimal impact on subsequent growth. On the other hand, a 5-log reduction HPP treatment (600 MPa for 3 minutes) was highly effective, reducing the concentration of *L. monocytogenes* to below 1 cell per contaminated package. This treatment achieved a level of inactivation close to pasteurization standards, effectively eliminating the risk of listeriosis. The combination of lactate and HPP did not provide additional risk reduction benefits compared to lactate or HPP alone, as the piezo-protective effect of lactate reduced the lethality of HPP (Figure 1). This finding challenges the assumption that combining post-lethality treatments with antimicrobial agents always results in better control of *L. monocytogenes*. The study underscores the need for product-specific validation of HPP efficacy, considering the interactions between different intervention strategies [7].

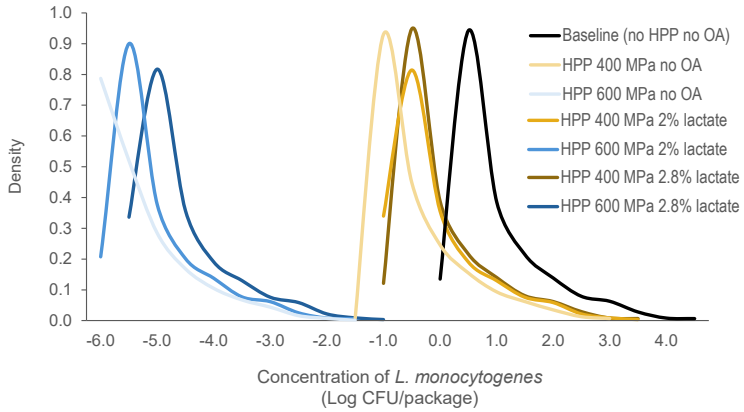


Figure 1. Concentration of *L. monocytogenes* in cooked ham formulated without and with potassium lactate before (i.e. baseline scenario) and immediately after the application of HPP.

IV. CONCLUSION

The study highlights the importance of selecting appropriate intervention strategies to reduce listeriosis risk in cooked ham. Reducing product shelf-life and using lactate as an antimicrobial agent are effective measures. High-pressure processing at 600 MPa or the use of bioprotective cultures can further enhance risk reduction. These findings can guide food business operators in designing and prioritising effective measures to control *L. monocytogenes* and ensure safer meat products.

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