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QUALIFIED NON-PATHOGENIC SURROGATES OF *SALMONELLA* FOR IN-PLANT VALIDATION OF DRY FERMENTED SAUSAGE SAFETY

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I. INTRODUCTION

Dry-fermented sausages (DFS) are shelf-stable thanks to their physicochemical characteristics, with water activity and/or pH levels below the threshold for the growth of spoilage or pathogenic bacteria. However, stability does not always guarantee food safety as pathogens present in raw materials can survive the production process and remain in the final product at levels exceeding legal limits, posing a health risk. In this respect, the Rapid Alert System for Food and Feed (RASFF) has recorded numerous alerts about DFS failing to meet the microbiological criteria as well as salmonellosis outbreaks linked to DFS. Additionally, in the last EFSA/ECDC zoonosis report [1], fermented sausages were the ready-to-eat food with higher rate of non-compliance with the microbiological safety criteria set by the Regulation (EC) 2073/2005, requiring no detection of *Salmonella* in 25 g of product and limiting *Listeria monocytogenes* to a maximum of 100 CFU/g.

To ensure food safety, producers must implement self-monitoring systems based on Hazard Analysis and Critical Control Points (HACCP). In this context, producers must identify and apply the most suitable control measures ensuring the safety of food products placed on the market. Central to HACCP is the validation of control measures, scientifically proving that they are effective against identified hazards.

The aim of this study was to assess the availability and suitability of tools to validate the food safety of DFS taking into account product's formulation and processing conditions.

II. MATERIALS AND METHODS

Internationally recognized validation procedures were reviewed, including the Codex Alimentarius guidance on validation [2] and the main reference documents providing guidelines for conducting experimental validation studies [3,4]. Additionally, two challenge tests were performed in chorizo to evaluate the behaviour of *Salmonella* in comparison with a qualified pool of enterobacteria used as non-pathogenic surrrogate (developed in the framowork of the VALSalm project). The assays were performed in the biocontained pilot plant of IRTA-Monells (Biosafety level 2) [5]. Chorizos were prepared using the producer's ingredients and additives. Meat batter was inoculated during mixing with approximately 6 log CFU/g of either a cocktail of three *Salmonella* strains (selected from the IRTA Food Safety and Functionality Program collection) or the pool of enterobacteria. The production process replicated the producer's production process conditions (35 days at 11°C and 100–74% relative humidity) and acidification and drying (a_w) profiles. *Salmonella* and entrobacteria counts were determined in cromogenic agars throughout the process. Finally, as a comparison of methodological approaches, predictive microbiology was applied to predict the behavior of *Salmonella* in DFS using the pH, a_w and temperature profiles from the challenge tests as input values for the mathematical model developed by Pin et al [6].

III. RESULTS AND DISCUSSION

Validation tools

There are three tools available for conducting validation studies, which can be used individually or in combination: scientific literature, experimental assays (challenge tests), and mathematical models from predictive microbiology. Scientific literature involves gathering bibliographic data on the behaviour of pathogens in foods with characteristics and production conditions equivalent to the ones under study. Literature data is often not conclusive enough to guarantee the safety of a specific production process, due to the unique formulations and production conditions. As a result, it is often necessary to carry out challenge tests. These are experimental assays to study the behaviour of deliberately inoculated pathogens. They can be conducted using either the target pathogen or a surrogate microorganism. Key factors to be considered in the design and execution of challenge tests include the reproduction of the industrial production process, the selection of the target microorganism and its inoculation, and the analytical determinations to be performed throughout the production process (physicochemical and microbiological parameters) [3,4]. Predictive microbiology can complement experimental validation studies; however, the suitability

of the applied models should be previously evaluated. As a final point, the execution and interpretation of validation studies requires experts who can properly assess its scope, application, and limitations.

Case study: Validation of *Salmonella* lethality during chorizo production

Challenge test results showed a progressive reduction of both *Salmonella* and enterobacteria levels along the production process of chorizo, reaching a final lethality of about 3.3 log units. The correlation between *Salmonella* reduction and the behaviour of the enterobacteria pool confirmed its suitability as a non-pathogenic surrogate for future in-plant validation studies. The prediction of the behaviour of *Salmonella* through predictive microbiology using pH, a_w, and temperature profiles from the challenge test as inputs showed that the model significantly underestimated pathogen reduction. It even predicted slight initial growth of *Salmonella*, which was not observed experimentally, and predicted only a 1.4 log reduction by the end of the process compared to the 3.3 log observed in the challenge test. Similar discrepancies have been described in other studies with fuet-type DSF [7,8], highlighting the current limitations of predictive models for assessing the behaviour of *Salmonella* during DFS production.

Observed *Salmonella* lethality aligns with typical reductions in Southern European DFS, which, due to their production methods and physicochemical characteristics, generally do not achieve the 5-log reductions in pathogens (*Salmonella* and *L. monocytogenes*) required by North American standards. Therefore, beyond validating the lethality of the production process, ensuring food safety may also involve verifying the no detection of contamination in the raw materials or meat batter through robust sampling plans. For example, North American guidelines recommend testing 15 to 30 samples per batch (n=15 or 30, c=0) [9,10].

IV. CONCLUSION

Currently available tools and methodologies make challenge tests the most reliable option for providing scientific evidence of *Salmonella* lethality in DFS and validating microbiological safety. If in-plant validation is needed, surrogate organisms such as the qualified enterobacteria pool developed in our study can be used. As part of the HACCP system, after the validation studies are run, routine monitoring of pH and a_w of each production batch and verification procedures through microbiological determinations of raw materials and final products should be implemented.

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