Foundation for Meat and Poultry Research and Education
Final report

Draft manuscript

The manuscript regarding this work has been accepted to Foods. A copy of the publication is attached as a separate document. The publication can also be found here: https://doi.org/10.3390/foods12214034.

Executive summary

Project Title

Development and validation of an antimicrobial database to predict microbial load reduction on raw pork components against *Salmonella.*

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Foods

Objectives:

- To develop a time-efficient screening tool for antimicrobials against *Salmonella* Typhimurium on pork loin ends.
- To use a statistical model (Response Surface Methodology) to study the effect of combinations of effective antimicrobial compounds to inhibit *Salmonella* Typhimurium cell counts on pork.
- To validate the results from the model using a laboratory-scale validation.

Conclusions:

Antimicrobial washes are common food safety practices used in the meat industry. However, there are many compounds that can be used as potential antimicrobials against *Salmonella* on pork. Therefore, it is important to develop screening tools to efficiently identify compounds that inhibit bacterial growth from those that do not. This study implemented a time- and cost-efficient screening tool, which was used to screen 35 compounds for their potential antimicrobial activity against *Salmonella* on pork. Pork loin ends were miniaturized by cutting cubes of pork of approximately 69 cm^3 that fit at the bottom of a test tube. Then, the pork was inoculated with *Salmonella* and the individual antimicrobial compounds were added to the surface of the

inoculated pork. The concentration of remaining bacteria on the meat was used to compared between treatments and their effectiveness. The results from this initial screening showed that only 31% (11/35) of the compounds that were screened were able to inhibit *Salmonella* from the surface of pork. Then, six of the effective compounds were selected to study any potential synergism or antagonism between them if added in combination to the meat, these compounds were lactic acid, formic acid, cumin oil, clove oil, peppermint oil, and spearmint oil. A statistical model known as Response Surface Methodology (RSM) was used to study potential interactions between the antimicrobial compounds. RSM is an alternative to the traditional approach for screening antimicrobial combinations. Traditionally, every possible combination between antimicrobials at different levels would have to be performed to find interactions between these compounds. However, with RSM it is possible to do a fraction of the experiments and still be able to find possible synergisms or antagonisms between antimicrobials, which makes RSM a novel and efficient tool to be used. With RSM, a linear model is built, and statistical analysis are performed to determine any possible interaction between antimicrobials. The RSM model in the present study showed good statistical quality parameters, which means that the model can be used to predict interactions confidently. However, the RSM model predicted that there is no interaction between the tested antimicrobials. This means that the tested antimicrobials do not have a significantly higher inhibitory effect against *Salmonella* on pork when added in combination which confirms that there is no advantage in using any of the 6 antimicrobials in combination. Regardless, other antimicrobials that are individually effective can be analyzed using the techniques implemented in this study to find potential interactions in the future. Finally, the RSM results were confirmed and validated in a laboratory-scale setting. The pork meat was handled mimicking a real-life case-scenario where the pork was inoculated with *Salmonella*, hung, and sprayed with single and combined antimicrobial compounds using a commercial sprayer. The results from the validation confirmed the RSM results, single antimicrobial treatments (lactic acid, formic acid, cumin, clove, peppermint, and spearmint oil) are just as effective as combinatorial treatments confirming that there was no synergism between the treatments.

Deliverable:

The present study represents a time- and cost-efficient alternative to antimicrobial screening. The protocols and techniques implemented can be used to continue to screen many antimicrobials alone or in combination that can be implemented by the meat industry. The obtained results also show that combining well-known and effective antimicrobials, such as lactic acid, with novel ones, such as essential oils, does not always result in a synergistic antimicrobial effect. However, the tools developed in this study can be used to screen other compounds that can be potentially effective alone and/or in combination to further our understanding of antimicrobial activity and to ensure the food safety of pork products in a time- and cost-effective manner. Furthermore, the techniques that we implemented can be easily replicated in any similar microbiology laboratory, making these tools available to research and industry laboratories alike as more and more potential antimicrobial compounds become available.

Presentations and publications:

1. Resendiz-Moctezuma, C. Corea, P. Stasiewicz, M.J., Miller, M.J. (2023, July 16-19). *Use of a Doehlert Matrix to Identify Antimicrobial Combinations on the Surface of Raw Pork Meat Against Salmonella* spp. [Poster presentation]. International Association of Food

Protection, Toronto, Canada. https://www.foodprotection.org/annualmeeting/archive/2023/events-meetings/.

2. Resendiz-Moctezuma, C, Fonville, A.P.L., Harsh, B.N., Stasiewicz, M.J. and Miller, M.J. 2023. *Use of Doehlert Matrix as a Tool for High-Throughput Screening of Organic Acids and Essential Oils on Miniaturized Pork Loins, Followed by Lab-Scale Validation that Confirmed Tested Compounds Do Not Show Synergistic Effects against Salmonella* Typhimurium. *Foods. 12(21), 4034.* **https://doi.org/10.3390/foods12214034**