Plant-based antimicrobials against *Salmonella* in turkey processing

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Diversity of *Salmonella* serotypes

Salmonella is a major foodborne pathogen causing significant disease and economic burden in the United States (CDC, 2022). A recent report on the source attribution of *Salmonella* using multiyear outbreak surveillance data shows that more than 75% of foodborne illness is attributed to seven categories of food, with a higher contribution from chicken, turkey, and eggs (CDC, 2021). In addition, analysis of the trends of past *Salmonella* outbreaks associated with poultry products indicates the emergence of serotypes with resistance to multiple clinically relevant antibiotics. Several *Salmonella* serotypes have acquired efficient mechanisms to attach and survive on complex foods like poultry meat, resulting in significant challenges to pathogen control. Moreover, some *Salmonella* serotypes have high survival capacity in various environmental conditions adding to the complexity of control approaches. Furthermore, the recent decade witnessed several outbreaks related to poultry meat and meat products, including turkeys, with serotypes like *Salmonella enterica* Heidelberg, *Salmonella enterica* Typhimurium, *Salmonella enterica* Enteritidis, *Salmonella enterica* Reading, *Salmonella enterica* Heidelberg, *Salmonella enterica* Schwarzengrund, and *Salmonella enterica* Hadar (CDC, 2022). Adding to the list, *Salmonella enterica* Agona and *Salmonella enterica* Saintpaul were present from several samples of ground meat, legs, and meat cuts (Erol et al., 2013), manifesting as serotypes of concern. The presence of multiple *Salmonella* serotypes on poultry meat and their unique evolutionary advancement must be considered while choosing appropriate postharvest interventions at the processing facilities.

Postharvest interventions:

Salmonella attached to the carcasses and present in the fecal matter serves as a source of cross-contamination during poultry processing. The contamination can occur at any processing stage, starting from transporting flocks to the slaughterhouse until the final product is packaged. Even though scalding temperature, quality water, airflow, good processing practices, and water pH lower the *Salmonella* load on the carcass to a certain extent, appropriate interventions applied at various processing points can ensure a better microbiologically safe product (USDA FSIS, 2021). Based on the USDA FSIS guidelines, interventions focusing against *Salmonella* can be broadly classified as chemical, biological, and physical methods. Chemical interventions include chlorine, acidified sodium chlorite, trisodium phosphate, organic acids and oxidizers, and quaternary ammonium compounds. The use of bacteriophage targeting *Salmonella* can be a unique

biological intervention. Electrolyzed oxidizing water, high-pressure pasteurization, and ionizing radiation are USDA-recommended physical interventions against *Salmonella*. The presence of organic matter, pH range, and high cost associated with infrastructure and maintenance are the major drawbacks to applying some of these interventions (USDA FSIS, 2021). Plant-based antimicrobials can be viable options to address the setbacks with the currently approved interventions since they could maintain their activity at a wide range of pH, organic content, and temperatures. This article discusses the outcomes of essential oil research in turkey processing and their potential implications for the industry's future.

Essential oils as postharvest interventions:

The application of essential oils at different stages of poultry processing has been actively investigated in the past few years. As with other chemical interventions, essential oils can be tested for spraying or dipping applications. Most of the essential oil-based studies revolve around dipping at scalding and chilling stages, given that dipping provides more contact area and prolonged duration of action of essential oils or their ingredients. Recently, the efficacy of three essential oils, namely, eugenol, carvacrol, and beta resorcylic acid, against *Salmonella enterica* Heidelberg and *Salmonella enterica* Enteritidis were compared with that of chlorine and peracetic acid, two USDA recommended antimicrobials in the poultry processing, at both scalding and chilling conditions on chicken wings. The study demonstrated that each tested essential oil reduced *Salmonella* counts in combination with peracetic acid, with carvacrol showing overall best results at scalding and chilling conditions (Nair et al., 2020). An extensive study using carvacrol against *Salmonella enterica* Enteritidis on organic chicken carcass resulted in significant reductions at chilling temperature, more than twice the reduction obtained with 200 ppm peracetic acid under similar conditions (Nair et al., 2021).

Our previous studies using a combination of 1% *trans*-cinnamaldehyde with 500 ppm peracetic acid in scalding conditions resulted in more than 2 $\log_{10}$ CFU/g reductions of *Salmonella enterica* Heidelberg on chicken drumsticks along with a complete reduction in scalding water (Peichel et al., 2019). Similarly, a study using 2% lemongrass essential oil against *Salmonella enterica* Heidelberg on chicken skin resulted in nearly 4 and 2.5 $\log_{10}$ CFU/sample reductions in scalding and chilling conditions, respectively (Dewi et al., 2021). Studies on plant-derived antimicrobials like lemongrass essential oil or *trans* cinnamaldehyde as a direct additive in ground turkey meat resulted in more than 2.5 $\log_{10}$ CFU/g reductions of *Salmonella enterica* Heidelberg after a storage period of 7 days at 4°C (Dewi et al., 2022). In the context of these results from our lab, our next aim was to identify and study essential oils with multiple active antimicrobial components for their efficacy against *Salmonella* at different processing stages.

Pimenta essential oil:

Pimenta essential oil is the source of several beneficial plant-derived antimicrobial compounds obtained from the pimenta tree, belonging to the *Myrtaceae* family. This oil, also known as allspice oil, is approved as a generally recognized as safe (GRAS)-status

compound by the FDA (FDA, 2016). Eugenol, β myrcene, and E caryophyllene are three major antimicrobial components extracted from different parts of this plant (Samuel Mérida-Reyes et al., 2020). It showed antibacterial activity against a broad spectrum of pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella enterica*, *Streptococcus*, *Candida*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterococcus faecalis* (Milenković et al., 2020; Lowe et al., 2017; Lorenzo-Leal et al., 2019; Ismail et al., 2020). The presence of phenolic compounds and flavonoids in pimenta essential oil could also contribute to its antioxidant properties (Milenković et al., 2020).

Published reports from our lab indicate that pimenta essential oil and its nanoemulsion can be used against multidrug-resistant *Salmonella enterica* Heidelberg (Nair and Kollanoor Johny, 2017). This study tested the compound as both scalding and chilling tank additives against *Salmonella enterica* Heidelberg on turkey skin. Three different concentrations of pimenta essential oil were tested at 3 time points and found that the antibacterial effect was concentration and time-dependent. Approximately 2 $\log_{10}$ CFU/inch^2 reductions of *Salmonella* were obtained when pimenta essential oil was used as a scald additive, whereas 2.4 $\log_{10}$ CFU/inch^2 reductions were obtained in chilling water for 5 minutes. In the same study, the reduction was maintained for 48 hours under storage at 4^{o}C. It was also found that pimenta nanoemulsion resulted in a comparable decrease in *Salmonella enterica* Heidelberg populations on turkey skin as with pimenta essential oil treatments (Nair and Kollanoor Johny, 2017).

A study determining the efficacy of pimenta essential oil against *Salmonella enterica* Reading showed promising effects for both direct and indirect applications in turkey processing (Peichel et al., 2021). In the direct application study, 0.25% of pimenta essential oil was directly added into ground turkey inoculated with *Salmonella enterica* Reading at two different inoculation doses. In both these studies, pimenta essential oil at 0.25% reduced more than 2 $\log_{10}$ CFU/g at day 0 and further maintained the reduction at a storage temperature of 4°C until day 7. Adding 0.25% of pimenta essential oil in water kept at 4°C as a post-chill dip for 30 seconds before grinding caused a reduction of approximately 2 $\log_{10}$ CFU/g *Salmonella enterica* Reading in ground turkey compared to controls (Peichel et al., 2021). Similarly, a recent study from our lab found that 2% pimenta essential oil can be used as a direct additive to obtain significant reductions of *Salmonella enterica* Agona and *Salmonella enterica* Saintpaul in ground turkey meat (Manjankattil et al., 2021). When tested as an antimicrobial additive for chilling purposes, the essential oil significantly reduced *Salmonella* recovery, comparable with peracetic acid. It was also noted that a decrease of approximately 1.5 and 3.5 $\log_{10}$ CFU/sample of the combinations of *Salmonella enterica* Agona and *Salmonella enterica* Saintpaul on meat and skin were obtained when dipped in chilling water containing both 2.5% pimenta essential oil and 500 ppm peracetic acid. Neither the direct nor the indirect application of pimenta essential oil affected the color, pH, or chemical attributes such as the oxidative stability of turkey meat over 7 days of storage at chilling conditions (Manjankattil et al., 2021).

The mechanism of action of pimenta essential oil is not reported yet. However, like other known essential oils, it can cause damage to the cell membrane and its ATP

machinery and thus cell death (Burt., 2004; Dewi et al., 2021). Moreover, the primary component of pimenta essential oil, eugenol, was proven to downregulate pathogenic genes of *Salmonella enterica* Enteritidis (Kollanoor-Johny et al., 2012; Johny et al., 2017).

Conclusion:

The diversity of *Salmonella* serovars and the emergence of some of them as causative agents of foodborne outbreaks have demanded research into developing innovative interventions such as plant-based antimicrobials. Plant options present unique opportunities to explore active components present in the essential oils with multiple mechanisms of action, reducing the chances of the development of resistance in bacteria. Our lab has been conducting extensive research on the efficacy of pimenta essential oil and other essential oils against different *Salmonella* serotypes, including outbreak strains of emerging serovars associated with turkey production and processing. The outcomes summarized in this article suggest consideration of essential oils for postharvest safety of turkey products, improving the industry's sustainability. We are scaling up the studies in processing plants before recommending their usage.

Acknowledgment

The authors would like to acknowledge the MnDRIVE GFV graduate fellowship 2020-2021; Minnesota Department of Agriculture Grant #81824 FY20-21; the USDA National Needs Fellowship (#2016-38420-25285); Minnesota Agricultural Experimentation Station Project # MIN-16-120, and USDA NIFA OREI Project #2017-51300-26815 for supporting the graduate training and research reported in this document.

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