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Evaluation of The Antimicrobial Effect of Aqueous Extracts of Plants or Fruit Dipping Solutions on Raw Meats Inoculated with *Salmonella* Enteritidis

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ABSTRACT

Meat and meat products are subject to microbial deterioration, leading to safety and quality issues. Several plants derived essential oils (EOs) can be effectively used as natural alternative antimicrobial agents in meat. The objectives of this study were to evaluate the antimicrobial effect of 4 plants or fruit aqueous extracts (AE) dipping solutions on raw meats (chicken, pork, and beef) inoculated with Salmonella Enteritidis (SE). The AE of thyme was more effective in killing SE in poultry meat, followed by pork meat. Thyme and garlic had no bactericidal effect on beef. The AE of grapefruit and oregano had a bactericidal impact on all three types of meats evaluated. All four AE significantly reduced SE in chicken and pork meat when compared with the control group. Only beef meat treated with grapefruit and oregano AE significantly reduced SE. The results of these experiments suggest that dipping raw chicken in AE of plant and fruit can significantly reduce populations of SE.

Keywords
Beef, Chicken, Essential oils, Pork, *Salmonella* Enteritidis.

Introduction
The poultry, pork, and beef industries have the challenge of controlling *Salmonella* spp. within processing and manufacturing facilities [1]. Infection and contamination by *Salmonella* on live animals and carcasses can take place during transport and processing [2]. Fecal bacteria from the gastrointestinal tract are the main source of carcass contamination following processing [3]. Hence, reliable methods are required to reduce populations of spoilage bacteria and foodborne enteropathogens. Chlorine (sodium hypochlorite) is commonly used during processing to reduce pathogen loads on carcasses. However, chlorine effectively reduces *Salmonella* and *Campylobacter* by as much as 1 to 2 log_{10} on poultry carcasses [4].

Nevertheless, organic matter such as blood or fat can neutralize chlorine, making it ineffective. Failure to optimize the disinfectant properties of chlorine (improper pH, concentration, or composition of incoming water) may reduce its efficacy, and chlorine treatment produces toxic gas and trichloramine [5]. Hence, alternative methods to disinfect meat carcass are needed. Essential oils (EOs) are natural products extracted from plant material (flowers, buds, herbs, roots, leaves, or fruits). Their use as natural preservatives in many foods is gaining interest because of their antibacterial, antifungal, antioxidant, and anti-carcinogenic properties [6]. Phenolic compounds, such as carvacrol, eugenol, thymol, and limonene, are mainly responsible for the antimicrobial activity of EOs to increase cell membranes' permeability and lead to loss of cellular constituents.

The combination of heat treatment and essential oils and their components has been researched and used to reduce microbial contamination in food, mainly because of the interesting synergistic effects [7]. This study's objectives were to evaluate the antimicrobial effect of aqueous extracts of plants or fruit dipping solutions on raw meats (chicken, pork, and beef) inoculated with *Salmonella* Enteritidis and incubated at 37 ºC for 24 hours.

Materials and Methods

Meat samples

Forceps and scissors were used to remove strips of chicken aseptically, pork, and beef (approximately 1 cm^3) purchased from a local supermarket.

Bacterial strain

A poultry isolate of *Salmonella enterica* subspecies *enterica* serovar Enteritidis (SE) was used for all experiments. This isolate was selected for resistance to nalidixic acid (Sigma, St. Louis, MO). The amplification and enumeration protocol for these isolates has previously been described [8].

Salmonella Enteritidis culture preparation

A frozen aliquot of SE was inoculated into 10 mL of brain heart infusion (BHI) broth (Difco, Sparks, MD, USA) and incubated at 37°C for 24 h in a shaking incubator (New Brunswick Scientific, Edison, NJ, USA) at 200 rpm. After 24 h, 10 mL of fresh BHI was inoculated with ten μL of this culture, vortexed, and incubated at 37°C for 18 h at 200 rpm to ensure that the bacterial culture was in the exponential growth phase. Finally, 10 mL of fresh BHI was inoculated with 20 μL of the 18 h culture to concentrate approximately 10^8 cfu/mL.

Natural antimicrobial dipping solutions

Screening of 4 plants or fruit aqueous (AE) extracts and acetic acid against SE was conducted. These extracts were obtained from thymus (*Thymus vulgaris);* grapefruit (*Citrus* × *paradisi*); garlic (*Allium sativum*), and oregano (*Origanum vulgare*). The AE was prepared by soaking 50 g of leaves or fruit in 200 ml distilled water 24 h individually. Materials were ground in a blender (Variable-speed blender JZ-04244-85; Cole-Parmer., Vernon Hills, United States) for 30 seconds and the suspension was filtered two times through a filter paper Nº 1 (Filter-paper 1001-055 grade 1; Whatman., Maidstone, United Kingdom) as described by Quevedo et al., 2010 [9].

Experimental design

Meat samples were dipped into a suspension of 10^8 cfu/mL of SE (n = 3) for 30 seconds. Samples were then removed and dipped into a solution of either phosphate-buffered saline (PBS) control; or each of the AE washes solutions for an additional 30 s as described previously [10]. Control and treated samples were placed in individual sample bags and incubated at 37°C for two hours. Following incubation, control and treated samples were removed from the incubator and cultured separately. Briefly, meat samples were homogenized within sterile sample bags using a rubber mallet. Sterile saline (5 mL) was added to each sample bag, and hand stomached. Serial dilutions were spread plated on brilliant green agar (Becton, Dickinson, and Co. Sparks, MD) plates containing 25 µg/mL novobiocin (Sigma, St. Louis, MO) and 20 µg/mL nalidixic acid for SE. Each sample was plated in triplicate. The plates were incubated at 37°C for 24 h, and viable colonies were observed and enumerated.

Statistical analysis

All data were analyzed using Analysis of Variance (ANOVA) with further separation of significantly different means using Duncan's Multiple Range test using SAS [11]. Significant differences were reported at $P < 0.05$.

Results and Discussion

The meat industry is constantly confronted by the demand of safe and high-quality meat and meat products. Furthermore, the recent demand for ready-to-eat meat products and novel notions of all-natural has rapidly increased [12]. However, meat and meat products are likely to microbial contamination due to the essential nutrients. Heat treatment is the most used method in the food industry to eliminate pathogenic microorganisms from food products. However, traditional pasteurization cannot eliminate some enteropathogens [13]. Another method used to reduce microbial contamination in food is chemical preservation. Essential oils, from aromatic plant materials, including flowers, buds, roots, bark, and leaves, are one of the best alternatives to chemical compounds, given their substantial antimicrobial activities. Hence, EOs from oregano, rosemary, thyme, clove, balm, ginger, basilica, coriander, marjoram, and basil have shown a more significant potential to be used as an antimicrobial agent in the meat industry [14]. The antimicrobial activity of EOs has been consistently linked to phenolic constituents such as carvacrol, eugenol, and thymol [15]. While the antimicrobial activity of EOs is not attributable to one specific mechanism, EOs can degrade the cell wall, disturb the membrane's phospholipid and proteins components, and disrupt the electron flow [14]. The main compound of EOs are terpenes and terpenoids.

In contrast, the other consists of aromatic compounds (phenylpropanoids), and several studies have demonstrated the antibacterial activity of essential oils against several enteric pathogenic bacteria [16,17]. Table 1 summarizes the antimicrobial effect of aqueous extracts of plants or fruit dipping solutions on different types of meat inoculated with *Salmonella* Enteritidis.

Treatment	Chicken	Pork	Beef
Control PBS	$9.25 \pm 0.43^{a,x}$	$9.50 \pm 0.86^{a,x}$	$9.57 \pm 1.0^{a,x}$
Thyme	$5.43 \pm 0.75^{c,y}$	$6.53 \pm 0.80^{b,y}$	$8.10 \pm 1.52^{a,x}$
Grapefruit	$6.52 \pm 0.19^{a,y}$	$6.69 \pm 0.53^{a,y}$	$6.23 \pm 0.26^{a,zy}$
Garlic	$5.10 \pm 0.17^{b,zy}$	$4.82 \pm 0.30^{cb,z}$	$8.20 \pm 1.90^{a,x}$
Oregano	$6.01 \pm 0.52^{a,y}$	$6.31 \pm 0.15^{a,y}$	$6.70 \pm 0.17^{a,y}$

Table 1: Antimicrobial effect of aqueous extracts of plants or fruit dipping solutions on different types of meat inoculated with *Salmonella* Enteritidis

Data expressed as $\log_{10}$ mean ± standard error.

^{abc} Values within rows for type of meat, or ^{xyz} Values within treatment column, with different superscripts differ significantly ($P < 0.05$).

The AE of thyme was more effective in killing SE in poultry meat, followed by pork meat. However, thyme had no bactericidal effect on beef. Similar results were noted in garlic. On the other hand, the AE of grapefruit has a bactericidal impact on all three types of meats evaluated. The same trend was observed with the oregano AE (Table 1). When assessing the antimicrobial effect of AE of plants or fruit by meat type, in chicken meat, all four AE significantly reduced SE when compared with the control group. Similar results were observed in pork meat; however, garlic showed the lowest SE numbers of all experimental groups. D-limonene is a vital flavor component in citrus essential oil (e.g., lemon, lime, orange, mandarin, etc.), generally regarded as safe (GRAS) [18].

Interestingly, only beef meat treated with grapefruit and oregano AE significantly reduced SE, whereas thyme and garlic did not affect SE (Table 1). Treatment of carcass rinse that decrease *Salmonella* by two $\log_{10}$ cfu/ml are considered *adequate* [19]. In these experiments, the AE wash solution showed significant antibacterial activity against SE, and an important foodborne pathogen commonly implicated in meat processing (Table 1).

Overall, the results of these experiments suggest that dipping different raw meat in AE of plant and fruit can greatly reduce populations of SE when incubated at 37ºC. Future studies will be directed at determining the effect of these AE solutions on the texture, color, oxidative stability, pH, as well as their effect on shelf life of these products over time using refrigeration temperatures.

Authors' contributions

AR-G, JEM-B, GT-I, Conceptualization; CG-C, OP-R, Data curation; HV-H, JA-L, FC, Formal analysis; AR-G, JEM-B, Investigation and Methodology; JEM-B, OP-R, GT-I, Supervision, Validation and Visualization; AR-G, JEM-B, Writing - original draft; GT-I, XH-V, Writing, review and editing.

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