Antimicrobial efficacy of white mustard essential oil and carvacrol against *Salmonella* in refrigerated ground chicken

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ABSTRACT Essential oils in combination with other antimicrobials can be added to food products to reduce the levels of target microbes lower than the infectious dose required to cause human illness. The purpose of this study was to investigate the antimicrobial efficacy of white mustard essential oil (**WMEO**) and carvacrol against *Salmonella* in ground chicken stored at 4 and 10°C. At 4°C, 0.75% WMEO +0.1% carvacrol treatment had significantly lower ($P < 0.05$) *Salmonell*a at the end of 12-day storage than the control, which contained no antimicrobials. A combination of 0.75% WMEO and 0.01% carvacrol had a bacteriostatic effect against *Salmonella* in ground chicken samples stored at 10°C for 7 D. The application of the antimicrobials controlled the growth of *Salmonella* by delaying the exponential phase at temperature abuse and reducing levels of *Salmonella* to less than the positive control at 4°C. The use of WMEO and carvacrol shows potential in reducing levels of *Salmonella* under refrigerated conditions and controlling its growth under temperature abuse conditions in raw poultry products. Further research is needed to investigate the toxicity of the compounds and the most efficient way to apply it to a food product to maximize antimicrobial activity.

Key words: essential oil, *Salmonella*, chicken, antimicrobial, natural

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INTRODUCTION

Foodborne nontyphoidal *Salmonella* causes approximately 1 million illnesses and 378 deaths per year in the United States (Hoffman et al., 2015). In particular, *Salmonella* outbreaks have been associated with consumption of contaminated poultry and eggs, while at the same time, poultry meat is becoming increasingly popular among US consumers and is expected to reach 94.3 lbs/capita consumption in 2019 (National Chicken Council, 2019). Moreover, consumers are concerned about the use of artificial and synthetic ingredients in foods (Burt, 2004). This consumer trend presents a unique challenge to the poultry industry as they have to ensure safety of poultry and poultry products while complying with consumer demands. Therefore, there is a need to explore natural alternatives to the artificial antimicrobials that are effective in ensuring food safety of poultry products.

Essential oils (**EO**) are natural compounds derived from plants and have been studied for their various chemical and biological properties, including antimicrobial activities (Cosentino et al., 1999; Burt, 2004; Erkan et al., 2008; Teixeira et al., 2013). White mustard EO (**WMEO**), derived from the plant *Sinapis alba* L., is an essential oil that has shown in vitro antimicrobial activity against *Salmonella* Enteritidis, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, and *Campylobacter jejuni* (Monu et al., 2014; Techathuvanan et al., 2014). An in vitro study in nutrient broth showed that WMEO at a concentration of 0.84% reduced counts of *Salmonella* Enteritidis by 4 logs, and at 0.42%, it had a bacteriostatic effect (Monu et al., 2014). White mustard essential oil shows potential as a natural preservative to be used in variety of products because of its broad antimicrobial activity.

Isothiocyanates are the main bioactive compounds with antimicrobial activity that are commonly derived from mustard and other plants found in the Brassicaceae family. In white mustard, the form of isothiocyanates produced in the plant is p-4-hydroxybenzyl isothiocyanates (**HBITC**), which is the main antimicrobial component of WMEO (Ekanayake et al., 2012; Angelino et al., 2015). Allyl isothiocyanate, derived from black and brown mustard, is another widely known isothiocyanate that possesses broad antimicrobial activity (Delaquis and Mazza, 1995). Much research has been carried out

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on this compound; however, it is highly volatile and possesses a strong sensory property, particularly at warm temperatures. This strong sensory profile may negatively affect the flavor of food to which it is applied, which is a limiting factor of allyl isothiocyanates in food use (Delaquis and Mazza, 1995). Another limiting factor of EO in general is their low dispersibility in food caused by their hydrophobic nature, which can lead to reduced antimicrobial activity (Shah et al., 2012). When used in food products, EO require higher concentrations than what is used in vitro to inhibit food pathogens. This has been a major issue for the broad use of EO in foodstuffs. EO have been used in combination with other antimicrobials to reach a synergistic effect which has been proven to be effective in reducing the concentrations needed to inhibit foodborne pathogens. Thymol and carvacrol are the active antimicrobial compounds that are found in thyme and oregano. These purified compounds have well-documented antimicrobial activity against a variety of foodborne bacteria but are costly and have extremely strong flavor and odor. Therefore, it is beneficial to determine if they can be combined with other plant extracts. It has been previously shown that thymol and carvacrol have increased efficacy when combined with compounds such as linalool (a terpene found in *Ocimum basilicum*) (Bouzouita et al., 2003) or cinnamaldehyde.

White mustard EO has been shown to be more stable at refrigeration and frozen temperatures making its application to a refrigerated or frozen product ideal (Ekanayake et al., 2006). The main antimicrobial component 4-HBITC has been evaluated in microwaved chicken pot pie. Not only was there a reduction of *Salmonella* spp. but at the end of the cook time, the presence of 4-HBITC was not detected (David et al., 2013). This further illustrates the potential of this compound to be used in controlling *Salmonella* spp. in a raw non–ready-to-eat poultry product without changing the sensory profile.

Raw poultry products are stored under refrigeration at 4°C or lower to prevent the growth of *Salmonella.* However, temperature abuse that could occur during improper refrigerated storage in restaurants and homes could render the product to temperature higher than 4°C, thus increasing *Salmonella* food safety risk. In addition, meal-kit delivery services are becoming increasingly popular, and there is the potential for temperature abuse during the shipping of these products. Murphy et al. showed that even thermally injured *Salmonella* cells are capable of growth at 8°C (Murphy et al., 2001). The purpose of this study was to evaluate the antimicrobial efficacy of WMEO in combination with carvacrol against *Salmonella enterica* and in ground chicken stored at at the recommended 4°C and and temperature abuse condition of 10°C.

MATERIALS AND METHODS

Culture Preparation

Salmonella serovars Enteritidis, Typhimurium, Heidelberg, Kentucky, and Montevideo (obtained from Nelson Cox, USDA-ARS, Athens, Georgia) were used in this study. Frozen cultures of each serovar were grown in 9 mL tryptic soy broth with an initial pH of 7.2 (Neogen, MI) at 37°C for 18 to 20 h. Nalidixic acid (**NAL**)–resistant (up to 100 ppm) serovars of each strain were used in a 5-serovar cocktail. The bacterial population of overnight cultures of each serovar was determined in 100 ppm NAL tryptic soy broth before performing the experiment. Individual cultures were washed twice by centrifuging at 9,391 × *g* for 2 min (Eppendorf centrifuge 5424R, Hauppauge, NY). Pellets were resuspended in 0.1% peptone water (Neogen, MI). The cocktail was prepared by combining washed cultures of *Salmonella* Typhimurium, Heidelberg, Montevideo, Kentucky, and Enteritidis to achieve a final bacterial load of ca. 10^7 CFU/mL (ca. 2×10^6 CFU/mL of each individual serovar).

Beforeexperiments, inoculum levels of each organism were verified in triplicate by serially diluting and spread plating overnight cultures on tryptic soy agar to determine the CFU/mL after 20 h of incubation at 37°C.

Application of Antimicrobials to Ground Chicken

Antimicrobial solutions were made by diluting WMEO and carvacrol (>99%; Acros Organics, NJ) solutions with propylene glycol (Amresco, LLC, OH) into stock solutions of 37.5% WMEO, 25% WMEO, 0.5% carvacrol, 37.5% WMEO with 0.5% carvacrol, and 25% WMEO with 0.5% carvacrol on the day of the experiment.

Ground chicken consisted of boneless chicken thighs with skin from the Auburn University Poultry Science Research Unit. Chicken thighs were ground using a Mini-32 Biro grinder (MFG Co., OH) and placed into aluminum trays. The positive and negative controls both consisted of 2,100 g and 1,800 g of ground chicken for the 4°C and 10°C trials, respectively. As for the treatments, 1,800 g and 1,500 g of ground chicken were placed in aluminum trays for 4°C and 10°C trials, respectively. Meat was inoculated by adding 1 mL of the NAL-resistant 5-serovar *Salmonella* cocktail per 100 g of ground chicken and was manually mixed in aluminum trays. After mixing, aluminum trays were stored in the refrigerator at 4°C for 30 min to allow for bacterial attachment.

There were 5 treatments applied to the ground chicken in addition to the positive and negative control. Antimicrobial treatments applied were as follows: 0.75% WMEO, 0.5% WMEO, 0.75 + 0.1% carvacrol, 0.5% WMEO +0.1% carvacrol, and 0.1% carvacrol. Propylene glycol was added to the positive and negative controls to ensure it did not have an antimicrobial effect against *Salmonella.* In addition, to account for the moisture added by the inoculum in the treatments, 1 mL of 0.1% peptone water per 100 g of chicken was added to the negative controls. Individual samples were prepared on the first day of each trial by taking 100 g from each treatment batch and sealing with polyethylene film in

styrofoam trays (Genpak, Glens Falls, NY) with DriLoc DLSA 100 "5.5 × 7.0" absorbent pads (Novipak Reading, PA).

Moisture, Fat, and pH Analysis of Ground Chicken

The forced air-dry oven method, AOAC 950.46B, was used to analyze the moisture content of ground chicken for each trial before application of the treatments. Approximately, 5 g of sample was weighed out and dried at 95°C to 105°C for 16 to 18 h in replicates of 3. Dried samples were then placed into a desiccator to allow cooling for 1 h. After cooling, weight was recorded. Dried samples (2–3 g/sample) were then analyzed for crude fat using AOAC 960.39 via the Goldfisch method using petroleum ether (50 mL) (Macron Fine Chemicals, Radnor, PA) in triplicates.

The pH of ground chicken samples was measured before and throughout the storage at both 4°C and 10°C. The pH meter was calibrated before taking measurements. Measurements were performed on each tray using an Accumet XL15 (Fisher Scientific, NH) resulting in 3 pH values for each treatment per sampling day.

Microbiological Analysis

The 4°C trial was monitored for 12 D, sampling every 2 D by taking individual 25 g portions of ground chicken from each triplicate tray and adding each 25 g sample to a stomacher bag filled with 225 mL of buffered peptone water (**BPW**) (Hardy Diagnostics, CA) and stomached at 230 rpm for 2 min. After stomaching, 10-fold serial dilutions were made using BPW and plated on 100 ppm NAL TSA (Hardy Diagnostics). Plates were incubated at 37°C for 24 h before colonies were counted and recorded. Sampling procedure was the same for the 10°C trial but with an 8-day storage period. In addition, sampling was performed every 2 D with additional sampling on the seventh day. A *Salmonella*-enrichment method as per the USDA was performed on negative samples on day 0 to verify that the chicken used was free of *Salmonella* (USDA, 2019). Ground chicken was stomached with 225 mL of BPW and incubated at 35°C ± 2°C for 20 to 24 h as per MLG 4.09 procedure 4.5.2 (USDA, 2019). After incubation, 0.5 mL aliquots from each stomacher bag were dispensed in 10 mL of tetrathionate broth and incubated at 42°C ± 0.5°C for 22 to 24 h in accordance with procedure 4.6 in the MLG 4.09 by the USDA. Samples were then streaked on XLT4 agar for identification of presumptive *Salmonella* (USDA, 2019). Each treatment was conducted in triplicate, and experiments were performed 3 times.

Statistical Analysis

Before statistical analysis, all microbiological data were transformed into $\log_{10}$ scale. All data shown in tables are reported as mean ± SD. Statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC). ANOVA was performed using the Student–Newman–Keuls test to compare the means at a confidence interval of 95%.

RESULTS AND DISCUSSION

Antimicrobial Effect of WMEO With Carvacrol in Ground Chicken

No *Salmonella* were detected in the negative control throughout storage; there were no colonies present after direct spread plating (in which the the detection limit was 2 log CFU/g), and furthermore, no viable cells were detected when enrichment procedures were applied. Table 1 shows survival of *Salmonella* (log CFU/g) in ground chicken during 12 D of storage at 4°C in the presence of different concentrations of WMEO and carvacrol. All treatments, with the exception of 0.1% carvacrol alone, had significantly lower counts of *Salmonella* ($P < 0.05$) than the positive control throughout 12 D of storage. Compared with control samples, there was an overall difference of 0.5 to 0.6 log CFU/g *Salmonella* at day 10 and 12 of storage when WMEO with and without carvacrol were applied to the ground chicken at 4°C but not when carvacrol alone was applied. The combination of WMEO and carvacrol shows potential in being used together as it lowered the concentrations of *Salmonella* compared with the positive control. *Salmonella* populations did not change over the 12-day storage period indicating the effectiveness of refrigerated temperatures (4°C) in controlling the pathogen growth in chicken meat (Pintar et al., 2007).

Table 2 shows the effect of WMEO and carvacrol over an 8-day storage period at 10°C. Results show that at

Table 1. Antimicrobial effect of WMEO and carvacrol on *Salmonella* spp. in ground chicken at 4°C.

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
Control	5.41 ± 0.33^{A}	5.15 ± 0.33^{A}	5.19 ± 0.40^{A}	5.14 ± 0.35^{A}	5.08 ± 0.34^{A}	5.10 ± 0.34^{A}	5.13 ± 0.34^{A}
0.75% WMEO		4.83 ± 0.28^{C}	4.78 ± 0.24^{C}	4.60 ± 0.44^{C}	4.53 ± 0.35^{C}	4.60 ± 0.36^{C}	$4.52 \pm 0.31^{B,C}$
0.5% WMEO		4.93 ± 0.22^{B}	4.90 ± 0.25^{B}	4.75 ± 0.35^{B}	4.81 ± 0.28^{B}	4.78 ± 0.35^{B}	4.48 ± 0.53^{B}
0.75% WMEO +0.1% carvacrol		4.58 ± 0.39^{E}	4.46 ± 0.31^{E}	4.52 ± 0.37^{D}	4.36 ± 0.39^{D}	4.30 ± 0.47^{D}	4.39 ± 0.32^{C}
0.5% WMEO +0.1% carvacrol		4.71 ± 0.40^{D}	4.64 ± 0.33^{D}	4.58 ± 0.33^{C}	4.58 ± 0.40^{C}	4.58 ± 0.39^{C}	$4.59 \pm 0.45^{B,C}$
0.1% carvacrol		5.21 ± 0.30^{A}	5.16 ± 0.36^{A}	5.16 ± 0.33^{A}	5.15 ± 0.32^{A}	5.10 ± 0.28^{A}	5.09 ± 0.35^{A}

[A-D]Within each column, values with different letters are significantly different ($P < 0.05$) N = 9.
Abbreviation: WMEO, white mustard essential oil.
Values are mean log CFU/g ± SD.

Table 2. Antimicrobial effect of WMEO and carvacrol on *Salmonella* spp. in ground chicken at 10°C.

	Day 0	Day 2	Day 4	Day 6	Day 7	Day 8
Control	5.18 ± 0.11^{A}	6.12 ± 0.16^{A}	6.92 ± 0.12^{A}	7.62 ± 0.46^{A}	8.02 ± 0.16^{A}	8.05 ± 0.20^{B}
0.75% WMEO		5.07 ± 0.14^{D}	5.43 ± 0.48^{B}	5.87 ± 0.73^{C}	5.54 ± 0.38^{D}	6.80 ± 0.80^{D}
0.5% WMEO		5.39 ± 0.32^{C}	5.53 ± 0.38^{B}	6.11 ± 0.44^{B,C}	6.38 ± 0.33^{C}	7.26 ± 0.55^{C}
0.75% WMEO +0.1% carvacrol		4.98 ± 0.22^{D}	5.08 ± 0.44^{C}	5.44 ± 0.59^{D}	5.53 ± 0.39^{D}	6.46 ± 0.81^{E}
0.5% WMEO +0.1% carvacrol		5.12 ± 0.15^{D}	5.40 ± 0.56^{B}	6.22 ± 0.41^{B}	6.34 ± 0.35^{C}	7.00 ± 0.30^{C,D}
0.1% carvacrol		5.92 ± 0.48^{B}	6.87 ± 0.13^{A}	7.62 ± 0.47^{A}	7.79 ± 0.27^{B}	8.42 ± 0.39^{A}

$^{A-D}$Within each column, values with different letters are significantly different ($P < 0.05$) N = 9.
Abbreviation: WMEO, white mustard essential oil.
Values are mean log CFU/g ± standard deviation.

the highest concentration of antimicrobial (0.75% WMEO + 0.1% carvacrol), a bacteriostatic affect was observed up to the seventh day of storage at temperature abuse of 10°C. After 8 D of storage at 10°C, a 3 log CFU/g increase in *Salmonella* was observed in the positive control. The behavior of *Salmonella* growth in this study is similar to previous studies in which a 2 to 3 log CFU/g increase has been seen with *Salmonella* at temperature abuse of 10°C in chicken thighs and breast (Pintar et al., 2007). As observed in Table 2, 0.75% WMEO, 0.5% WMEO +0.1% carvacrol, and 0.75% WMEO +0.1% carvacrol were statistically different ($P < 0.05$) than 0.5% WMEO after 2 D of storage. Although, over the course of the 8 D, 0.75% WMEO + 0.1% carvacrol was the most effective treatment against *Salmonella* and statistically different than all other treatments on day 2, 4, 6, and 8. In addition, on the final day of storage, all treatments were approximately 1 log CFU/g less than the positive control and 0.1% carvacrol.

Fat, Moisture, and pH of Ground Chicken

Fat content was analyzed as fat is one major food component that greatly reduces the efficacy of EO activity in addition to high moisture content (Gutierrez et al., 2008). The average moisture and fat content for the ground chicken thighs with skin used at 4°C and 10°C were 68.6% moisture and 12.8% fat and 69.0% moisture and 11.9% fat, respectively. As for pH, a range of 6.54 to 6.86 was seen for ground chicken thighs throughout storage at 10°C and 4°C; there was no significant difference in pH between treatments throughout the storage of chicken at both temperatures. These results are similar to what was found in other experiments at 4°C (Brannan, 2008). The pH increased to final reading of 6.7 for the positive control and all treatments on day 8 at 10°C.

CONCLUSION

In comparison with previous studies in nutrient broth, the anti-*Salmonella* efficacies of WMEO were reduced when applied to ground chicken meat. The application of 0.75% WMEO +0.1% carvacrol in ground chicken at 4°C showed a 0.7 log CFU/g reduction after 12 D of storage, whereas all other treatments, except the positive control and 0.1% carvacrol, showed a 0.5 log CFU/g reduction. In addition, the refrigeration temperature had a bacteriostatic effect allowing the antimicrobial to put more stress on the organism to reduce levels up to 1 log CFU/g in all treatments containing WMEO. At 10°C, after 2 D, *Salmonella* grew approximately 1 log CFU/g in the positive control and samples with 0.1% carvacrol. In comparison, the growth of *Salmonella* was controlled for 7 D using a 0.75% WMEO +0.1% carvacrol treatment. At the final day of storage, all treatments containing WMEO had significantly lower levels of *Salmonella* than the control, and treatments with 0.75% WMEO were ca. 2.5 log CFU/g lower than the control. The addition of 0.1% carvacrol did not appear to enhance the efficacy of WMEO. These results illustrate the potential usefulness of WMEO as an antimicrobial in chicken stored at abusive temperatures.

In conclusion, WMEO shows potential as a natural preservative in controlling *Salmonella* in chicken products. However, further research is needed to investigate the toxicity of the essential oil and the most efficient way to apply it to a food product.

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