

Plant Extracts and Essential Oils at Concentrations Acceptable to a Sensory Panel Inactivate *Salmonella* Typhimurium DT104 in Ground Pork

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Abstract

A potential method to inhibit pathogenic bacterial growth in meat is through the introduction of plant-derived antimicrobials. Because these antimicrobials may also adversely affect the sensory characteristics of the meat, the objectives of this study were 1) to define the appropriate concentrations of olive extract, apple extract, oregano oil, and cinnamon oil added to ground pork that are acceptable to a sensory panel, and 2) to determine their antimicrobial activities against *Salmonella* Typhimurium DT104 in inoculated ground pork. Plant extracts were evaluated against two initial inoculum levels (6 and 4 log CFU/g of pork) of *Salmonella*. Sensory tests showed that acceptable concentrations of oregano and cinnamon oils were 0.5% and of olive and apple extracts were 3%, respectively. Ground pork samples were inoculated with *Salmonella*, treated with antimicrobials at various concentrations (0.1% - 0.5% cinnamon and oregano essential oils and 3% - 5% olive and apple extracts), and stored at 4°C for 7 days. Survivors were enumerated at days 0, 3, 5, and 7. Cinnamon oil at 0.5% and olive extract at 3% induced a 1.0 and a 0.9 log CFU/g (from 6-log CFU/g initial inoculum) reduction, respectively, at day 7. At 3%, olive extract showed a 1.06 log CFU/g maximum reduction of *Salmonella* from a 4-log CFU/g initial inoculum. Pork samples containing oregano oil and apple extract did not show a significant reduction compared to the control without the antimicrobials. The results indicate that cinnamon oil and olive extract can potentially be applied at consumer-acceptable concentrations against low levels of *S. Typhimurium* DT104 in ground pork.

Keywords

Essential Oils, Ground Pork, Plant Extracts, *Salmonella* Typhimurium DT104, Sensory Panel

1. Introduction

Salmonella Typhimurium DT104 is well known for its resistance to multiple antibiotics and is one of the major foodborne pathogens worldwide [1] [2] [3] [4] [5]. The organism can be isolated from a wide range of animals, mostly cattle and pigs [4]-[11].

Humans can be infected by *Salmonella* via consumption of contaminated food such as pork sausage [12]. Epidemiological studies in Germany reported that consumption of raw pork products is the major risk factor in sporadic *Salmonella* infections of both adults and children [13] [14].

Meat is an ideal medium for growth and survival of pathogens and spoilage microbes due to its high moisture and nutrient content and moderate pH. Numerous antimicrobial compounds from plant sources, such as essential oils and plant extracts can inhibit microbial growth. Therefore, a potential method of inhibiting pathogenic bacterial growth in meat could be through the introduction of plant-derived antimicrobials. Many studies have demonstrated the antimicrobial activity of plant essential oils and plant extracts against different foodborne pathogens *in vitro* and in ground beef, juice, and vegetables [9] [10] [11] [15]-[24]. While the addition of antimicrobials may improve microbiological safety, it may simultaneously adversely affect the sensory characteristics of the meat [25] [26].

To help overcome this aspect, the objectives of this study were 1) to define the appropriate concentrations of oregano oil (OO), cinnamon oil (CO), olive extract (OE) and apple extract (AE) that are acceptable to consumers when added to ground pork; and 2) to evaluate their antimicrobial activity against *S. Typhimurium* DT104 in ground pork at concentrations acceptable to a sensory panel.

2. Materials and Methods

2.1. Bacterial Culture and Media

Salmonella Typhimurium DT 104 H3278 (provided by Dr. V. K. Juneja, U. S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA) was the target pathogen in the experiments. The stock culture was maintained at -80°C in cryovials (Microbank, Austin, TX). One hundred microliter stock was activated in 9.9 ml tryptic soy broth (TSB; EMD Chemicals, Darmstadt, Germany) and maintained at 4°C with monthly transfers. For each experiment, 100 μl stock culture was taken, mixed with 9.9 ml TSB and grown at 37°C for 18 to 24 hours. The bacterial population was approximately 8 log CFU/ml after overnight incubation. On the day of the experi-

ment, 1 ml overnight culture was taken and mixed with 9 ml of peptone water (PW; Becton, Dickinson and Company, Sparks, MD, USA) to obtain the working culture. In this working culture the bacterial population was approximately 7 log CFU/ml (high inoculum level). For the experiments requiring low inoculum level of *Salmonella*, the overnight culture was further diluted to 5 log CFU/ml working culture. Enumeration for *S. Typhimurium* was performed by diluting in buffered peptone water (BPW; EMD Chemicals), plating on xylose lysine desoxycholate (XLD; EMD Chemicals) agar and counting after incubating the plates at 37°C for 24 - 48 hours.

2.2. Food Product and Plant-Derived Antimicrobials

Ground pork used in the study was provided by the Meat Science Lab (currently the Food Products and Safety Lab) at the University of Arizona. The animals were harvested and processed in a federally inspected plant (Meat Science Lab; Identification No. 966). The fat portion of the ground pork was about 20%. The meat was stored at -20°C and thawed at 4°C for 24 hours before the experiment.

Oregano oil (OE) and cinnamon oil (CO) needed for sensory analysis were purchased from the local grocery stores. According to the labels, the products contained 100% oregano (*origanum*) and 100% cassia bark (Chinese cinnamon) essential oils, respectively. For laboratory experiments, oregano and cinnamon oils were obtained from Lhasa Karnak Herb Company (Berkeley, CA), olive extract (OE) from CreAgri, Inc. (Hayward, CA), and apple extract (AE) from Apple Poly, LLC (Morrill, NE).

2.3. Sensory Panel Test

All the materials used in the sensory panel tests were consumable. The tests were conducted over a period of 4 days. Each day one antimicrobial mixed in the ground pork was tested. Eight panel members (employees in the Meat Science Lab, who were very familiar with meat attributes) participated in each test and evaluated the color, aroma, taste and acceptability differences among three treated and one regular pork samples. The concentrations of CO and OO added to the pork were 0.1%, 0.5% and 1.0%. The concentrations of OE and AE added to the pork were 3%, 5% and 7%.

Raw ground pork (272 g) was mixed with one of the plant essential oils or extracts at appropriate concentrations. The untreated ground pork was used as a control which was labeled with a numerical ID and the identity of this sample was not revealed to the panelists. Each treated sample was also labeled with a specific numerical ID. All the samples were broiled to an internal temperature of 160°F, which was measured using a kitchen thermometer. Panel members evaluated the meat color by vision, aroma by smell, and taste by mastication using an 8-point scale (**Table 1**). Acceptability was also evaluated by mastication using a 7-point scale (**Table 1**).

Table 1. Assigned scores of sensory characteristics evaluated in treated and control non-inoculated ground pork.

Assigned score	Sensory characteristics			
	Color	Aroma	Taste	Acceptability
1	Extremely light	Extremely weak	Extremely bland	Totally unacceptable
2	Very light	Very weak	Very bland	Moderately unacceptable
3	Moderately light	Moderately weak	Moderately bland	Slightly unacceptable
4	Slightly light	Slightly weak	Slightly bland	Neutral
5	Slightly dark	Slightly strong	Slightly strong	Slightly acceptable
6	Moderately dark	Moderately strong	Moderately strong	Moderately acceptable
7	Very dark	Very strong	Very strong	Totally acceptable
8	Extremely dark	Extremely strong	Extremely strong	

2.4. Antimicrobial Activity of Plant Compounds at Consumer Acceptable Concentrations against *S. Typhimurium* in Ground Pork

The concentrations of OO and CO added to the pork were 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. A positive control that was inoculated without any antimicrobials added was also included. Ground pork (60 g) was taken and mixed with one of the test essential oils at appropriate concentrations. The pork was heated on an electric skillet (West Bend Housewares LLC, West Bend, WI) at 70°C for 5 minutes. The main purpose of this step was to reduce the background bacteria present in the pork. This practice also simulated the usual cooking of ground pork by consumers (Food Safety and Inspection Service, 2011). The cooked pork was then cooled to room temperature and 45 g pork was inoculated with 1 ml of 7 log CFU/ml *S. Typhimurium* DT104 working culture. Inoculated samples were homogenized by hand massage and then pummeled using a stomacher (Lab-Blender 400; Seward, London, UK) for 1 min. Samples were stored at 4°C and analyzed for the surviving *Salmonella* populations at 0, 3, 5, and 7 days of storage.

The concentrations of OE and AE added into the meat were 3% each, and a positive control (inoculated but without any added antimicrobials) was also included. Sample preparation procedures were similar to that of the essential oil treatments with one exception. Since the powder extracts were hard to mix with ground pork using a stomacher, they were added and mixed using a spatula during the heating process. The antimicrobial activity of plant extracts against two initial inoculums levels (5 and 7 log CFU/ml of working culture which resulted in 4 and 6 log CFU/g of ground pork) of *S. Typhimurium* was evaluated. Samples were stored at 4°C and analyzed for surviving *Salmonella* populations at 0, 3, 5, and 7 days of storage.

2.5. Microbial Analysis of *S. Typhimurium* in Ground Pork Samples

For determining the surviving populations of *S. Typhimurium* after treatments,

samples (10 g) were pummeled using a stomacher in 90 ml BPW. Appropriate serial dilutions were further done in BPW and aliquots plated on XLD. Plates were incubated at 37°C for 24 - 48 h and enumerated. Control samples were also analyzed similarly.

2.6. Statistical Analysis

Three or more replicates were done for each experiment. Means and standard deviations of surviving bacterial populations were calculated using Excel (Microsoft Corporation, Redmond, WA). Data were analyzed by one-way analysis of variance (ANOVA) using Minitab (Minitab Inc., State college, PA) 16.0 software. The significant differences among treatment means were calculated by Tukey's test ($P < 0.05$).

3. Results and Discussion

3.1. Effect of Plant Antimicrobials on the Sensory Characteristics of Ground Pork

The scores for each sensory characteristic of pork with various concentrations of antimicrobials are shown in **Table 2**. Color scores of OO and CO treated ground

Table 2. Influence of oregano oil, cinnamon oil, olive extract and apple extract on the sensory characteristics of non-inoculated ground pork.

Antimicrobials	Treatment	Scores of different sensory characteristics			
		Color	Aroma	Taste	Acceptability
Oregano oil	0%	3.88 ± 0.83 ^a	4.25 ± 0.89 ^a	4.38 ± 0.52 ^a	4.8 ± 1.2 ^a
	0.1%	4.3 ± 1.3 ^a	4.8 ± 1.3 ^a	4.13 ± 0.83 ^a	5.0 ± 1.1 ^a
	0.5%	4.8 ± 1.3 ^a	6.6 ± 1.2 ^b	7.0 ± 1.2 ^b	1.8 ± 1.2 ^b
	1.0%	3.88 ± 0.99 ^a	6.6 ± 1.2 ^b	7.88 ± 0.35 ^b	1.13 ± 0.35 ^b
Cinnamon oil	0%	4.9 ± 1.5 ^a	3.8 ± 1.7 ^a	3.4 ± 1.2 ^a	4.5 ± 1.9 ^{ab}
	0.1%	3.5 ± 1.1 ^a	4.6 ± 1.6 ^{ab}	4.38 ± 0.92 ^a	5.1 ± 1.6 ^a
	0.5%	4.88 ± 0.64 ^a	6.0 ± 1.1 ^b	6.3 ± 1.2 ^b	2.4 ± 1.4 ^{bc}
	1.0%	4.1 ± 1.5 ^a	6.3 ± 1.6 ^b	7.38 ± 0.92 ^b	1.6 ± 1.1 ^c
Olive extract	0%	4.3 ± 1.6 ^a	3.25 ± 0.89 ^a	3.9 ± 1.1 ^a	4.8 ± 1.9 ^a
	3%	3.5 ± 1.7 ^a	6.1 ± 1.5 ^b	7.57 ± 0.79 ^b	1.00 ± 0.00 ^b
	5%	5.25 ± 0.89 ^a	7.13 ± 0.99 ^b	7.75 ± 0.46 ^b	1.0 ± 0.0 ^b
	7%	5.5 ± 1.1 ^a	7.25 ± 0.46 ^b	7.75 ± 0.46 ^b	1.0 ± 0.0 ^b
Apple extract	0%	3.6 ± 1.2 ^a	4.3 ± 1.4 ^a	4.0 ± 1.3 ^a	4.4 ± 1.9 ^a
	3%	4.63 ± 0.92 ^{ab}	6.13 ± 0.99 ^b	6.1 ± 1.3 ^b	2.50 ± 0.76 ^b
	5%	6.00 ± 0.76 ^{bc}	6.3 ± 1.0 ^b	7.00 ± 0.76 ^b	1.63 ± 0.74 ^b
	7%	6.88 ± 0.83 ^c	7.38 ± 0.74 ^b	7.63 ± 0.52 ^b	1.13 ± 0.35 ^b

Scores presented as mean ± standard deviation; n = 8. Meat color scores ranged from 8 (extremely dark) to 1 (extremely light); aroma scores ranged from 8 (extremely strong) to 1 (extremely weak); taste scores ranged from 8 (extremely strong) to 1 (extremely bland); acceptability scores ranged from 7 (totally acceptable) to 1 (totally unacceptable). Within the column of each treatment, means that do not share the same letter (a, b, c) are significantly different ($P < 0.05$).

pork ranged from 3 to 5 (moderately light to slightly dark). They were not significantly different from the control ground pork ($P > 0.05$). When the oil concentrations reached 0.5%, significant differences in aroma, taste and acceptability between treated and control samples were found ($P < 0.05$). Aroma and taste scores increased from 4 to 7 (slightly weak/bland to very strong), and consumer acceptability decreased to “moderately unacceptable”. Although OO and CO did not alter the meat color, considering their influence on aroma, taste and acceptability, we decided that the threshold should not exceed 0.5% in ground pork.

Olive extract did not influence the meat color, but a significant color difference was observed in 5% AE treated pork ($P < 0.05$) compared to the control. When both powder extracts reached 3%, significant differences in aroma, taste and consumer acceptability were detected between treated and control ground pork ($P < 0.05$). The aroma and taste scores of 3% OE and 3% AE treated pork increased to “very strong”, and the consumer acceptability decreased to almost “totally unacceptable”. Hence, we decided that the thresholds of OE and AE should not exceed 3%. The results indicated that high concentrations of these antimicrobials adversely affected the ground pork sensory characteristics.

To place the present findings on sensory properties in perspective, we will briefly mention related studies on reported sensory aspects of meats with added plant-based antimicrobials.

Ahn, *et al.* [27] reported that the addition of 1% Acti Vin (grape seed extract), and 1% pycnogenol (pine bark) in raw ground beef during 9-day refrigerated storage increased the redness; 1% pycnogenol, and 1% oleoresin rosemary increased the lightness and yellowness. Shan, *et al.* [28] also found that the addition of 16% cinnamon stick, oregano, clove, pomegranate peel and grape seed on raw pork made the meat color become darker. In our study, the color of OO, CO and OE treated pork at various concentrations was not different from the control. However, pork samples treated with 5% and 7% AE were darker. Differences in the results with regard to the meat color between our study and that of the others could be due to the different types of antimicrobials used in the studies, the volume and concentrations added into the meat, and the test protocols applied during the sensory test.

Sensory effects of carvacrol and cinnamaldehyde in carrot broth were evaluated for acceptance by Valero and Giner [29]. Cinnamaldehyde at 2 $\mu\text{l}/100\text{ ml}$ of carrot broth was the most acceptable level and when the concentration was increased to 6 $\mu\text{l}/100\text{ ml}$ of carrot broth, it had a negative effect. Carrot broth with added carvacrol at concentrations of 2.5 - 10 $\mu\text{l}/100\text{ ml}$ was unacceptable to the panelists. The concentrations of antimicrobials used by Valero and Giner [29] were relatively low compared to our study. However, low consumer acceptability was reported. The influence of antimicrobials on the food sensory attributes could vary based on the test matrix, presence of fats and other nutrients, and the forms of antimicrobials used (single chemical compounds versus a plant extract that contains various compounds).

Skandamis and Nychas [30] reported that raw minced beef mixed with oregano oil at 1% (v/w), stored in air and modified atmospheres at 5°C, maintained acceptable color up to 7 and 18 days, respectively. However, raw minced beef mixed with 1% oregano oil stored in air showed a change in odor starting at day 5 of storage. We also observed that OE did not change the color of cooked ground pork but altered the odor.

Ntzimani, *et al.* [31] studied the sensory properties (odor and taste) of semi-cooked, vacuum-packed chicken fillets marinated using the combination of ethylenediaminetetraacetate (EDTA; 1.5%), lysozyme (1.5%) and oregano oil (0.2%)/rosemary oil (0.2%). They reported that the taste of cooked chicken coated with EDTA, lysozyme and oregano oil was bitter than those coated with rosemary oil. Despite using a different meat matrix, we found that 0.1% OO and CO did not affect the taste and acceptability of cooked ground pork, whereas we observed significant adverse effects by essential oils at 0.5%.

3.2. Antimicrobial Activity of Plant-Derived Compounds at Consumer-Acceptable Concentrations against *S. Typhimurium* DT104 in Ground Pork

Table 3 and Table 4 show the surviving *Salmonella* populations in OO and CO treated pork during storage of up to 7 days. At day 0, the bacterial populations in all samples were about 6 log CFU/g. Very slight growth variations were found in each of the OO treated samples when comparing their final bacterial populations at day 7 to the initial population at day 0, as well as to the control (Table 3). These changes did not represent any significant difference between treated samples and the control ($P > 0.05$). The *Salmonella* populations remained constant regardless of antimicrobial concentrations. It was evident that concentrations of OO lower than 0.5% did not exhibit antimicrobial activity at 4°C.

Slight variations (non-significant; $P > 0.05$) were seen with 0.1%, 0.2% and 0.3% CO when compared to the control and their initial bacterial population at day 0 (Table 4). Significant decreases were found in 0.4% CO treated pork after

Table 3. Population of *S. Typhimurium* DT104 in ground pork with added oregano oil at various concentrations and stored at 4°C up to 7 days.

Day	Mean population of <i>S. Typhimurium</i> (Log CFU/g)						Bac. change 0.5% ^c
	Positive control	0.1%	0.2%	0.3%	0.4%	0.5%	
0	5.66 ± 0.34 ^{ay}	5.64 ± 0.29 ^{ay}	5.70 ± 0.21 ^{ay}	5.59 ± 0.19 ^{ay}	5.76 ± 0.28 ^{ay}	5.51 ± 0.07 ^{ay}	−0.15
3	5.78 ± 0.22 ^{ay}	5.97 ± 0.34 ^{ay}	5.91 ± 0.30 ^{ay}	5.91 ± 0.34 ^{ay}	5.64 ± 0.30 ^{ay}	5.91 ± 0.24 ^{ay}	+0.13
5	5.93 ± 0.50 ^{ay}	6.03 ± 0.64 ^{ay}	6.07 ± 0.33 ^{ay}	6.01 ± 0.06 ^{ay}	5.91 ± 0.54 ^{ay}	5.97 ± 0.36 ^{ay}	+0.04
7	5.82 ± 0.38 ^{ay}	5.63 ± 0.34 ^{ay}	5.78 ± 0.30 ^{ay}	5.86 ± 0.20 ^{ay}	5.74 ± 0.21 ^{ay}	5.87 ± 0.47 ^{ay}	+0.05
Bac. change ^d	+0.16	−0.01	+0.08	+0.27	−0.02	+0.36	

Data presented as mean ± SD. N = 3. Within a row, means that do not share a letter (superscript a and b) are significantly different ($P < 0.05$). Within a column, means that do not share a letter (superscript x and y) are significantly different ($P < 0.05$). ^c*Salmonella* population change between 0.5% OO treated sample and control. “+” represent log increase; “−” represent log reduction. ^d*Salmonella* population change at day 7 from day 0. “+” represent log increase; “−” represent log reduction.

Table 4. Population of *S. Typhimurium* DT104 in ground pork with added cinnamon oil at various concentrations and stored at 4°C up to 7 days.

Day	Mean population of <i>S. Typhimurium</i> (Log CFU/g)						Bac. change 0.5% ^c
	Positive control	0.1%	0.2%	0.3%	0.4%	0.5%	
0	5.49 ± 0.23 ^{ax}	5.57 ± 0.52 ^{ax}	5.65 ± 0.77 ^{ax}	5.72 ± 0.22 ^{ax}	5.86 ± 0.17 ^{ax}	5.75 ± 0.23 ^{ax}	+0.26
3	6.34 ± 0.48 ^{ax}	6.29 ± 0.58 ^{abx}	5.78 ± 0.29 ^{abx}	5.34 ± 0.21 ^{abx}	5.57 ± 0.24 ^{abxy}	5.14 ± 0.42 ^{bxy}	-1.20
5	5.80 ± 0.11 ^{ax}	5.79 ± 0.27 ^{ax}	5.75 ± 0.32 ^{ax}	5.57 ± 0.41 ^{ax}	5.37 ± 0.05 ^{ay}	5.27 ± 0.14 ^{axy}	-0.53
7	6.07 ± 0.38 ^{ax}	5.89 ± 0.24 ^{abx}	5.71 ± 0.36 ^{abx}	5.49 ± 0.48 ^{abx}	5.26 ± 0.18 ^{aby}	5.03 ± 0.24 ^{by}	-1.04
Bac. change ^d	+0.58	+0.32	+0.06	-0.23	-0.60	-0.72	

Presented as mean ± SD. N = 3. Within a row, means that do not share a letter (superscript a and b) are significantly different ($P < 0.05$). Within a column, means that do not share a letter (superscript x and y) are significantly different ($P < 0.05$). ^c*Salmonella* population change between 0.5% CO treated pork and control. “+” represent log increase; “-” represent log reduction. ^d*Salmonella* population change at day 7 from day 0. “+” represent log increase; “-” represent log reduction.

Table 5. Population of *S. Typhimurium* DT104 in ground pork (high initial inoculum-6 log CFU/g) with added olive extract and apple extract and stored at 4°C up to 7 days.

Day	Mean population of <i>S. Typhimurium</i> (Log CFU/g)				Bacterial population change (OE control) ^c	Bacterial population change (AE control) ^c
	Olive extract		Apple extract			
	Positive control	Olive extract 3%	Positive control	Apple extract 3%		
0	5.48 ± 0.25 ^{ax}	5.68 ± 0.29 ^{ax}	5.49 ± 0.23 ^{ax}	5.77 ± 0.38 ^{ax}	+0.20	+0.28
3	5.62 ± 0.02 ^{ax}	5.45 ± 0.17 ^{axy}	6.34 ± 0.48 ^{ax}	5.55 ± 0.29 ^{ax}	−0.17	−0.79
5	5.68 ± 0.31 ^{ax}	5.25 ± 0.29 ^{axy}	5.80 ± 0.11 ^{ax}	5.54 ± 0.24 ^{ax}	−0.43	−0.26
7	5.70 ± 0.29 ^{ax}	4.80 ± 0.32 ^{by}	6.07 ± 0.38 ^{ax}	5.61 ± 0.12 ^{ax}	−0.90	−0.46
Bac. change ^d	+0.22	−0.88	+0.58	−0.16		

Presented as mean ± SD. N = 3. Within a row, means that do not share a letter (superscript a and b) are significantly different ($P < 0.05$). Within a column, means that do not share a letter (superscript x and y) are significantly different ($P < 0.05$). ^c*Salmonella* population change between treated pork and control. “+” represent log increase; “-” represent log reduction. ^d*Salmonella* population change at day 7 from day 0. “+” represent log increase; “-” represent log reduction.

day 5, which led to a 0.60 log CFU/g reduction from day 0 ($P < 0.05$). A 1.04 log CFU/g reduction at day 7 was caused by 0.5% CO compared to the control, and a 0.72 log CFU/g reduction from the initial population ($P < 0.05$). Increasing the amounts of CO in the pork showed a greater decrease in the *Salmonella* population (concentration-dependent activity) when stored at 4°C for 7 days.

Table 5 shows the surviving *Salmonella* populations in pork treated with 3% OE and 3% AE at higher initial inoculum levels (6 log CFU/g). OE demonstrated a significant difference at day 7 compared to day 0 and the control and showed 0.8 and 0.9 log CFU/g reductions, respectively ($P < 0.05$). Treatment with AE did not result in any significant difference between the treated samples and controls up to 7 days ($P > 0.05$).

Based on these results, 0.5% CO and 3% OE demonstrated nearly 1 log CFU/g reduction in *Salmonella* population in ground pork stored at 4°C for 7 days when the initial bacterial population was 6 log CFU/g. Our results are consistent with other findings. For example, Juneja, *et al.* [32] reported that 0.1% and 0.5%

of oregano oil, carvacrol and cinnamaldehyde did not inhibit *Clostridium perfringens* growth in ground beef during 12 and 15 hours of the chilling process. Apostolidis, *et al.* [33] found that 0.075% (750 ppm) of oregano extract, cranberry extract and their combinations induced 0.2 to 0.5 log CFU/g reduction of *Listeria monocytogenes* in ground beef compared to the control after 5 days. Schirmer and Langsrud [34] concluded that 0.2% of cinnamaldehyde in raw pork did not have an antimicrobial effect against *Lactobacillus algidus*, *Leuconostoc mesenteroides*, *Leuconostoc carnosum*, *Carnobacterium maltaromaticum*, *Carnobacterium divergens*, *Brochothrix thermosphacta*, and *Serratia proteamaculans*. In our experiment, there were >4 log CFU/g *S. Typhimurium* DT104 in the pork samples stored at 4°C for 7 days.

Yin and Chao [35] studied the antimicrobial effects of roselle calyx extract against *Campylobacter* species in ground beef stored for 6 days at 15°C. Chao and Yin [36] investigated the effects of roselle calyx extracts against foodborne pathogens (*S. Typhimurium*, *Escherichia coli* O157:H7, *L. monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*) in ground beef and apple juice after 3 days of storage at 25°C. Both studies showed a reduction of about 2 - 4 log CFU/ml or g in the pathogen populations in both ground beef and apple juice. In both studies, the beef samples used were trimmed off all visible extra-muscular fat. Differences observed in the results between our study in ground pork and those obtained in other studies with ground beef and apple juice [35] [36] could be due to the utilization of different food matrices and the variation in the fat content of the meats.

3.3. Antimicrobial Activity of Olive and Apple Extracts in Ground Pork against Lower Inoculum Levels of *S. Typhimurium* DT104

Table 6 shows the results of the antimicrobial effects of OE and AE against lower inoculum levels of *Salmonella* (4 log CFU/g). OE at 3% demonstrated significant

Table 6. Population of *S. Typhimurium* DT104 in ground pork (low initial inoculum 4 log CFU/g) with added olive extract and apple extract and stored at 4°C up to 7 days.

Day	Mean population of <i>S. Typhimurium</i> (Log CFU/g)			Bacterial population change (OE control) ^c	Bacterial population change (AE control) ^c
	control	olive extract 3%	apple extract 3%		
0	3.87 ± 0.19 ^{ax}	3.84 ± 0.07 ^{ax}	3.84 ± 0.07 ^{ax}	-0.03	-0.03
3	4.12 ± 0.25 ^{ax}	3.42 ± 0.17 ^{by}	3.56 ± 0.38 ^{bx}	-0.70	-0.56
5	4.03 ± 0.20 ^{ax}	3.44 ± 0.26 ^{ay}	4.11 ± 0.33 ^{ax}	-0.59	+0.08
7	4.22 ± 0.33 ^{ax}	2.79 ± 0.08 ^{bz}	3.98 ± 0.42 ^{bx}	-1.43	-0.24
Bac. change ^d	+0.35	-1.05	+0.14		

Data presented as mean ± SD. N = 3. Within a row, means that do not share a letter (superscript a and b) are significantly different (P < 0.05). Within a column, means that do not share a letter (superscript x and y) are significantly different (P < 0.05). ^c*Salmonella* population change between treated pork and control. “+” represent log increase; “-” represent log reduction. ^d*Salmonella* population change at day 7 from day 0. “+” represent log increase; “-” represent log reduction.

decline from its initial population starting at day 3, leading to a 1.05 log CFU/g reduction at day 7 ($P < 0.05$), and a 1.43 log CFU/g reduction on day 5 compared to the control ($P < 0.05$). Thus, olive extract demonstrated greater activity against lower inoculum levels of *S. Typhimurium* DT104 compared to the higher inoculum levels.

AE at 3% exhibited minimal activity even against low level of *Salmonella*. Although at day 3, a 0.56 log CFU/g reduction was observed; subsequently bacterial growth increased and by day 7 there was only a 0.24 CFU/g log reduction compared to the control. Similar results were observed by Uhart, Maks and Ravishankar [18] using two inoculum levels of *S. Typhimurium*. They reported that adding 5% garlic, 7% ginger and 5% turmeric to heated ground beef inoculated with 5 log CFU/g *S. Typhimurium* DT104, resulted in 0.34, 0.26 and 0.23 log CFU/g reductions, respectively, at day 10. With an 8 log CFU/g inoculum, the reduction was only 0.12 logs CFU/g with 5% garlic. These results showed that garlic, ginger and turmeric showed better reductions with a lower initial pathogen population.

The normal background microflora population in pork/ground meat is about 3 log CFU/g [25] [37]. This population is lower than the inoculum level of *Salmonella* used in the current study (6 and 4 log CFU/g). According to Gill, *et al.* [38], the total number of *E. coli* recovered from cooled pork carcasses was <2.2 log CFU/2500 cm². Bohaychuk, *et al.* [39] also reported that 92.7% of pork carcasses sampled from 34 facilities had levels of *E. coli* < 10 CFU/cm². On pork carcasses, the average count was 0.1 log/cm² for *E. coli*, 0.57 log CFU/cm² for *S. aureus* 0.66 most probable number (MPN)/cm² for *Campylobacter jejuni* and *Campylobacter coli*, and 0.18 MPN/cm² for *Salmonella* [40]. These findings indicate the low level of pathogen contaminations on pork carcasses.

Since we found that the olive extract is more effective against a low initial pathogen level, it is likely that it might have greater activity against pathogenic bacteria in store-bought pork/ground pork with low initial numbers of bacteria. Furthermore, studies have shown the additive effect of a mixture of various plant antimicrobials [41] [42] [43]. These findings suggest another direction for further investigation, which could be the evaluation of combined effect of two or more of the plant antimicrobials used in this study.

4. Conclusion

Sensory properties of ground pork were adversely affected by the added high concentrations of OO, CO, OE and AE. Our results suggest that for a ground pork product to be sensorily acceptable by consumers, the maximum concentration of OO and CO should be lower than 0.5%, while for OE and AE the maximum concentration should be lower than 3%. Our results also suggest that the antimicrobials were more effective against *Salmonella* at lower initial bacterial populations (4 log CFU/g) in comparison to higher initial bacterial levels of 6 log CFU/g in ground pork. OO and AE did not show antimicrobial activity at con-

centrations of 0.5% and 3%, respectively, in pork. Discovering the proper balance between desirable antimicrobial effects of plant compounds and extracts and the concurrent ability to maintain the food sensory qualities requires further investigation. Because 1 log reduction in ground pork and beef might be significant, CO and OE merit further evaluation by the meat industry for their antimicrobial efficacy.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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