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Efficacy of plant-derived antimicrobials for controlling *Salmonella* Schwarzengrund on dry pet food

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ABSTRACT

Salmonella enterica is a major human pathogen that is responsible for 23,000 hospitalizations annually in the United States. Contact with contaminated pet food and infected companion animals can transmit salmonellosis to humans. Recent multistate human outbreaks of salmonellosis linked to commercial contaminated dry dog foods underscore the need for controlling the pathogen in pet foods for protecting pet and public health. In this study, the efficacy of five Generally Recognized as Safe (GRAS) status, plant-derived antimicrobials (PDAs), namely trans-cinnamaldehyde (TC), carvacrol (CR), thymol (TY), eugenol (EG), and caprylic acid (CA) applied as a vegetable oil or chitosan based antimicrobial spray on dry pet food for reducing *Salmonella* Schwarzengrund was investigated. Three hundred gram portions of a commercial dry dog food were inoculated with a two-strain mixture of nalidixic acid (NA) resistant *S.* Schwarzengrund (~6 log CFU/g), followed by a spray treatment with 0%, 0.5%, 1% or 2% of TC, CR, TY, EG or CA in combination with 5% vegetable oil or 1% chitosan as a carrier. The control and treated dog food samples were stored at 25 °C for 28 days. On days 0, 1, 3, 5, 7, 14, 21, and 28, *Salmonella* on pet food was enumerated by serial dilution and plating on xylose lysine desoxycholate (XLD) agar. All PDAs at 1% and 2% applied in vegetable oil or chitosan reduced *S.* Schwarzengrund by at least ~2 log CFU/g on day 3 of storage when compared to control ($P < 0.05$). No significant reductions in *Salmonella* were observed on feed sprayed with only vegetable oil or chitosan ($P > 0.05$). Overall, 2% TC in vegetable oil or chitosan was the most effective treatment, where at least 3 to 3.5 log CFU/g reduction in bacterial populations was observed during storage ($P < 0.05$). Results suggest that the aforementioned PDAs could potentially be used as an antimicrobial spray to reduce *S.* Schwarzengrund on dry dog food. However, further studies on the acceptance of PDA-treated dry food by dogs are needed.

1. Introduction

Salmonella enterica is a major foodborne pathogen that causes an estimated 1.2 million human illnesses, 23,000 hospitalizations and 450 deaths annually in the United States (CDC, 2018). Salmonellosis is also a zoonotic disease that can occur due to exposures other than the consumption of contaminated food. For example, salmonellosis in humans has been reported from handling contaminated pet food (Freeman et al., 2016; Jackson et al., 2013). *Salmonella* contaminated pet food leads to infections in companion animals, where the infected animals shed *Salmonella* in the feces and saliva for a prolonged time, thereby making them a viable carrier for the pathogen (Apanavicius et al., 2007; Singh et al., 2007). Further, dogs and cats could shed *Salmonella* asymptomatically for 3 to 6 weeks, and up to 3 months (Imanishi et al., 2014). *Salmonella* infections in humans could be linked to direct contact with contaminated pet foods, exposure to cross-contaminated human food products, direct or indirect contact with infected pets or house environment (Lambertini et al., 2012). From 2007 to 2012, two major outbreaks of human salmonellosis were associated with contaminated pet food products, where > 100 people were reported ill from 20 different states, and about 40% of the infected patients were one year of age or younger (CDC, 2007, 2008, 2011; Li et al., 2012). In light of these outbreaks, the US Food and Drug Administration conducted a nationwide survey in 2012 to determine *Salmonella* prevalence on dry

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pet foods, pet treats and supplements (FDA, 2012). Currently, chemicals such as organic acids and formaldehyde are used for decontamination of animal feeds, including pet food (Jones, 2011). However, they are found to be minimally effective in reducing pathogen load (Carrique-Mas et al., 2007). Thus, there is a critical need for identifying novel and safe strategies for inactivating *Salmonella* on dry pet foods.

Plant-derived antimicrobials (PDAs) are a group of natural plant compounds that have traditionally been used as food preservatives and flavor enhancers. In the past decade, the use of PDAs as effective antimicrobials has gained significant attention due to their non-toxic nature, increasing concern over the safety of synthetic chemicals, and emerging antibiotic-resistant microorganisms (Salamci et al., 2007). Carvacrol (CR) and thymol (TY) are phenolic isomers present as active ingredients in oregano oil (*Origanum glandulosum*). *trans*-Cinnamaldehyde (TC) is an aromatic aldehyde obtained from the bark extract of cinnamon (*Cinnamomum zeylandicum)*. Eugenol (EG) is another polyphenolic compound that is a component of clove oil (*Eugenia caryophillis*). Caprylic acid (CA) is an eight-carbon medium chain fatty acid present in vegetable oil and coconut oil (Jensen, 2002; Sprong et al., 2001). All the aforementioned compounds are classified as GRAS (generally recognized as safe) by the FDA (Arrebola et al., 1994; Leriche and Carpentier, 1995; Venkitanarayanan et al., 2013). Previous studies conducted in our laboratory revealed that these natural molecules possess significant antimicrobial properties against *Salmonella in vitro* and *in vivo* (Kollanoor-Johny et al., 2010; Upadhyaya et al., 2013; Venkitanarayanan et al., 2013).

Chitosan (CH) is a biodegradable, GRAS-status polymer derived from the deacetylation of chitin, a natural polysaccharide present as the main component of exoskeletons of crustaceans (Kumar, 2000). Chitosan possesses antimicrobial properties against Gram-positive and Gram-negative bacteria (No et al., 2002; Sagoo et al., 2002). In addition, chitosan is used as an antimicrobial carrier coating or film on foods due to its emulsification and gelation properties (Knorr, 1984; No et al., 2002; Upadhyay et al., 2015, 2016; Upadhyaya et al., 2016). In the pet food industry, vegetable oil is commonly used as a carrier and diluent of additives in dry pet foods (Aldrich and Koppel, 2015). Therefore, this study investigated the efficacy of aforementioned PDAs in combination with 5% soybean vegetable oil or 1% chitosan as an antimicrobial spray for reducing *Salmonella* Schwarzengrund on dry dog food.

2. Materials and methods

2.1. Preparation of bacterial culture

Two strains of *S.* Schwarzengrund (CVM 19633 and DBS-GA-F25499) obtained from BEI resources (Manassas, VA) were used in this study. All bacteriological media were purchased from Difco (Becton Dickinson, Sparks, MD). *Salmonella* strains were pre-induced for resistance to 50 μg/ml of nalidixic acid (NA; Sigma Aldrich, St. Louis, MO) for selective enumeration (Kollanoor-Johny et al., 2010; Niemira and Lonczynski, 2006; Taormina and Beuchat, 1999; Upadhyaya et al., 2013). Each strain was cultured separately in 10 ml tryptic soy broth (TSB) containing 50 μg/ml of NA, and incubated at 37 °C for 24 h. After 3 passages, the inoculum was prepared by centrifuging each *S.* Schwarzengrund culture at 3600 × *g* for 15 min at 4 °C. The pellet of each *Salmonella* strain was washed twice and resuspended in 10 ml of 0.1% peptone water, and 0.1 ml of the resuspension was spread plated onto xylose lysine desoxycholate (XLD) agar plates containing 50 μg/ml of NA (XLD + NA). After incubation at 37 °C for 24 h, 10 ml of 0.1% peptone water was added onto the XLD + NA plate containing colonies of each *Salmonella* strain, and the agar surface was gently washed to collect the bacteria (Beuchat and Mann, 2011). Equal portions of the two *S.* Schwarzengrund cultures were combined and used as the inoculum (~8 log CFU/ml). The bacterial count in the two-strain cocktail were confirmed by plating 0.1 ml portions of appropriate dilutions on XLD + NA plates, followed by incubation at 37 °C for 48 h.

2.2. Preparation of PDA treatments

All PDAs (TC, CR, EG, TY, and CA) and low molecular weight chitosan (~5 to 15 kDa) were purchased from Sigma-Aldrich (99% purity, SAFC grade; Sigma-Aldrich). Pure soybean vegetable oil was procured from Fisher Scientific (Asheville, NC). To prepare 1% chitosan solution (*w/v*), 1 g chitosan was dissolved in sterile deionized water containing 0.1% acetic acid (Sigma Aldrich), heated at 60 °C, and stirred for 6 h to fully dissolve chitosan (Chen et al., 2012; Martínez-Camacho et al., 2010; Wu et al., 2008). Subsequently, each PDA was added to 5% vegetable oil or 1% chitosan solution to prepare 15%, 30%, and 60% (vol/vol) treatment solutions and the solutions were vortexed thoroughly for proper mixing of the PDAs.

A commercially available dry dog food was purchased from a local pet store. Prior to the experiment, duplicate 10 g portions of dog food were placed in a sterile WhirlPak bag (Sigma-Aldrich) containing 100 ml of cysteine selenite broth and incubated at 37 °C for 48 h. The enriched culture was streaked on XLD plates and incubated at 37 °C for 48 h to determine the presence of any *Salmonella* spp. on dry dog food.

For each treatment, 300 g portions of dry dog food were spray inoculated with 15 ml of *S.* Schwarzengrund cocktail culture to obtain ~ 6 log CFU/g of the pathogen using an air atomizer (Master air brush, Eco kit-17, TCP global, San Diego, CA) in a biosafety cabinet. Following inoculation, dog food was placed in a biosafety cabinet for 1 h to facilitate bacterial attachment. Ten milliliters of soybean vegetable oil based or chitosan-based-PDA treatments at 0%, 15%, 30%, and 60% (vol/vol) were sprayed onto the inoculated dry dog food to obtain final concentrations of 0%, 0.5%, 1%, and 2% (vol/wt) of each PDA. Dry dog food inoculated with *S.* Schwarzengrund, but not subjected to any PDA spray treatment served as the baseline. Moreover, vegetable oil and 1% chitosan without any PDA were included as controls to test if they exerted any antimicrobial effect on *S.* Schwarzengrund. After treatment, 10 g portions of dry dog food were transferred to a sterile WhirlPak bag and stored at 25 °C for 28 days. On days 0, 1, 3, 5, 7, 14, 21, and 28, a volume of 20 ml of neutralizing broth (Sigma-Aldrich) was added to each bag containing 10 g of PDA-treated or untreated dry dog food, and pummeled in a stomacher (Stomacher 400 Circulator, Seward, Davie, FL) for 1 min. The dog food homogenate was serially diluted (1:10) in 0.1% peptone buffer, and 0.1 ml aliquots from appropriate dilutions were surface plated on duplicate XLD-NA plates, and incubated at 37 °C for 48 h. In addition, 1 ml of the homogenate was enriched in 50 ml of cysteine selenite broth at 37 °C for 48 h. Following enrichment, the culture was streaked on XLD-NA plates, incubated at 37 °C for 48 h, and observed for typical *Salmonella* colonies.

2.3. Water activity and pH measurement

The pH and water activity of dog food from all treatments and control were measured, as previously described by Oni et al. (2016). Briefly, dry dog food samples were treated with PDAs in combinations of vegetable oil or chitosan as described above but with no *Salmonella* inoculation. Untreated dry dog food served as control. Immediately after PDA treatments, all treated and untreated dry pet food samples were ground and kept in the biosafety hood for 2 h. The pH was determined at 25 °C by weighing 1 g portions of ground dry dog food sample from each treatment, pulverizing with mortar and pestle, and hydrating with 2.5 ml distilled water. The pH of each sample was measured using a pre-calibrated pH meter (Horiba, Baltimore, MD). For water activity measurement, 4 g portions of ground dry dog food from each sample were used to determine the water activity using a water activity meter (Rotronic, Hauppauge, NY) as per the manufacturer's instructions.

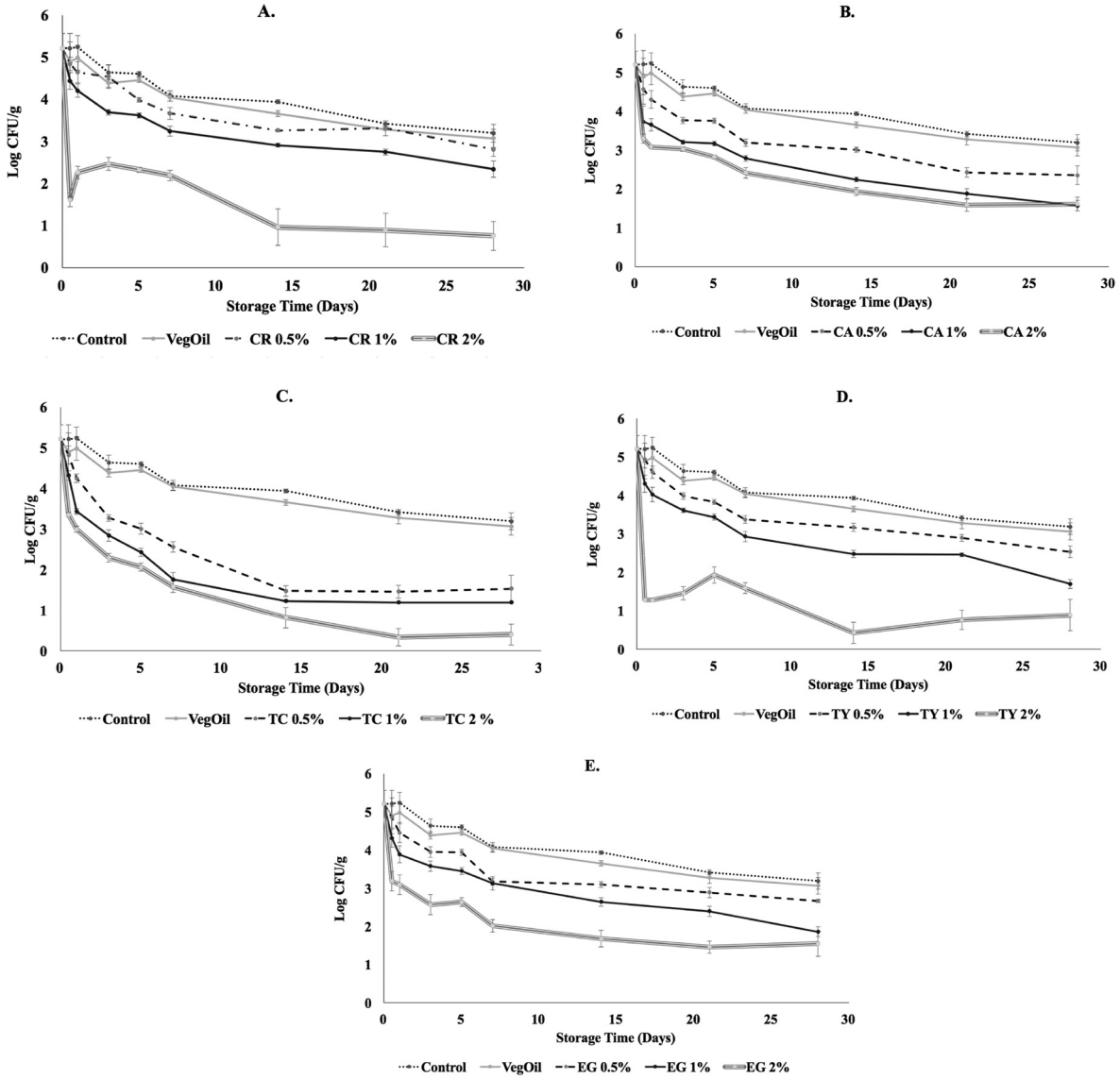

Fig. 1. Effect of plant-derived antimicrobials in combination with vegetable oil on *Salmonella* Schwarzengrund populations on dry dog food. A–E show the effect of carvacrol (CR), caprylic acid (CA), trans-cinnamaldehyde (TC), thymol (TY), and eugenol (EG), respectively.

2.4. Statistical analysis

A completely randomized design was used with a 5 × 4 × 8 factorial treatment structure. The factors included 5 PDAs (CR, CA, TC, TY, and EG), 4 concentrations (0%, 0.5%, 1%, and 2%) and 8 time points (days 0, 1, 3, 5, 7, 14, 21, and 28). The data were analyzed using the PROC-MIXED procedure of the Statistical Analysis Software (SAS Institute Inc., Cary, NC). Triplicate samples were assayed on each sampling day, and the study was replicated twice. Differences among the means were analyzed at $P < 0.05$ using Fisher's least significance difference test with appropriate corrections for multiple comparisons.

3. Results

3.1. Effect of PDAs in combination with vegetable oil on S. Schwarzengrund

Fig. 1A–E show the effect of various PDAs at 0%, 0.5%, 1%, and 2% applied in vegetable oil on *Salmonella* survival on dry dog food. On day 0, ~5 to 5.5 log CFU/g of *Salmonella* was recovered from treated and control samples. In all samples, *Salmonella* populations gradually decreased over the 28-day storage period. On day 28, ~4.0 log CFU/g of *Salmonella* was present on control samples. Vegetable oil did not significantly affect the survival of *Salmonella* as compared to the control throughout the storage period ($P > 0.05$). However, all PDAs at 1% and 2% significantly reduced *Salmonella* populations on dry dog food in a concentration dependent manner ($P < 0.05$). Dry dog food treated with 0.5% of each aforementioned PDA decreased *Salmonella* populations by ~0.4 to 2.0 log CFU/g as compared to untreated control and vegetable oil control on day 28; TC was the most effective treatment followed by TY, CA, EG, and CR. Among the PDAs, TC and TY at 2% exerted the greatest antimicrobial effect against *Salmonella* with ~ 3.0 log CFU/g and 2.5 log CFU/g reductions when compared to the control on day 28, respectively (Fig. 1C–D).

3.2. Effect of PDAs in combination with 1% chitosan on S. Schwarzengrund

Salmonella recovered from untreated (baseline) and 1% chitosan control samples on day 28 ranged from 3.2 to 3.8 log CFU/g. All PDA treatments in 1% chitosan exhibited a similar inhibitory effect on *Salmonella* survival on dry dog food as observed in combination with vegetable oil (Fig. 2A–E). Treatment with 1% chitosan by itself did not significantly reduce *Salmonella* counts on dog food ($P > 0.05$);

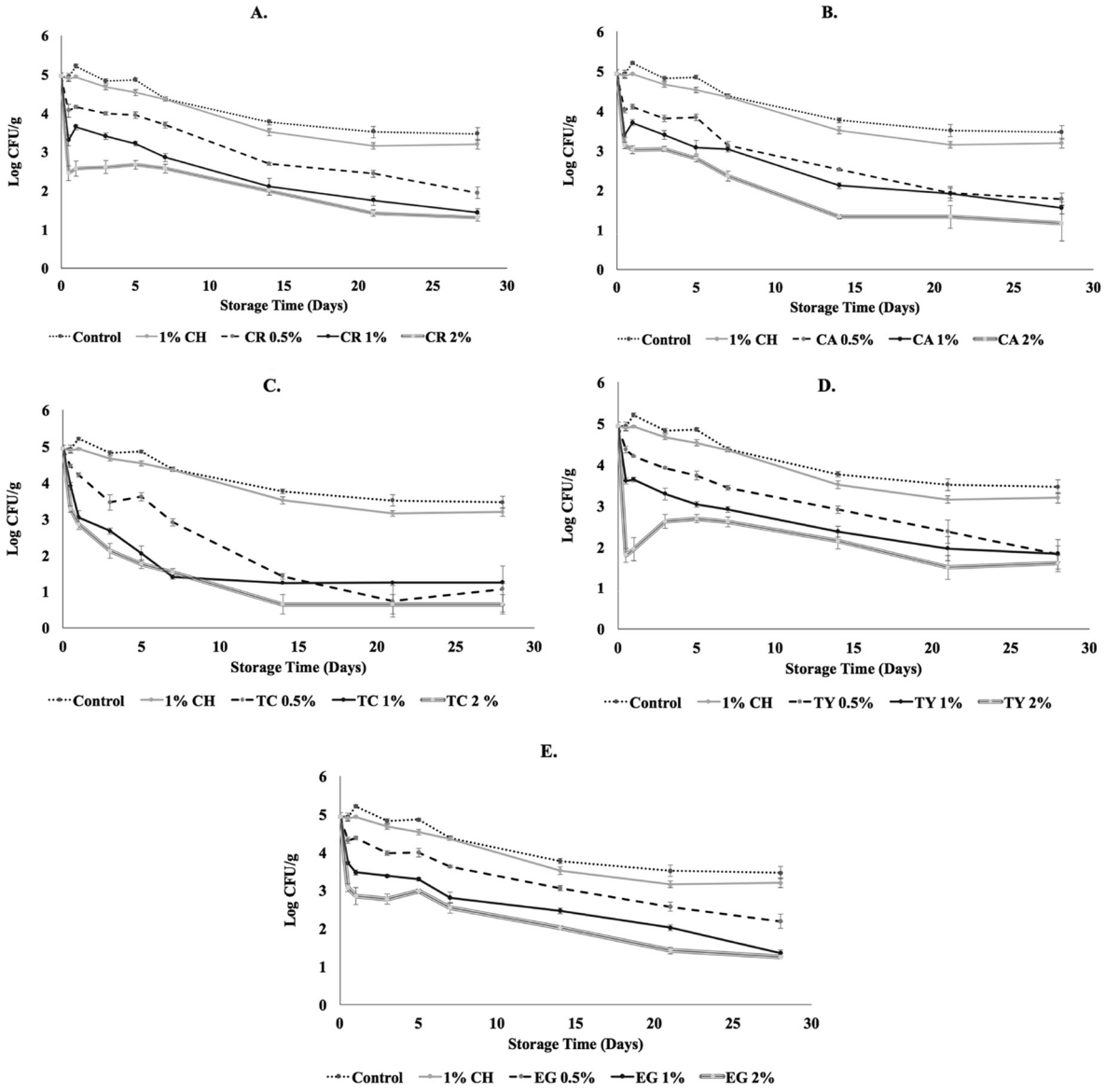

Fig. 2. Effect of plant-derived antimicrobials in combination with 1% chitosan on *Salmonella* Schwarzengrund on dry dog food. A–E show the effect of carvacrol (CR), caprylic acid (CA), trans-cinnamaldehyde (TC), thymol (TY), and eugenol (EG), respectively.

however, PDAs at 0.5%, 0.1%, and 0.2% in combination with 1% chitosan significantly decreased the bacterial population throughout the storage period ($P < 0.05$). Treatments containing PDAs at 0.5% in combination with 1% chitosan resulted in ~1.3 to 2.4 log CFU/g reduction in *Salmonella* counts on day 28 compared to the control, where maximum reduction (~ 2.4 log CFU/g reduction) was observed with 0.5% TC. *Trans*-cinnamaldehyde at the concentration of 2% was the most effective treatment, where *Salmonella* population was decreased to as low as 0.5 log CFU/g on day 14.

All PDA treatments at 0.5% in combination with 1% chitosan were generally found to be more effective in reducing *Salmonella* than their combination with vegetable oil. For instance, the PDAs at 0.5% with vegetable oil decreased *Salmonella* on dog food by ~ 0.4 to 2.0 log CFU/g (Fig. 1), whereas the same concentration of PDAs sprayed in 1% chitosan yielded 1.3 to 2.4 log CFU/g reduction in *Salmonella* counts compared to controls on day 28 (Fig. 2). Similarly, on day 28, ~1 log CFU/g reduction in *Salmonella* populations was observed on dog food sprayed with 0.5% CR in vegetable oil (Fig. 1A); however, 0.5% CR in combination with 1% chitosan decreased *Salmonella* counts by ~1.8 log CFU/g (Fig. 2A). Likewise, TY at 0.5% with vegetable oil resulted in a reduction of ~1 log CFU/g *Salmonella* on day 28 (Fig. 1D), whereas 0.5% TY with 1% chitosan decreased pathogen load by ~1.7 log CFU/g (Fig. 2D).

3.3. Effect of PDA treatments on pH and water activity

Table 1 shows the pH and water activity of dry dog food treated with or without PDAs in combination with vegetable oil, where it can be observed that the PDAs at 0.5%, 1%, and 2% in combination with vegetable oil did not significantly affect these parameters. Although PDAs in combination with 1% chitosan did not significantly change the pH values of dog food samples, these treatments significantly increased the water activity as compared to the untreated control (Table 2).

4. Discussion

Approximately 43 million households in the United States own at least one dog, and majority of these pet owners rely on dry pet food as the primary source of nutrition for their pets (AAFCO, 2012; AVMA, 2012). However, two multi-state outbreaks of human salmonellosis

Table 1
Water activity and pH of dry dog food treated with plant-derived antimicrobials in combination with vegetable oil.

Treatments	a_w[1]	pH[2]
Control	0.30 ± 0.004[a]	5.89 ± 0.015[a]
Vegetable oil	0.30 ± 0.005[a]	5.81 ± 0.026[a]
TC 0.5%	0.29 ± 0.006[a]	5.92 ± 0.032[a]
TC 1%	0.31 ± 0.008[a]	5.80 ± 0.067[a]
TC 2%	0.31 ± 0.009[a]	5.84 ± 0.061[a]
TY 0.5%	0.31 ± 0.003[a]	5.82 ± 0.031[a]
TY 1%	0.31 ± 0.006[a]	5.83 ± 0.057[a]
TY 2%	0.32 ± 0.004[a]	5.80 ± 0.050[a]
CR 0.5%	0.29 ± 0.008[a]	5.82 ± 0.037[a]
CR 1%	0.28 ± 0.003[a]	5.80 ± 0.043[a]
CR 2%	0.29 ± 0.001[a]	5.80 ± 0.067[a]
CA 0.5%	0.31 ± 0.004[a]	5.85 ± 0.057[a]
CA 1%	0.31 ± 0.007[a]	5.70 ± 0.099[a]
CA 2%	0.32 ± 0.004[a]	5.76 ± 0.040[a]
EG 0.5%	0.32 ± 0.014[a]	5.84 ± 0.032[a]
EG 1%	0.31 ± 0.001[a]	5.72 ± 0.080[a]
EG 2%	0.31 ± 0.012[a]	5.84 ± 0.084[a]

[a]Means with the same superscript in a column are not significantly different ($P < 0.05$).
[1,2]Values represent the mean ± SEM of three samples.

Table 2
Water activity and pH of dry dog food treated with plant-derived antimicrobials in combination with 1% chitosan.

Treatments	a_w[1]	pH[2]
Control	0.27 ± 0.004[h]	5.89 ± 0.014[a]
Chitosan 1%	0.70 ± 0.018[a]	5.83 ± 0.043[a]
TC 0.5%	0.57 ± 0.007[e]	5.76 ± 0.055[a]
TC 1%	0.53 ± 0.004[f]	5.90 ± 0.040[a]
TC 2%	0.49 ± 0.009[g]	5.79 ± 0.040[a]
TY 0.5%	0.70 ± 0.005[a]	5.81 ± 0.072[a]
TY 1%	0.67 ± 0.003[b]	5.82 ± 0.041[a]
TY 2%	0.65 ± 0.006[c]	5.79 ± 0.026[a]
CR 0.5%	0.65 ± 0.010[c]	5.90 ± 0.005[a]
CR 1%	0.64 ± 0.003[c]	5.74 ± 0.089[a]
CR 2%	0.62 ± 0.006[d]	5.88 ± 0.041[a]
CA 0.5%	0.64 ± 0.003[c]	5.84 ± 0.071[a]
CA 1%	0.63 ± 0.002[cd]	5.75 ± 0.072[a]
CA 2%	0.62 ± 0.005[d]	5.74 ± 0.045[a]
EG 0.5%	0.56 ± 0.006[e]	5.74 ± 0.078[a]
EG 1%	0.56 ± 0.004[e]	5.86 ± 0.080[a]
EG 2%	0.55 ± 0.005[ef]	5.78 ± 0.068[a]

[1,2]Values represent the mean ± SEM of three samples.
[a–d]Means with different superscripts in a column differ significantly ($P < 0.05$).

linked to contaminated dry dog food and numerous recalls of contaminated pet foods and treats highlight the need for an effective strategy to control *Salmonella* on dry pet food. Since *Salmonella* would not survive under the extrusion conditions applied during dry pet food manufacturing process, the presence of *Salmonella* is usually due to post-extrusion contamination in the processing plant. Therefore, the present study investigated the efficacy of the PDAs in combination with vegetable oil or 1% chitosan for potential application as a post-extrusion spray treatment for reducing *S.* Schwarzengrund contamination on dry dog food.

Antimicrobial activity of PDAs against *Salmonella* has been previously demonstrated with potential applications in the food systems. Upadhyaya et al. (2015) reported that TC and EG at 1% were effective in reducing *Salmonella* Enteritidis on eggshells by fumigation treatment. Another study done by Ravishankar et al. (2010) confirmed that CR and TC at 1% significantly inactivated *Salmonella* Newport on celery and oysters. Results of the present study indicate that the PDAs especially at concentrations of 1% and 2% in combination with vegetable oil or chitosan significantly reduced *S.* Schwarzengrund on dry pet food. Further, we observed the increased antimicrobial activity of PDAs in combination with chitosan against *Salmonella* on dry dog food as compared to their combination in vegetable oil. The increased antimicrobial efficacy of PDAs with chitosan observed in this study concurs with that by Wang et al. (2011), who observed synergistic antimicrobial effects between chitosan and a variety of antimicrobials, including plant compounds. Similarly, Anacarso et al. (2011) reported that a combination of chitosan with essential oils produced greater anti-listerial activity than chitosan alone on vegetables and fruits.

Water activity is one of the critical factors that affect the survivability of pathogens on dry pet foods. In our study, dry dog food sprayed with PDAs in vegetable oil did not significantly change the water activity as compared to that of untreated control ($P > 0.05$). Treatments with PDAs in chitosan increased water activity of the dry pet food (0.49–0.73) as compared to control (0.27). Himathongkham et al. (1999) and Koutsoumanis et al. (2004) stated that a water activity above 0.90 was required for the growth of *Salmonella*, which was not observed in any of the samples in the current study. Further, Oni et al. (2016) reported that rehydration of dry dog food with 35–50% of additional water (water activity > 0.95) increased *Salmonella* populations for up to 4.6 log CFU/g within 3 days, whereas *Salmonella* counts declined with 20% rehydration level possibly due increased stress of active bacterial metabolism under water activity ranging from 0.92 to 0.97.

On the contrary, it is noteworthy that water activity has been documented to exert variable effects on bacterial survival in foods. For example, a study done by Gurtler and Beuchat (2007) showed that populations of *Enterobacter sakazakii* decreased significantly at water activity 0.44 as compared to water activity 0.26 after a month of storage at 30 °C in powdered infant formula. However, with regards to *Salmonella*, the bacterial counts were similar at water activity 0.33 and 0.53 within the first month of storage at 37 °C in skim milk powder (Lian et al., 2015). In this study, *Salmonella* populations on chitosan-treated dry pet food were not significantly different as compared to control throughout the entire storage ($P > 0.05$), suggesting that the lethality of PDAs in combination with chitosan against *Salmonella* may not because of the increased water activity.

Although the elevated water activity with PDA/chitosan treatments might increase the susceptibility of dry pet food to xerophilic spoilage microorganisms (de Silóniz et al., 2000; Matan et al., 2006), previous studies have shown that PDAs, including TC, TY, CR, and EG effectively inhibited the growth of molds and yeasts *in vitro* or in different food applications (Azzouz and Bullerman, 1982; Conner and Beuchat, 1984; Matan et al., 2006; Singh and Chittenden, 2010; Yin et al., 2015).

The hydrophobicity of PDAs allows them to target the lipid-containing bacterial cell membrane and makes the membrane more permeable, leading to leakage of ions and other cell contents (Cox et al., 2000; Sikkema et al., 1995; Ultee et al., 2002). Devi et al. (2010) investigated the mechanism of bactericidal action of EG against *Salmonella* Typhi through Scanning Electron Microscopy and confirmed that the antibacterial activity of this compound was due to the interaction of antimicrobial on bacterial cell membrane. On the other hand, chitosan has also been documented to weaken the membrane barrier properties of the outer membrane of Gram-negative bacteria, where the interaction between the positively charged chitosan molecules and negatively charged microbial cell walls plays a critical role in its antimicrobial activity (Helander et al., 2001). Thus, the combination of PDAs and chitosan could be more detrimental on bacteria than their combination with vegetable oil.

In the United States, the usage of additives for pet food products must be GRAS or approved by the FDA (FDA, 2018). Toxicological effects of PDAs to dogs and cats have been previously studied with lethal doses (LD50) ranging 250 to 500 mg/kg body weight through intravenous, stomach tube, or oral administrations (BG Chemie, 2000; Coujolle and Franck, 1944; IARC, 1985; Lauber and Hollander, 1950; Suntres et al., 2015). Although acute exposure to PDAs at high

concentrations might be hepatoxic or even lethal to companion animals and humans, data on the toxicity of PDAs at lower concentration given with foods to dogs and cats are currently lacking. Based on the recommendation of the daily food consumption for dogs and cats, the concentrations used in the current manuscript are below the LD50 levels of these compounds to dogs and cats.

In the current study, we observed an aroma of PDAs, especially with TC treatments on dry pet food immediately after the PDA treatments. However, this aroma declined steadily and PDA-treated samples were comparable to untreated control at the end of the storage. Multiple brands of cinnamon flavored dog treats are commercially available, and a combination of TY and CR supplementation to canned or dry pet food products has been patented as a palatability enhancer (Qvyjt, 2005). However, detailed palatability studies on PDA-treated pet food are necessary to ensure acceptability to dogs.

In conclusion, results of the current study suggest that all tested PDAs, especially at 1% and 2% in combination with 5% vegetable oil or 1% chitosan could potentially be used as an antimicrobial spray treatment to reduce *S.* Schwarzengrund on dry pet foods. Despite the effectiveness of PDA treatments against *S.* Schwarzengrund on dry pet food, the importance of a HACCP plan that includes good plant hygiene and efficient cleaning practices should not be neglected. Follow-up investigations on the palatability and toxicology of PDA-treated pet food and large scale efficacy studies under commercial settings are warranted.

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