

# Evaluation of peroxyacetic acid, liquid buffered vinegar, and cultured dextrose fermentate as potential antimicrobial interventions for raw chicken livers

Leslie Pearl M. Cancio<sup>1,2</sup>  | Mary-Grace C. Danao<sup>1</sup> | Gary A. Sullivan<sup>3</sup> | Byron D. Chaves<sup>1</sup> 

<sup>1</sup>Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, USA

<sup>2</sup>Office of the Regional Director, Department of Science and Technology (DOST) XI, Davao City 8000, Philippines

<sup>3</sup>Department of Animal Science, University of Nebraska-Lincoln, Lincoln, Nebraska 68583, USA

## Correspondence

Byron D. Chaves, Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE 68588, USA.  
Email: [byron.chaves-elizonado@unl.edu](mailto:byron.chaves-elizonado@unl.edu)

## Abstract

This study aimed to evaluate the use of peroxyacetic acid (PAA), buffered vinegar (BV), and cultured dextrose fermentate (CDF) to reduce *Salmonella* on artificially inoculated raw chicken livers, one of the most consumed offal around the world. Samples were inoculated with a 5-strain cocktail of poultry-borne *Salmonella* to obtain 10<sup>6</sup> CFU/g and immersed for 90 s with agitation in one of the following treatments: distilled water (control), 450 ppm PAA, 2.0% (w/v) BV, or 1.5% (w/v) CDF, prior to storing at 4°C. *Salmonella* was enumerated on XLD agar and monitored for 14 days. Data were analyzed using analysis of covariance. After immersion, there was a significant *Salmonella* reduction ( $p < .05$ ) with all treatments, including the control. PAA resulted in the greatest numerical reduction at 0.65 ± 0.12 log; however, there were no significant differences in the reductions among all other treatments ( $p > .05$ ). After 14 days, higher numerical reductions were observed for PAA, but only when compared to CDF. Although similar reductions ( $p > .05$ ) were noted after 14 days except for CDF, *Salmonella* counts were lowest in all timepoints when PAA was used. PAA and CDF inhibited the growth of aerobic bacteria until day 3 while BV inhibited the growth up to 7 days. Regarding objective color, chicken livers immersed in PAA became lighter, but the difference was not sustained over time. No differences were observed in redness or yellowness values across any treatments.

## 1 | INTRODUCTION

Handling contaminated pet food has emerged as a transmission risk factor for human salmonellosis (Davies, Lawes, & Wales, 2019; Nemser et al., 2014). Cases associated with contaminated pet food, such as kibble, have been reported (Behravesh et al., 2010; Hassan et al., 2019; Imanishi et al., 2014; Schnirring, 2018). Additionally, recent trends in pet food products, particularly raw meat-based diets (RMBDs), present an even higher risk for *Salmonella* transmission because they are formulated with raw ingredients such as fish, meat, or poultry, including chicken livers (Freeman, Chandler, Hamper, &

Weeth, 2013; Finley et al., 2007; Morelli, Bastianello, Catellani, & Ricci, 2019; Nüesch-Inderbilen, Treier, Zurfluh, & Stephan, 2019; Seong et al., 2015). Consumption of contaminated chicken liver has been associated to human disease, one of which is the 2011 *Salmonella enterica* serovar Heidelberg outbreak that sickened 39 individuals in the United States (CDC, 2012), and a relatively recent study found a very high prevalence of *Salmonella* (59.4%, 148/249) in retail chicken livers in three United States (Jung et al., 2019).

Livers are often marketed by raw pet food manufacturers and specialty pet food shops for pet owners who choose to prepare homemade RMBDs, but the risk of *Salmonella* is not minimized during

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preparation. Pet food is considered adulterated if it is contaminated with *Salmonella* as there is no subsequent lethality step to eliminate it (U.S. FDA, 2013, 2018). Therefore, there is a need to evaluate and validate the use of antimicrobial interventions to mitigate the risk of pathogen transmission to the pets and within the household. When chicken liver is used in ground meat blends, interventions should precede mixing and grinding as these processes allow for surface pathogens to be spread into the final product (Stelzleni, Ponrajan, & Harrison, 2013).

At present, the U.S. FDA does not have a regulatory definition for “clean label.” However, clean label products have been described as those that are free from additives, artificial colors, and flavors (Grant & Parveen, 2017). One example of clean-label product commonly used as an antimicrobial is buffered vinegar (BV), acetic acid combined with a buffer, either sodium or potassium-based alkali, to reduce the negative impact on the functional properties of the product (Badvela, Dickson, Sebranek, & Schroeder, 2016). There are studies reporting its effectiveness in controlling microbial contamination (Badvela et al., 2016; Desai et al., 2014; Ponrajan et al., 2011). Another widely used antimicrobial approved by the FDA in the United States is Microgard (Al-Zoreky, Ayres, & Sandine, 1991). This patented antimicrobial is comprised of metabolites from milk, dextrose, or wheat with propionic bacteria or specific *Lactococci* (Von Staszewski & Jagus, 2008). Microbial inhibitory activities of the fermentate on dairy products, dressings, and some vegetables have been reported (Samapundo, de Baenst, Eeckhout, & Devlieghere, 2017; Serna-Jiménez, Uribe-Bohórquez, Rodríguez-Bernal, Klotz-Ceberio, & Quintanilla-Carvajal, 2020; Von Staszewski & Jagus, 2008; Yang et al., 2021). BV and fermentates can be listed on the product labels as “vinegar” and “cultured milk” or “cultured dextrose,” respectively.

Peracetic acid (PAA), on the other hand, is an increasingly popular antimicrobial agent used in poultry decontamination (Cano, Meneses, & Chaves, 2021; Zhang, Garner, McKee, & Bilgili, 2018). The U.S. Department of Agriculture Food Safety and Inspection Service does not require labeling for PAA if its use does not exceed 2,000 ppm of peroxyacids and 1,435 ppm of hydrogen peroxide, as it is considered a processing aid (U.S. Department of Agriculture Food Safety and Inspection Service, 2021). A Joint FAO/WHO Expert Committee on Food Additives (2005, 2006) and a European Food Safety Authority (EFSA, 2014) risk assessment showed that there is no potential health concern in using PAA if it is prepared within the conditions for which it has been evaluated. These include PAA treatment preparations for pre-chill (spray washing or short-duration dip treatment), chill (chiller baths) and post-chill (short-duration dip treatment) steps in poultry processing. The concentrations evaluated were 400–700 ppm for spray washes, up to 230 ppm in the long duration chiller baths, and concentrations not exceeding 2,000 ppm in the short-term baths (EFSA, 2014).

With the current guidelines and trends in raw pet food manufacturing in mind, the objective of this study was to evaluate the efficacy of PAA, BV, and cultured dextrose fermentate (CDF) to inactivate *Salmonella* artificially inoculated on raw chicken livers. Additionally, the effect of PAA, CDF, and BV on chicken livers' aerobic bacteria population and color were evaluated. Because PAA is an

effective antimicrobial intervention in poultry processing, PAA was hypothesized to yield the highest *Salmonella* reduction compared to BV and CDF.

## 2 | MATERIALS AND METHODS

### 2.1 | Inoculum preparation and sample inoculation

Five poultry-borne strains of *Salmonella enterica* subsp. *Enterica*, namely Braenderup (NVSL 96-12528), Enteritidis (IV/NVSL 94-13062), Hadar (JE 322 2013 MI), Heidelberg (2247-1), and Typhimurium (ATCC 14028), were incubated individually at 35°C for 24 hr in 9 mL of tryptic soy broth (TSB; Remel, Lenexa, KS). Using poultry-borne strains may better represent the *Salmonella* serovars that circulate in naturally contaminated samples. From each suspension, 0.1 mL was transferred to 200 mL of TSB and incubated individually at 35°C for 24 hr. Subsequently, cell cultures were pooled together to make a bacterial cocktail with a final concentration of 10<sup>8</sup> CFU/mL as determined by decimal serial dilutions followed by plating onto Xylose Lysine Deoxycholate agar (XLD; BD, Franklin Lakes, NJ).

Chicken livers were procured from a commercial processor and stored frozen at –20°C until further use. Approximately 24 hr before inoculation, the livers were thawed at 4°C. Background aerobic microbiota and *Salmonella* counts were determined by plating onto Petrifilms Aerobic Plate Count plates (3 M, Saint Paul, MN) and XLD agar, respectively. The mean APC obtained was 2.57 ± 0.25 log CFU/g while *Salmonella* was not detected in any of the triplicate batch samples at a 10 CFU/g limit of quantification. Chicken livers were dipped in the bacterial cocktail for 30 s, drained on a stainless-steel grill grid and air-dried for 20 min. The inoculated samples were placed in a cooler at 4°C for 24 hr to allow for further microbial attachment. Prior to applying the antimicrobial treatments, three subsamples of inoculated livers were obtained from every batch to determine the initial mean *Salmonella* counts, resulting in 6.79 ± 0.09 log CFU/g.

### 2.2 | Preparation and application of antimicrobial treatments

One-liter solutions of 450 ppm PAA (Birkoside MP-2, Birko Corp., Henderson, CO); 1.5% w/v CDF (MicroGARD 200, International Flavors & Fragrances Inc., New Century, KS); and 2.0% w/v powdered BV Corbion (Verdad Powder N6 Vinegar, Lenexa, KS) were prepared by diluting the concentrated solution (for PAA) or dissolving powder (for CDF and BV) in cold (4°C) sterile distilled water. PAA concentration was tested using a PAA test kit (Peracetic Acid VACUettes kit K-7904B, CHEMetrics, Inc., Midland, VA).

Chicken livers inoculated with *Salmonella* spp. were then immersed in 4°C solutions of distilled water (control), PAA, CDF, or BV for 90 s with agitation at 40 rpm in a shaker incubator (SHKE6000-7, Thermo Scientific, Marietta, OH). After immersion of samples, extra liquid was allowed to drip for 3 min prior to vacuum packing (Multivac C200, Multivac Inc., Kansas City, MO). The treated

samples were individually packaged, stored at 4°C and were used subsequently for microbial analysis. Distilled water was used as control to determine reductions due to immersion and mechanical agitation.

## 2.3 | Microbiological analysis

Chicken livers were aseptically removed from their packaging on days 0, 3, 7, and 14 post-treatment. Two subsamples were analyzed for each treatment and day. Samples were weighed and placed in a sterile stomacher bag (Whirl-Pak®, Thomas Scientific LLC, Swedesboro, NJ) then mixed with the corresponding amount of 0.1% buffered peptone water (BPW; Sigma-Aldrich, St. Louis, MO) to prepare a 1:10 dilution. Samples were then stomached for 90 s at 200 rpm (Stomacher 400 Circulator, Seward Ltd., Bohemia, NY). Decimal serial dilutions were performed and duplicate-plated onto XLD agar. Plates were incubated at 37°C for 24 ± 2 hr. After enumeration, *Salmonella* counts were reported as log CFU/g and reductions computed using the initial *Salmonella* count (pre-treatment) and the average count of the subsamples at a specific sampling timepoint. Non-inoculated chicken livers were treated as described above. APC were enumerated on days 0, 3, 7, and 14 post-treatment. Two subsamples from each treatment were plated on duplicate APC Petrifilm and incubated at 35 ± 1°C for 48 ± 3 hr. Microbial counts were reported as log CFU/g.

## 2.4 | Liver color evaluation

The same liver samples used for APC were tested for color prior to plating. Color measurements were conducted using a handheld portable colorimeter (Model BC-10, Minolta Camera Co Ltd., Osaka, Japan) and expressed as  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness). Calibration was initially performed by placing a standard white Minolta calibration plate inside the same packaging bag used for the chicken liver to nullify the color and light reflectance properties of the packaging material (Petracci & Fletcher, 2002). Color measurements were taken at three different spots on the chicken liver surface that were free from noticeable defects (e.g., uneven surface, bruises) and were averaged. Meat color measurements were recorded on days 0, 1, 3, 7, and 14 post-treatment.

## 2.5 | Statistical analysis

Three independent replications were performed for each set of treatments using freshly prepared solutions of antimicrobial treatments and bacterial cocktails. Data were analyzed using a four-by-four factorial two-way analysis of variance with covariate (ANCOVA) wherein treatment and time were the independent variables, replications were treated as blocks, and weight as covariate. For color, data were analyzed using four-by-five factorial two-way analysis of variance (ANOVA) with treatment and time as independent variables and replications as block. When there was no interaction among variables,

the main effects were analyzed. When there was significant difference ( $p < .05$ ), Tukey–Kramer's post hoc test was applied to separate means between treatments. All statistical analyses were conducted using SAS Version 9.4 (SAS Institute, Cary, NC).

## 3 | RESULTS AND DISCUSSION

Statistical analysis showed there was a significant interaction between treatment and day ( $p = .04$ ) but there was no association between liver weight and log reduction ( $p = .19$ ). Therefore, *Salmonella* reductions were estimated using the mean weight (31.37 g) of the chicken liver samples. Immediately after treatment, results showed that there was a significant *Salmonella* reduction when using PAA ( $p < .0001$ ), BV ( $p = .0021$ ), CDF ( $p = .0016$ ), and the water control ( $p = .0012$ ). However, there were no difference in the reduction of *Salmonella* among the treatments and the control (Table 1) indicating that immersing chicken livers in antimicrobials was just as effective as immersing or washing in distilled water. Still, higher but non-significant log reductions were observed with PAA compared to BV ( $p = .2536$ ), CDF ( $p = .2894$ ), or the water control ( $p = .3505$ ). PAA affects growth of microorganisms in the food matrix by denaturing proteins, disrupting the cell membrane and obstruction of enzymatic and transport process (Block, 2011; Cano et al., 2021; King et al., 2005). Nagel, Bauermeister, Bratcher, Singh, and McKee (2013) observed reductions of 2.02 and 2.14 log CFU/mL rinsate in broiler carcasses when dipping for 20 s in 4 ± 2°C post-chill immersion tank using 400 ppm and 1,000 ppm PAA concentrations, respectively. Chen et al. (2014) also reported greater than a one-log reduction in *Salmonella* populations in ground chicken parts, specifically 1.5 and 1.3 log CFU/g. Higher concentrations (700 and 1,000 ppm) were used in a continuous in-line pathogen elimination tank with an immersion time of 23 s and a water temperature ranging from 10 to 15°C (4°C potable water was used to bring the treatments to the desired concentration). Although longer contact time was used in this study, the higher reductions observed in other studies could also be attributed to the design of their decontamination tank wherein rotation is employed to add more mechanical force to the immersion treatment as compared to using a shaking incubator.

For BV and CDF, low *Salmonella* reductions have been observed in other raw poultry and meat matrices. Stelzleni et al. (2013) studied the effects of two types of BV coupled with sodium dodecyl sulfate and levulinic acid against *S. Typhimurium* on ground beef patties and obtained reductions ranging from 0.36 to 0.70 log CFU/g after 7 days. The difference in the reductions between the BVs used and control (no intervention) were minimal, ranging from 0.17 to 0.36 logs. In the case of fermentates, a cultured milk fermentate (Microgard 100) used in an acidified chicken model showed no significant effect on *Escherichia coli* and *Brochothrix thermosphacta* when compared to the control (Lemay et al., 2002). However, these results contradict those reported by Ponrajan et al. (2011) where beef injected with brine and 2% BV resulted in a 1.0 log CFU/g reduction of *E. coli* O157:H7.

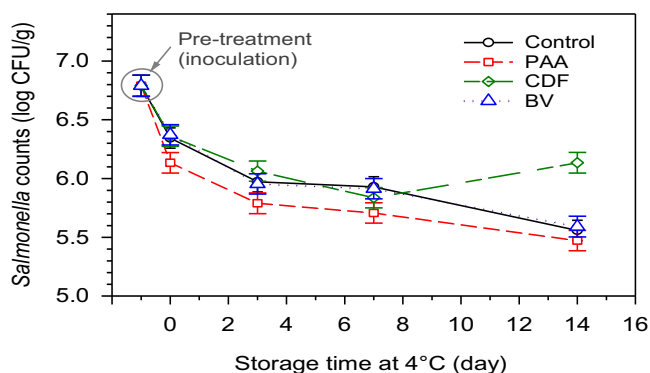
Overall, over the 14-day period after antimicrobial treatment, *Salmonella* populations decreased for the control, PAA and BV, but

**TABLE 1** *Salmonella* reductions ( $\log_{10}$  CFU/g) in chicken livers treated with different antimicrobials over 14 days of storage at 4°C.

Storage time (days)	Log reduction in <i>salmonella</i> spp. (mean $\pm$ SE)			
	Distilled water (control)	PAA	CDF	BV
0	0.44 $\pm$ 0.12 <sup>a,x</sup>	0.65 $\pm$ 0.12 <sup>a,x</sup>	0.43 $\pm$ 0.12 <sup>a,x</sup>	0.41 $\pm$ 0.12 <sup>a,x</sup>
3	0.81 $\pm$ 0.12 <sup>a,y</sup>	1.00 $\pm$ 0.12 <sup>a,x,y</sup>	0.72 $\pm$ 0.12 <sup>a,x,y</sup>	0.83 $\pm$ 0.12 <sup>a,y</sup>
7	0.85 $\pm$ 0.12 <sup>a,y</sup>	1.08 $\pm$ 0.12 <sup>a,y</sup>	0.95 $\pm$ 0.12 <sup>a,y</sup>	0.87 $\pm$ 0.12 <sup>a,y,z</sup>
14	1.22 $\pm$ 0.12 <sup>a,z</sup>	1.31 $\pm$ 0.12 <sup>a,y</sup>	0.65 $\pm$ 0.12 <sup>b,x,y</sup>	1.20 $\pm$ 0.12 <sup>a,z</sup>

Note: <sup>ab</sup>Least squares means within a row without common superscripts are different  $p < .05$ . <sup>xyz</sup>Least squares means within a column without common superscripts are different  $p < .05$ .

Abbreviations: BV, 2.0% powdered buffered vinegar (Verdad Powder N6 Vinegar); CDF, 1.5% cultured dextrose fermentate (Microgard 200); PAA, 450 ppm peracetic acid; SE, standard error.

**FIGURE 1** *Salmonella* populations on inoculated chicken livers treated with various antimicrobials over 14 days of refrigerated storage. Bars represent standard error.

not for CDF (Figure 1). Counts immediately after treatment (day 0) were significantly higher ( $p < .05$ ) compared to counts obtained on subsequent sampling timepoints for control and BV treatment. For PAA, *Salmonella* counts on day 0 became significantly different after day 7 ( $p < .05$ ). For CDF, the difference was only observed between days 0 and 7 ( $p = .001$ ). Although the decrease in *Salmonella* population at day 0 may be attributed to the different treatments, the decreasing trend on counts could also be attributed to storage temperature. Chicken livers were kept at 4°C which generally allow

*Salmonella* to survive but not grow. Pradhan et al. (2012) evaluated the effect of refrigerated and frozen storage temperature on the growth and survival of *S. Typhimurium* in chicken breast and observed a similar trend but the change in *Salmonella* populations did not vary significantly until day 7. Comparable observations were reported by Osaili et al. (2020) in ground camel meat wherein *S. Typhimurium* counts from the initial population had declined slightly after 7 days.

Figure 1 shows that *Salmonella* counts were almost identical between control and BV until the 14th day of storage, indicating that it is not effective in controlling *Salmonella* in chicken livers for prolonged refrigerated storage. Although the BV used in this study does not have bactericidal effect, it is marketed to extend the lag phase of microbial growth (i.e., a bacteriostatic effect) thus extending product shelf life (Corbion, 2022). While the bacteriostatic effect of BV in *Salmonella* was not evident in this study, this may be because a lower concentration and a shorter immersion time compared to previous studies were used. Heir et al. (2021) experimented with different concentrations (2.5–18%) and immersion times (300 s) of the same BV used in this study on raw salmon and reported complete inhibition of *Listeria monocytogenes* after 12 days. As for CDF-treated chicken liver, *Salmonella* populations decreased similarly with those in chicken livers treated with water until the seventh day of storage. Although the *Salmonella* counts had increased by day 14, this was not significantly higher than the counts obtained on day 7 ( $p = .10$ ).

With PAA, even though differences were not significant compared to the water control, *Salmonella* counts were numerically lower

**TABLE 2** Aerobic plate count (APC) ( $\log_{10}$  CFU/g) in chicken livers treated with different antimicrobials after 14 days of storage at 4°C.

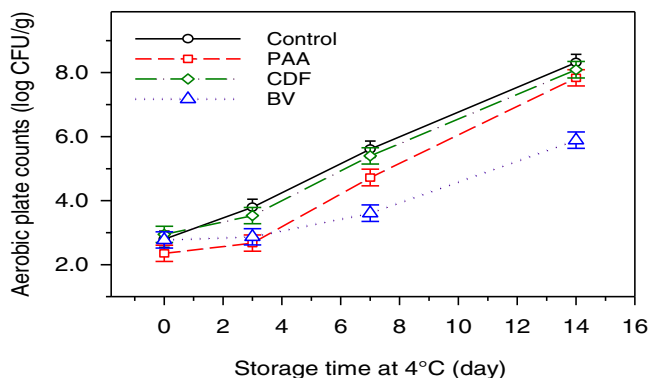
Storage time (days)	Aerobic plate counts ( $\log$ CFU/g) (mean $\pm$ SE)			
	Distilled water (control)	PAA	CDF	BV
0	2.80 $\pm$ 0.25 <sup>a,w</sup>	2.36 $\pm$ 0.25 <sup>a,x</sup>	2.95 $\pm$ 0.25 <sup>a,x</sup>	2.77 $\pm$ 0.25 <sup>a,x</sup>
3	3.79 $\pm$ 0.25 <sup>c,x</sup>	2.68 $\pm$ 0.25 <sup>a,x</sup>	3.53 $\pm$ 0.25 <sup>b,x</sup>	2.87 $\pm$ 0.25 <sup>a,b,x,y</sup>
7	5.61 $\pm$ 0.26 <sup>c,y</sup>	4.72 $\pm$ 0.26 <sup>b,y</sup>	5.40 $\pm$ 0.25 <sup>b,c,y</sup>	3.61 $\pm$ 0.26 <sup>a,y,z</sup>
14	8.32 $\pm$ 0.25 <sup>b,z</sup>	7.84 $\pm$ 0.25 <sup>b,z</sup>	8.09 $\pm$ 0.26 <sup>b,z</sup>	5.89 $\pm$ 0.25 <sup>a,z</sup>

Note: <sup>abc</sup>Least squares means within a row with different superscripts are different  $p < .05$ . <sup>wxyz</sup>Least squares means within a column with different superscripts are different  $p < .05$ .

Abbreviations: BV, 2.0% powdered buffered vinegar (Verdad Powder N6 Vinegar); CDF, 1.5% cultured dextrose fermentate (Microgard 200); PAA, 450 ppm peracetic acid; SE, standard error.



regardless of storage time. Additionally, *Salmonella* populations in PAA-treated samples demonstrated similar trends with other studies wherein *Salmonella* did not continue to grow exponentially under refrigerated conditions. In a study by Park, Harrison, and Berrang (2017) comparing 1,200 ppm PAA and 50 ppm of chlorine, the results showed that PAA was the more effective treatment. Additionally, the observed reduction using PAA was significantly higher compared to

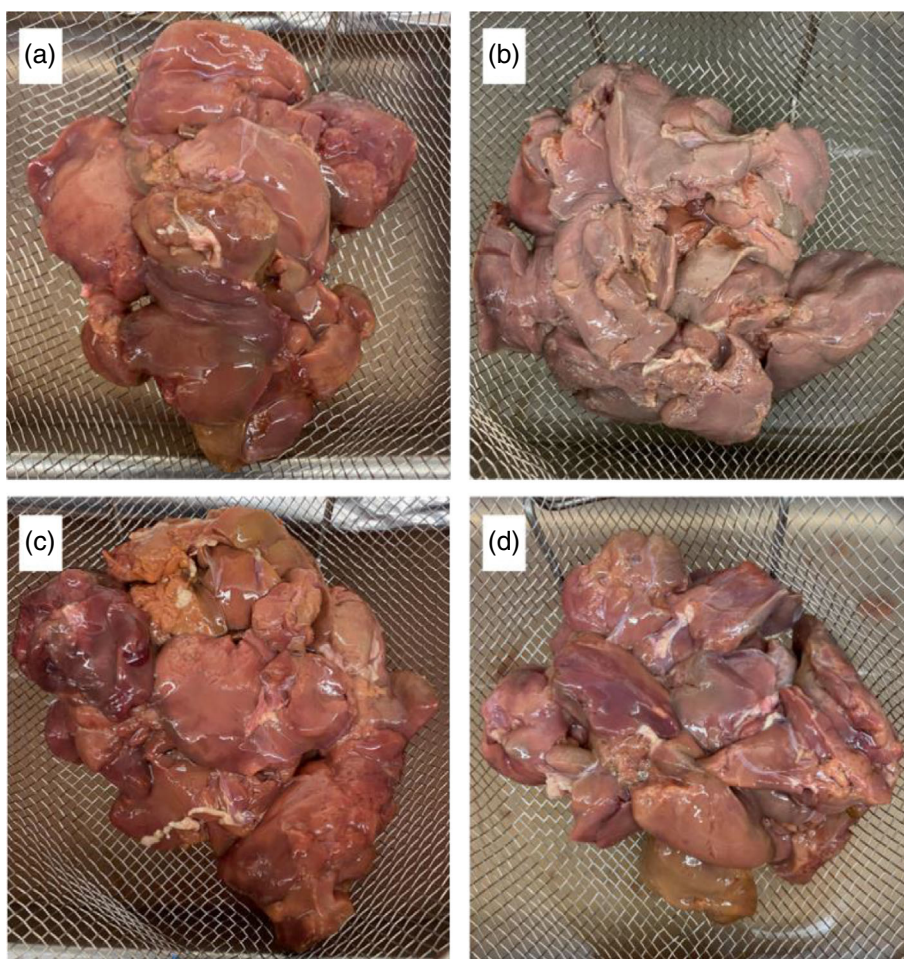


**FIGURE 2** Aerobic plate counts on chicken livers treated with various antimicrobials over 14 days of refrigerated storage. Bars represent standard error.

water-treated ground chicken. In terms of the effect on *Salmonella* population after 9 days of storage, observed values over time did not change (Park et al., 2017).

For an intervention to be considered practical in the meat and poultry industries, the accepted criterion is at least one-log reduction of the pathogen of interest (Brashears & Chaves, 2017). The mean estimates of *Salmonella* reduction after 14 days of storage were greater than 1 log CFU/g for PAA, BV and the control, but only samples treated with PAA demonstrated reduction that will likely be greater than one log (95% CI = 1.06, 1.56 log CFU/g). While this technically meets the one-log reduction criteria, the recommended duration for storage at 4°C of chicken livers for animal consumption is typically 4–7 days to preserve microbiological quality. By the time PAA-treated chicken livers reach 1 log reduction when stored at 4°C, the product may already be beyond its intended shelf life.

Similar to the *Salmonella* challenge study, there was a significant treatment by day interaction ( $p < .0001$ ) for aerobic plate counts (APCs) but no interaction between liver weight and the achieved microbial counts ( $p = .86$ ). Therefore, simple effects of treatment and day were further assessed. Table 2 and Figure 2 show the APC counts using different antimicrobial interventions. Immediately after treatment (day 0), no differences in the APC were observed. However, on day 3, the difference was now seen as APC of PAA-treated samples



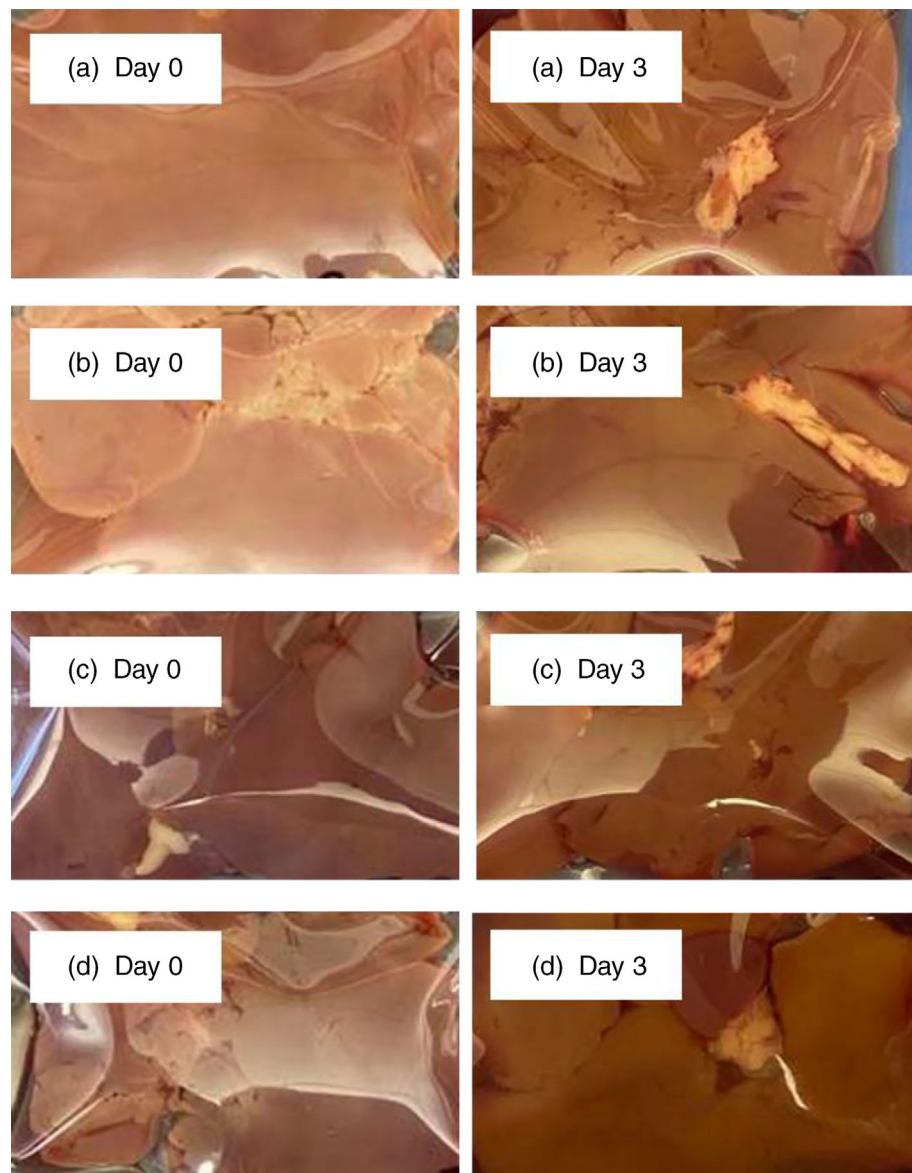
**FIGURE 3** Photos of chicken livers after dipping in (a) water, (b) 450 ppm PAA, (c) 1.5% CDF, and (d) 2.0% BV.

was significantly lower compared to CDF ( $p = .0234$ ) and the water control ( $p = .0024$ ). Additionally, APC in chicken livers treated with BV was significantly different compared to that of the control ( $p = .0146$ ). On day 7, BV continued to show lower microbial counts compared to PAA ( $p < .0004$ ), CDF ( $p < .0001$ ) and the water control ( $p < .0001$ ). While there was already a difference between chicken livers treated with BV and PAA, the latter was still lower than the control ( $p < .05$ ). According to ICMSF (1986), 5.70 log CFU/g APC value is considered an upper microbiological limit for fresh poultry products quality. The data showed that chicken livers treated with water and CDF are already nearing spoilage levels by day 7 while PAA and BV continued to maintain lower levels of aerobic bacteria. By day 14, however, counts for all treatments were greater than 5.70 log CFU/g with BV still having significantly lower APC levels than the other treatments.

APC counts on chicken livers treated with distilled water continued to increase significantly from day 0 to day 3 ( $p = .0071$ ), day 7 ( $p < .0001$ ), and day 14 ( $p < .0001$ ). This was in contrast with

chicken livers treated with antimicrobial treatments wherein growth was much slower. No differences were observed in the APC between the day of treatment and the third day of storage ( $p > .05$ ) but counts increased as the storage time reached the 7th and 14th day. Of the three antimicrobials, CDF was the least effective at inhibiting bacterial growth as a marginal difference between counts of day 0 and day 3 ( $p = .18$ ) was observed.

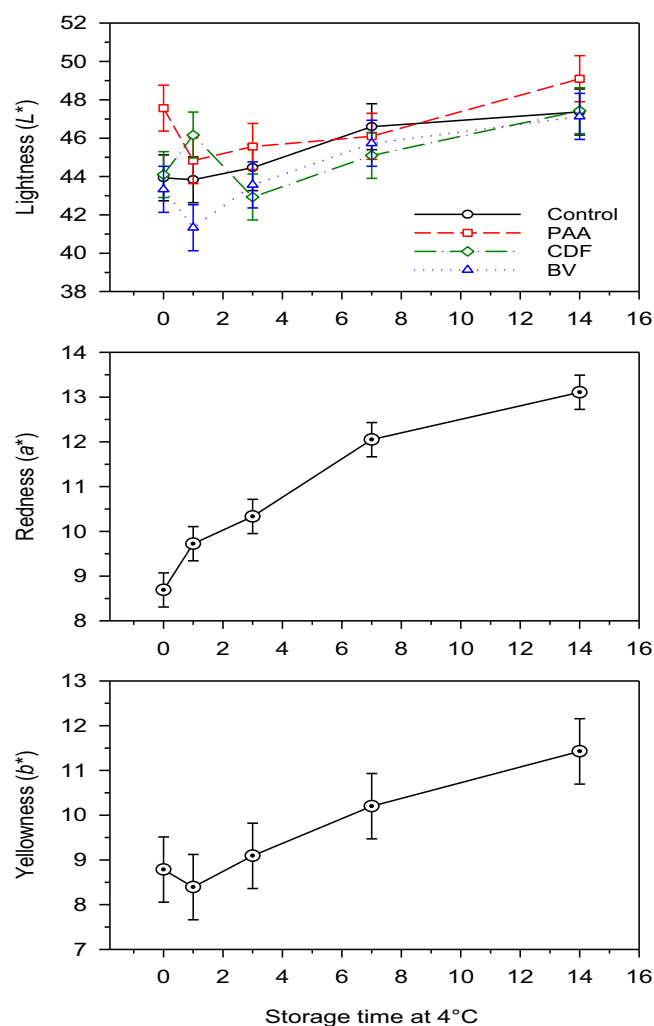
The most effective treatment was BV as growth of aerobic bacteria was inhibited until day 7 and counts were not approaching spoilage level until the 14th day of storage. These results agreed with previous observations in chicken retail cuts treated with 1.0% BV, where product shelf life was extended from approximately 12–20 days (Desai et al., 2014). Organic acids such as acetic acid or vinegar are effective at reducing aerobic bacteria in meat and poultry through disruption of the normal cellular process in microorganisms, thus slowing growth (Badvela et al., 2016). Apart from aerobic bacteria, other researchers have measured spoilage by evaluating



**FIGURE 4** Photos of chicken livers inside the packaging material after dipping in (a) water, (b) 450 ppm PAA, (c) 1.5% CDF, and (d) 2.0% BV at day 0 and day 3.

psychrotrophic microorganisms and the results were similar to those observed in this study. Harris and Williams (2019) observed that 1.0–3.0% BV retarded the growth of psychrotrophs for 7 days in ground chicken breast meat, while Ponrajan et al. (2011) reported delayed growth for 21 days in beef top rounds and top sirloin steak using 2.0% BV.

When it comes to meat color, there was no treatment by day interaction observed for lightness ( $L^*$ ), redness ( $a^*$ ), or yellowness ( $b^*$ ) values. The type of antimicrobial treatment influenced the lightness ( $p = .005$ ) but not the redness ( $p = .7381$ ) or yellowness ( $p = .2536$ ) of the chicken livers. Refrigerated storage time influenced all color parameters ( $p < .05$ ). The use of CDF and BV showed no distinct differences when compared to the water control. However, chicken livers treated with PAA were significantly lighter than those treated with BV at day 0 ( $p = .0229$ ) although the difference between



**FIGURE 5** Color ( $L^*a^*b^*$ ) measurements of raw chicken livers treated with different antimicrobials and stored for 14 days at 4°C. Lightness ( $L^*$ ) values are presented for each antimicrobial treatment to show simple effects of treatment per day, while redness ( $a^*$ ) and yellowness ( $b^*$ ) values have been averaged for all treatments due to lack of statistical differences across treatments. Bars represent standard error.

treatments became marginal by day 1 ( $p = .0778$ ). Prior to packing, the difference in lightness was visibly noticeable between PAA-treated chicken livers and the other treatments (Figure 3). This could be due to presence of hydrogen peroxide in the antimicrobial agent which have been reported to cause a bleached appearance (Lillard & Thomson, 1983). However, between days 3 and 14, there were no differences observed among all treatments, suggesting that the initial lightening effect by PAA was temporary (Figures 4 and 5). Bauermeister, Bowers, Townsend, and McKee (2008) also reported lighter appearance of poultry carcasses treated with 100 and 150 ppm PAA, but differences were no longer observed by day 7 compared to the control. Although there were changes observed in some of the treatments as the days in storage increases, generally, chicken livers in this study became lighter which was also observed by Petracchi and Fletcher (2002) in broiler skin and meat. For redness ( $a^*$ ) and yellowness ( $b^*$ ), the main effects of prolonged storage also showed increasing values of these two parameters (Figure 5).

## 4 | CONCLUSIONS

*Salmonella* reductions in inoculated raw chicken livers after immersion in peroxyacetic acid (PAA), CDF, or BV were not different from the water control under the conditions used in this study. No difference in reductions among treatments was observed on the third or seventh day of storage. However, on the 14th day, a higher reduction was observed for PAA, BV, and the water control but not for CDF. Additionally, the trend showed a decrease in *Salmonella* population throughout storage of chicken livers at 4°C. Nevertheless, *Salmonella* counts in PAA-treated samples were numerically lower from day 0 to day 14 compared to other treatments indicating its potential to achieve moderate *Salmonella* reductions in raw chicken livers after treatment and prolonged storage at refrigerated conditions. Moreover, it was seen that all the antimicrobial treatments could be used to inhibit growth of aerobic bacteria as PAA and CDF were able to demonstrate control until the third day of storage and BV inhibited growth until the seventh day of storage. Overall, no significant differences in  $L^*$ ,  $a^*$ , or  $b^*$  values were observed in extended storage of chicken livers at 4°C. It is recommended to explore the use of PAA concentrations higher than 450 ppm to determine whether reductions may be higher. Furthermore, seeing BV as the most effective treatment in delaying the growth of aerobic bacteria, it may be worthwhile to investigate possible synergistic effect of PAA and BV in controlling pathogens and background microbiota of chicken livers.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

Leslie Pearl M. Cancio  <https://orcid.org/0000-0003-3154-5604>

Byron D. Chaves  <https://orcid.org/0000-0003-1899-6515>

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