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# Inhibition of *Listeria monocytogenes* by Buffered Dry Vinegar in Reduced-Sodium Ready-to-Eat Uncured Turkey Stored at 4°C

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#### **ABSTRACT**

A reduced-sodium ready-to-eat (RTE) uncured turkey was manufactured with buffered dry vinegar treatments to validate the inhibition of *Listeria monocytogenes* and spoilage microflora and to determine the effects on sensory and quality attributes. Samples were stored at 4°C for 12 weeks, and the study was independently replicated three times. Two different five-strain inocula of *L. monocytogenes* obtained from different sources were used for evaluating the efficacy of the buffered dry vinegar treatments. The results showed that 0.6 and 0.8% buffered dry vinegar with a sodium base (BDV-SB) and buffered dry vinegar with a potassium base (BDV-PB) at 0.7 and 0.9% controlled *L. monocytogenes* for 12 weeks. The untreated control product containing no buffered dry vinegar showed >1 log increase in *L. monocytogenes* populations counts at the end of 2 weeks. Statistical analysis confirmed that the dry vinegar treatments inhibited (P < 0.05) the growth of *L. monocytogenes* compared with the untreated control. No significant differences (P > 0.05) were seen in the inhibition of *L. monocytogenes* between the two different five-strain inocula. Instrumental color results showed no significant differences between the treatments. Purge loss results showed no significant differences between the dry vinegar treatments, but significant differences were seen between the untreated control and dry vinegar treatments at a few testing intervals. The overall results indicated that the dry vinegar ingredients (6.66 to 8.83 mM acetic acid in the finished product) were effective in inhibiting *L. monocytogenes* obtained from multiple sources in reduced-sodium RTE uncured turkey stored at 4°C without adversely impacting the quality attributes.

Key words: Listeria monocytogenes; Low sodium; Turkey; Vinegar

Listeria monocytogenes is one of the major foodborne pathogens that continue to be a serious threat to public health despite a decrease in number of cases annually (8, 24). Consuming food contaminated with L. monocytogenes can result in a serious infection, leading to fetal loss in pregnant women and fatalities in the elderly and people with weakened immune systems (25, 26). L. monocytogenes is one of the top five pathogens contributing to domestically acquired foodborne illnesses resulting in death (24). Among selected categories of ready-to-eat (RTE) meat and poultry products, deli meats and frankfurters without antimicrobials pose the greatest per serving risk of illness or death from L. monocytogenes because they are often consumed directly from the refrigerator without reheating (10, 25, 32). To inhibit the growth of L. monocytogenes, the U.S. Department of Agriculture, Food Safety and Inspection Service has approved a variety of antimicrobial agents that can be added to RTE meat and poultry products, and among them, lactates and diacetate are widely used (10, 13, 19, 33). It is estimated that if all listeria-prone deli products were reformulated with a growth inhibitor that 96% of the predicted listeriosis illnesses associated with RTE products sold at the retail deli could be prevented (30).

Demand for natural and organic foods in the United States is continuously increasing, as evidenced by increasing sales of these products, which rose to \$39.1 billion in 2014, and the organic market is experiencing double-digit growth of 11.3% (21). Research studies have shown preferences for natural and organic foods based on concerns about pesticides, antibiotics, hormones, genetic modifications, and chemical additives (17, 18). Hence, the development of clean label ingredients (e.g., no chemical-sounding names, no ingredient listed as artificial, and ingredients that consumers cannot understand) to inactivate L. monocytogenes and to inhibit its growth in RTE meats represents a high priority for the meat industry. Another challenge faced by the processed meat industry is sodium reduction because high sodium intake results in increased blood pressure and is a risk factor for cardiovascular disease (1, 20). The term "reduced-sodium" may be used if the individual food contains at least a 25% reduction in sodium as compared with an appropriate reference food (29). In the past few years, the U.S. food industry and the U.S. government have made many efforts to reduce the sodium content in processed foods (11, 12, 27). In 2008, the New York City Department of Health and Mental Hygiene started a voluntary National Salt Reduction Initiative, with the overall goal of reducing dietary salt consumption by 20% over 5 years (9, 14). To help the public reach this goal, the National

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TABLE 1. Composition of brine solutions used to manufacture reduced-sodium, RTE deli-style turkey breast containing different levels of buffered dry vinegar

	Ingredients (lb) (1 lb = $0.45 \text{ kg}$ )							
Treatments <sup>a,b</sup>	Water	Salt	Dextrose	Sodium phosphate	Potato starch	Buffered dry vinegar		
Untreated control	8.22	0.49	0.49	0.10	0.70	0.00		
0.4% BDV-SB	8.08	0.49	0.49	0.10	0.70	0.14		
0.6% BDV-SB	8.01	0.49	0.49	0.10	0.70	0.21		
0.8% BDV-SB	7.94	0.49	0.49	0.10	0.70	0.28		
0.5% BDV-PB	8.00	0.49	0.49	0.10	0.70	0.18		
0.7% BDV-PB	7.97	0.49	0.49	0.10	0.70	0.25		
0.9% BDV-PB	7.91	0.49	0.49	0.10	0.70	0.31		

<sup>&</sup>lt;sup>a</sup> BDV-SB and BDV-PB designate dry vinegar buffered with a sodium base and a potassium base, respectively. Both buffered dry vinegar ingredients were supplied by Kemin Industries, Inc. (Des Moines, IA).

Salt Reduction Initiative challenged food manufacturers to reduce the salt content of packaged and prepared foods by 25% over the same period. They developed a database containing 62 packaged and 25 restaurant food categories that contributed to salt intake and established targets for sodium content to be achieved by the end of 2012 and 2014 (9). Lunch meats fell into one of the processed food categories that were targeted. Since March 2011, 28 major food manufacturers (e.g., Kraft Heinz Company, Unilever, and Campbell Soups) and leading restaurant chains (e.g., Subway and Starbucks) have agreed to pursue salt reduction targets in one or more food categories (9). In 2013, it was announced that 21 companies met one or more of their voluntary commitments to reduce sodium content in prepackaged or restaurant foods (6). While sodium chloride imparts flavor and texture to foods, it also plays a critical role in food safety by reducing water activity, thereby diminishing the growth of spoilage and pathogenic microorganisms (1, 11). Hence, when developing low-sodium meats, precautions should be taken to avoid compromising flavor, texture, shelf life, and safety.

Buffered vinegar has attracted considerable attention from the meat industry for inhibiting L. monocytogenes in RTE meat and poultry products. Nonbuffered vinegar has limited usage in RTE meat and poultry products because of its low pH that could denature the meat proteins, thereby impacting the water retention and textural characteristics (28). Buffering the vinegar by using sodium- or potassiumbased alkali raises the pH and creates a minimal impact on the functional properties of the processed meat and poultry products. The advantage of using a potassium-based buffer is that it does not add sodium to the final food product, but excess use can impart bitter or metallic taste. Also, when compared with sodium salt, potassium salt has to be used at a higher application rate due to its high molecular weight. The current study highlights the antimicrobial efficacy of two buffered dry vinegar-based ingredients: one with a sodium base (BDV-SB) and the other with a potassium base (BDV-PB). The objectives of this study were to validate the inhibition of L. monocytogenes (two five-strain inocula obtained from different sources) and spoilage microflora (aerobic mesophilic populations and lactic acid bacteria

[LAB]) on reduced-sodium RTE uncured turkey manufactured with the two different dry vinegar ingredients, stored at 4°C for up to 12 weeks, and to determine the effect of the dry vinegar treatments on quality attributes, such as color and purge.

#### MATERIALS AND METHODS

Production of sliced turkey treatments. Seven treatment formulations of sliced, reduced-sodium, uncured, deli-style turkey breast were manufactured in the meat laboratory of Iowa State University, Ames. Treatments included an untreated control, 0.4, 0.6, and 0.8% BDV-SB, sodium-based buffered dry vinegar (dry vinegar described as white distilled vinegar buffered with sodium carbonate, sodium bicarbonate, or sodium hydroxide or a combination of them to a pH of 5.7 to 6.1, 67.2% acetic acid [BactoCEASE NV Dry, Kemin Industries, Inc., Des Moines, IA]) and 0.5, 0.7, and 0.9% BDV-PB, potassium-based buffered dry vinegar (dry vinegar described as white distilled vinegar buffered with potassium hydroxide to a pH of 5.7 to 6.1, 58% acetic acid [BactoCEASE NVK Dry, Kemin Industries, Inc.]). The application rates of the two dry vinegar ingredients were adjusted based on the actual acetic acid concentration to provide equivalent concentrations of acetic acid in the products. Turkey breasts were purchased from Turkey Valley Farms (Marshall, MN) and kept frozen until use. The turkey breasts were thawed at 4°C for 3 days before use. Turkey breasts were coarsely ground (model 7.5 424852, Biro Manufacturing Co., Marblehead, OH) through a kidney plate and 10% of the coarsely ground product was subsequently finely ground through a 0.3-cm plate. For each treatment, 10.2 kg of coarsely ground turkey and 1.1 kg of finely ground turkey was used to achieve effective protein binding and adhesion. The ground turkey was enhanced to 40% of original weight by adding 4.5 kg of brine solution (Table 1) containing water, salt (1.4%), dextrose, sodium phosphate, potato starch, and dry vinegar and was tumbled under vacuum for 30 min (DVTS 50, Dupey Equipment Co., Clive, IA). After tumbling, the breast meat was stuffed (Risco Vacuum Stuffer, model 1040C, Stoughton, MA) into plastic casings (15 cm diameter by 50 cm length; Dupey Equipment Co.) and cooked in a smokehouse (thermal processor, Maurer-Atmos, Reichenau, Germany) by using a three-step process: 1 h at 60°C, 1 h at 65.5°C, and finish until the internal temperature reached 75.5°C (168°F). After cooking, the turkey logs were transported to a 4°C cooler and stored overnight. The casings were removed the next day, and the turkey logs were sliced (model A-500, Bizerba, Piscataway, NJ),

<sup>&</sup>lt;sup>b</sup> Corresponding increasing concentrations of BDV-SB and BDV-PB ingredients provided equivalent acetic acid concentrations in the final RTE turkey breast product.

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with the individual slices weighing approximately  $25 \pm 0.5$  g each. Four slices were then vacuum packaged (Ultravac 2100, Ultrasource LLC, Kansas City, MO) by using high barrier vacuum pouches (B2175, Cryovac Sealed Air Corporation, Duncan, SC; oxygen transmission rate of 3 to 6 cm³/cm², 24 h at 4.4°C, and 0% relative humidity; and water vapor transmission rate of 5 to 6 g/cm², 24 h at 37.7°C, and 100% relative humidity). The desired concentration of salt in the final product was 1.4%. The sliced product was transported to Kemin Industries, Inc. under refrigerated conditions for inoculation and testing. The study was independently replicated three times by manufacturing the treatments on three different days.

Inoculum preparation. Two different five-strain inocula of L. monocytogenes were used in this study and inoculated on different sets of turkey samples separately, thus resulting in two parallel challenge studies for each replication. The purpose of using two different five-strain inocula was to check if there is any difference in the antimicrobial efficacy of dry vinegar ingredients against different strains. Inoculum 1 consisted of L. monocytogenes 101 (hard salami isolate, serotype 4b), L. monocytogenes 108 (hard salami isolate, serotype 1/2a), L. monocytogenes 310 (goat's milk cheese isolate, serotype 4), FSL-C1-109 (deli turkey isolate associated with illness, serotype 4b), and V7 (raw milk isolate, serotype 1). These strains were provided by Dr. Kathleen Glass (Food Research Institute, University of Wisconsin, Madison). Inoculum 2 consisted of H7762 (frankfurter isolate, serotype 4b), H7764 (deli turkey isolate, serotype 1/2a), H7769 (serotype 4b), H7976 (source not known), and Scott A (clinical isolate, serotype 4b), and these strains were obtained from Dr. James Dickson (Department of Animal Science, Iowa State University, Ames). One hundred microliters of each strain from the stock culture cryovials (stored at -80°C) containing 10% glycerol was aseptically transferred to 10 ml of tryptic soy broth (TSB; Bacto, BD, Sparks, MD) and incubated at 37°C for 18 to 20 h. A transfer of the overnight culture was made by transferring  $100~\mu l$  into 10~mlof fresh TSB in an Erlenmeyer flask and incubated at 37°C for 18 to 24 h. Cells were harvested by centrifugation  $(1,174 \times g, 20 \text{ min})$ at 21°C) and suspended in 4.5 ml of 0.1% buffered peptone water (pH 7.2). Approximately equivalent populations of each isolate were combined to provide a five-strain mixture of L. monocytogenes. Populations of each strain and the mixture were verified by plating on Trypticase soy agar (BBL, BD, Sparks, MD) and modified Oxford agar (listeria selective agar base, Difco, BD, Sparks, MD).

Inoculation and testing. Uncured turkey was surface inoculated with L. monocytogenes inoculum 1 or inoculum 2 to provide approximately 5 log CFU/100-g package (equivalent to 3 log CFU/ml of rinse material when using 100 ml of rinse for testing). For each package containing four slices, a total of 1.005 ml of liquid inoculum was added by distributing 0.335 ml over the surface of each slice, excluding the top one, and slices were stacked such that the inoculum was between the slices. Inoculated products were vacuum packaged (C100 Multivac, Sepp Haggemuller KG, Wolfertschewenden, Germany) in gas-impermeable pouches (3 mil high barrier nylon vacuum pouch with a water vapor transmission rate of 10 g/liter/m<sup>2</sup> for 24 h at 37.8°C and 100% relative humidity and an oxygen transmission rate of 3,000 cm<sup>3</sup>/liter/m<sup>2</sup> for 24 h at 23°C and 0% relative humidity) and stored at 4°C for up to 12 weeks. Triplicate-inoculated samples for each treatment were assayed for changes in L. monocytogenes populations, and duplicate uninoculated samples were assayed for changes in LAB and pH at 0, 2, 4, 6, 7, 8, 9, 10, 11, and 12 weeks.

L. monocytogenes populations were determined in rinse material obtained after adding 100 ml of sterile Butterfield phosphate buffer to the package and massaging the contents externally by hand for approximately 2 min. Serial (1:10) dilutions of rinse material were spread plated on duplicate plates of modified Oxford agar and incubated at 37°C for 48 h. The acceptance criterion for an effective dry vinegar treatment in this study was that it should not show >1 log increase in L. monocytogenes counts throughout the testing period. For plotting the results, the L. monocytogenes counts of each treatment at each storage point were averaged for three replications, and the change in L. monocytogenes population level from the initial (time zero) sampling was determined.

pH, LAB, and APC. Changes in pH and populations of natural microflora were evaluated in uninoculated samples to determine the effect of the experimental treatments on the growth of spoilage microorganisms that may ultimately affect the growth of L. monocytogenes. The pH of turkey slices from each treatment (Inlab Expert Pro ISM probe, S220, Mettler Toledo Inc., Columbus, OH) was measured on the slurry obtained by removing 10 g of the uninoculated sample and homogenizing with 90 ml of deionized water by using a blender (Stomacher 400, A. J. Seward, London, UK). To enumerate LAB and aerobic plate counts (APC) populations, the remaining portions of the uninoculated samples were rinsed with sterile Butterfield phosphate buffer (quantity equal to the weight of the turkey slices), and the serial dilutions of the rinse material was plated on all-purpose Tween agar (Difco, BD) with 0.002% bromocresol purple (25°C for 48 to 72 h) and plate count agar (Difco, BD; 37°C for 48 h), respectively. Mesophilic APC populations were enumerated at 0, 4, 8, and 12 weeks.

**Proximate and active ingredient analysis.** Triplicate uninoculated samples of each treatment for each replication were analyzed at Kemin Industries, Inc., for moisture (5 h at 100°C, AOAC Method 950.46; 2) water activity (Aqualab, model series-3, Decagon Devices, Inc., Pullman, WA), and pH. Duplicate samples of each treatment for each replication were analyzed for protein (AOAC Method 990.03 (3)), fat (AOAC Method 960.39 (4)), and sodium content (ICP-AOAC-965.17/985.01 mod. (5)) by Eurofins Scientific (Des Moines, IA). Acetic acid was analyzed by gas chromatography method at Kemin Industries, Inc., for duplicate samples of each treatment at weeks 0 and 12 for each replication.

**Instrumental color measurement.** Commission Internationale de l'Eclairage (CIE) L\*, a\*, b\* values (lightness, redness, yellowness, respectively) were measured on each treatment by using a Hunterlab ColorFlex Colorimeter (Hunter Associates Laboratory, Reston, VA), with Illuminant D65, 10° standard observer, and 1.25" viewing area and port. Color was measured on duplicate uninoculated turkey samples for each treatment after removing the slices from the package at four different times postprocessing (0, 4, 8, and 12 weeks).

**Purge loss (water holding capacity).** Purge loss was determined on duplicate samples of each treatment at four different times postprocessing (0, 4, 8, and 12 weeks) by a weight difference method. Each prepackaged treatment sample was measured to determine gross weight. The samples were removed from the package, blotted dry with paper towels for 10 s, and a net sample weight was recorded. The package was dried with a paper towel and reweighed to determine net packaging weight. Differences were calculated to determine percent purge loss:

TABLE 2. Sodium, potassium, and acetic acid results of reducedsodium, RTE deli-style turkey breast containing different levels of buffered dry vinegar

Treatment <sup>a,b</sup>	Sodium (%)	Potassium (%)	Acetic acid (%)
Untreated control	$0.66 \pm 0.04$	$0.25 \pm 0.01$	$0.02 \pm 0.01$
0.4% BDV-SB	$0.74 \pm 0.04$	$0.25 \pm 0.01$	$0.30 \pm 0.01$
0.6% BDV-SB	$0.77 \pm 0.03$	$0.25 \pm 0.01$	$0.43 \pm 0.03$
0.8% BDV-SB	$0.83 \pm 0.04$	$0.25 \pm 0.01$	$0.57 \pm 0.03$
0.5% BDV-PB	$0.62 \pm 0.06$	$0.42 \pm 0.01$	$0.35 \pm 0.03$
0.7% BDV-SB	$0.63 \pm 0.04$	$0.50 \pm 0.02$	$0.49 \pm 0.03$
0.9% BDV-SB	$0.63 \pm 0.03$	$0.54 \pm 0.02$	$0.52 \pm 0.05$

<sup>&</sup>lt;sup>a</sup> BDV-SB and BDV-PB designate dry vinegar buffered with a sodium base and a potassium base, respectively. Both buffered dry vinegar ingredients were supplied by Kemin Industries, Inc. (Des Moines, IA).

Purge loss (%) = [(gross weight(with packaging)   
- packaging weight - sample weight)   
$$\div$$
 gross weight]  $\times$  100 (1)

**Statistical analysis.** The microbiological data were reported as average values and standard deviations (log CFU per milliliter of rinse) for triplicate samples and three independent trials (n=3) for each test formulation. Differences between the experimental treatments and the untreated control for each five-strain inoculum, as well as between the two five-strain inocula, were analyzed by multifactor analysis of variance (ANOVA) by using the STAT-GRAPHICS Centurion XV software package (Statpoint Technologies, Inc., Warrenton, VA). Color and purge loss results were subjected to multifactor ANOVA. All statistically significant differences in the study were reported at P < 0.05 level.

## RESULTS

**Proximate and active ingredient results.** No appreciable differences were observed in the results for pH, moisture, water activity, fat, and protein among the treatments (data not shown). The pH values of the treatments ranged from  $6.27 \pm 0.04$  to  $6.34 \pm 0.06$ . Moisture contents ranged from  $75.23\% \pm 0.96\%$  to  $75.93\% \pm 0.84\%$ . Water activity of the treatments ranged from  $0.9796 \pm 0.0027$  to  $0.9840 \pm 0.0043$ . Fat and protein content ranged from  $0.50\% \pm 0.07\%$  to  $0.62\% \pm 0.12\%$  and  $17.48\% \pm 0.74\%$  to  $18.33\% \pm 0.85\%$ , respectively. Sodium and potassium contents (Table 2) were in the range of  $0.62\% \pm 0.06\%$  to  $0.83\% \pm 0.04\%$  and  $0.25\% \pm 0.01\%$  to  $0.54\% \pm 0.02\%$ , respectively. The acetic acid results (Table 2) of the dry vinegar treatments were in the expected range of 0.27 to 0.54%.

Inhibition of L. monocytogenes (five-strain inocula 1 and 2). Results from three replications (Table 3) showed that all dry vinegar treatments significantly (P < 0.05)inhibited the growth of L. monocytogenes compared with the untreated control. The untreated control showed an average log increase of 1.11  $\pm$  0.36 and 3.00  $\pm$  0.58 log CFU/ml of rinse by the end of 2 and 4 weeks, respectively. The acceptance criterion for an effective dry vinegar treatment in this study was that it should not show >1 log increase in L. monocytogenes counts throughout the testing period. The 0.4% BDV-SB showed an average increase of  $0.89 \pm 1.07$ log CFU/ml of rinse at the end of 8 weeks. The 0.5% BDV-PB showed an average increase of  $0.36 \pm 0.65 \log \text{CFU/ml}$ of rinse at the end of 9 weeks. The higher application levels of BDV-SB (0.6 and 0.8%) and BDV-PB (0.7 and 0.9%) consistently showed <1 log increase in L. monocytogenes counts throughout the testing period. No significant differences were seen between 0.6% BDV-SB and 0.7%

TABLE 3. Pooled average change in Listeria monocytogenes (five-strain inocula 1 and 2) levels on inoculated reduced-sodium, uncured turkey breast stored at 4°C for 12 weeks<sup>a</sup>

Week	Untreated	0.4% BDV-SB <sup>b,c</sup>	0.6% BDV-SB	0.8% BDV-SB	0.5% BDV-PB	0.7% BDV-PB	0.9% BDV-PB
0	$0 \pm 0$	$0 \pm 0$	$0 \pm 0$	$0 \pm 0$	$0 \pm 0$	$0 \pm 0$	0 ± 0
2	$1.11 \pm 0.36 \text{ A}^d$	$0.01 \pm 0.37 \; \mathrm{B}$	$-0.13 \pm 0.24 \text{ B}$	$-0.06 \pm 0.15 \; \mathrm{B}$	$-0.02 \pm 0.11 \text{ B}$	$-0.05\pm0.14\;\mathrm{B}$	$-0.27\pm0.13$ B
4	$3.00 \pm 0.58 \text{ A}$	$-0.19 \pm 0.22 \; \mathrm{B}$	$-0.22\pm0.29$ B	$-0.36 \pm 0.23 \; \mathrm{B}$	$-0.12 \pm 0.21 \text{ B}$	$-0.18 \pm 0.31 \; \mathrm{B}$	$-0.42\pm0.36$ B
6	$4.88 \pm 0.73 \text{ A}$	$0.43 \pm 0.59 \text{ B}$	$-0.24 \pm 0.32 \; \mathrm{BC}$	$-0.40 \pm 0.14 \text{ C}$	$-0.03 \pm 0.26 \text{ c}$	$-0.35 \pm 0.15 \text{ c}$	$-0.54 \pm 0.14 \text{ C}$
7	$5.36 \pm 0.60 \text{ A}$	$0.33 \pm 0.49 \; \mathrm{B}$	$-0.19 \pm 0.33 \; \mathrm{BC}$	$-0.35 \pm 0.20 \text{ c}$	$0.15 \pm 0.31 \; \text{BC}$	$-0.47\pm0.32\;{\rm C}$	$-0.60 \pm 0.28 \text{ c}$
8	$5.79 \pm 0.27 \text{ A}$	$0.89 \pm 1.07 \text{ B}$	$-0.05 \pm 0.61 \text{ c}$	$-0.37 \pm 0.15 \text{ c}$	$0.35 \pm 0.50 \text{ BC}$	$-0.55 \pm 0.35 \text{ c}$	$-0.56 \pm 0.17 \text{ c}$
9	$5.75 \pm 0.19 \text{ A}$	$1.37 \pm 1.30 \text{ B}$	$-0.20 \pm 0.34 \text{ CD}$	$-0.59 \pm 0.30 \text{ CD}$	$0.36 \pm 0.65 \text{ c}$	$-0.52 \pm 0.19 \text{ CD}$	$-0.79 \pm 0.31 \text{ D}$
10	$5.85 \pm 0.23 \text{ A}$	$1.42 \pm 1.36 \text{ B}$	$0.05 \pm 0.55$ CD	$-0.49 \pm 0.23 \text{ D}$	$0.74 \pm 0.77 \; \text{BC}$	$-0.47 \pm 0.42 \text{ d}$	$-0.74 \pm 0.35 \text{ D}$
11	$5.78 \pm 0.15 \text{ A}$	$1.37 \pm 1.42 \text{ B}$	$-0.02\pm0.64~\mathrm{CD}$	$-0.58 \pm 0.24 \text{ D}$	$0.42 \pm 0.89 \; \text{BC}$	$-0.58 \pm 0.24 \text{ D}$	$-0.73\pm0.19$ D
12	$5.92 \pm 0.05 \text{ A}$	$1.89 \pm 1.84 \text{ B}$	$0.08 \pm 0.77$ CD	$-0.51 \pm 0.25$ CD	$0.71 \pm 1.00 \text{ c}$	$-0.54 \pm 0.32 \; \mathrm{D}$	$-0.84 \pm 0.32 \; \mathrm{D}$

<sup>&</sup>lt;sup>a</sup> RTE turkey breast formulated with BDV-SB (0.4, 0.6, and 0.8%) or BDV-PB (0.5, 0.7, and 0.9%) was inoculated with *Listeria* monocytogenes to a target of 3 log CFU/ml of rinse. Untreated, inoculated RTE turkey breast served as a negative control. Changes in *L.* monocytogenes population levels were determined during vacuum-packaged storage. Values represent the mean  $\pm$  standard deviation of three replications (three samples per testing interval in each replication, n = 3).

<sup>&</sup>lt;sup>b</sup> Corresponding increasing concentrations of BDV-SB and BDV-PB ingredients provided equivalent acetic acid concentrations in the final RTE turkey breast product.

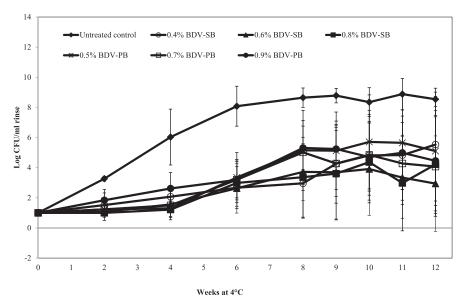
<sup>&</sup>lt;sup>b</sup> BDV-SB and BDV-PB designate dry vinegar buffered with a sodium base and a potassium base, respectively. Both buffered dry vinegar ingredients were supplied by Kemin Industries, Inc. (Des Moines, IA).

<sup>&</sup>lt;sup>c</sup> Corresponding increasing concentrations of BDV-SB and BDV-PB ingredients provided equivalent acetic acid concentrations in the final RTE turkey breast product.

<sup>&</sup>lt;sup>d</sup> Within each row, means with different letters are significantly different (P < 0.05).

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FIGURE 1. Average log populations of LAB in uninoculated, uncured RTE turkey breast samples stored at  $4^{\circ}$ C for 12 weeks. RTE turkey breast products formulated with BDV-SB (0.4, 0.6, and 0.8%) and BDV-PB (0.5, 0.7, and 0.9%) were analyzed for LAB levels during storage. Untreated turkey breast samples served as a negative control. Error bars represent the mean  $\pm$  standard deviation of three replications (two samples per testing interval in each replication, n = 3).

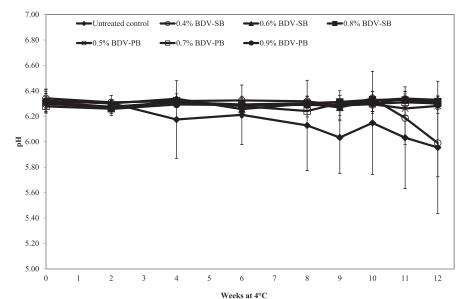


BDV-PB, as well as 0.8% BDV-SB and 0.9% BDV-PB, thus indicating no difference in the antimicrobial activity between sodium and potassium salts of dry vinegar.

Lactic acid bacteria counts and pH. Lactic acid bacteria counts (Fig. 1) at time zero for all the treatments were less than levels detectable by direct plating (<1 log CFU/ml of rinse). At the end of 12 weeks, counts increased to  $8.54 \pm 0.47 \log \text{CFU/ml}$  of rinse for the untreated control samples and significant differences were seen compared with dry vinegar treatments. The 0.4, 0.6, and 0.8% BDV-SB showed 5.53  $\pm$  3.75, 2.94  $\pm$  3.16, and 4.22  $\pm$  3.26 log CFU/ml of rinse, respectively, and no significant differences were seen among the three treatments. The BDV-PB treatments at 0.5, 0.7, and 0.9% showed 5.10  $\pm$  3.62, 4.07  $\pm$  3.30, and 4.46  $\pm$  3.33 log CFU/ml of rinse, respectively, and no significant differences were seen among the three treatments. The BDV-SB treatments at 0.6 and 0.8% differed significantly compared with the 0.5 and 0.9% BDV-PB treatments. No significant differences were seen between 0.4% BDV-SB and 0.7% BDV-PB treatments. These results showed that both dry vinegar ingredients did not inhibit the spoilage microbes. The pH results (Fig. 2) of the dry vinegar treatments showed no significant differences, except 0.4% BDV-SB was significantly lower than the other dry vinegar treatments at week 12, whereas untreated control samples were significantly lower than dry vinegar treatments from 8 to 12 weeks.

**APC.** The initial mesophilic aerobic count (Fig. 3) for the untreated control slices was  $1.69 \pm 1.07 \log$  CFU/ml of rinse. By the end of 12 weeks, counts reached  $7.43 \pm 0.63 \log$  CFU/ml of rinse. The APC for untreated control samples differed significantly with the vinegar treatments across all the testing intervals. Initial counts for the dry vinegar treatments were <1 log CFU/ml of rinse. By the end of 12 weeks, BDV-SB at 0.4, 0.6, and 0.8% showed  $3.73 \pm 2.40$ ,  $1.63 \pm 1.41$ , and  $2.20 \pm 1.91 \log$  CFU/ml of rinse, respectively. BDV-PB at 0.5, 0.7, and 0.9% showed  $1.56 \pm 0.61$ ,  $1.70 \pm 1.00$ , and  $2.90 \pm 1.76 \log$  CFU/ml of rinse,

FIGURE 2. pH determinations of uninoculated, uncured, RTE turkey breast samples stored at  $4^{\circ}$ C for 12 weeks. Turkey breast samples were formulated with BDV-SB (0.4, 0.6, and 0.8%) or BDV-PB (0.5, 0.7, and 0.9%) and analyzed for pH. Turkey breast without dry vinegar ingredients served as an untreated control. Error bars represent the mean  $\pm$  standard deviation of three replications (two samples per testing interval in each replication, n = 3).



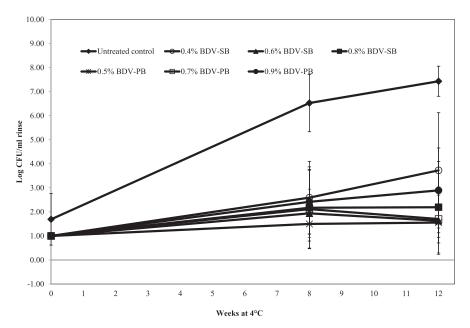


FIGURE 3. Average log populations of mesophilic aerobic populations in uninoculated, uncured RTE turkey breast samples stored at  $4^{\circ}$ C for 12 weeks. Turkey breast products were formulated using BDV-SB (0.4, 0.6, and 0.8%) and BDV-PB (0.5, 0.7, and 0.9%) and analyzed for APC throughout storage. Turkey breast formulated with no dry vinegar ingredients served as an untreated control. Error bars represent the mean  $\pm$  standard deviation of three replications (two samples per testing interval in each replication, n = 3).

respectively. No significant differences in APC were seen among the dry vinegar treatments across all the testing intervals, except 0.4% BDV-SB, which was higher than the other dry vinegar treatments at week 12. These results showed that dry vinegar treatments did not inhibit the growth of mesophilic aerobic bacteria but delayed the growth better than the untreated control.

**Instrumental color.** No differences (P > 0.05) were observed for L\* (76.81 to 78.68), a\* (1.57 to 2.83), and b\* (11.93 to 14.60) values among the seven treatments.

**Purge loss.** Purge loss values ranged from 1.4 to 5.4%. Statistical analysis of purge loss values showed no significant differences among the dry vinegar treatments, but significant differences were seen between untreated and few dry vinegar treatments. There were no significant differences among untreated 0.4 and 0.8% BDV-SB treatments, whereas significant (P < 0.05) differences were observed between untreated and the remaining dry vinegar treatments at weeks 0 and 8. The 0.9% BDV-PB treatment resulted in significantly (P < 0.05) greater purge when compared with 0.4 and 0.6% BDV-SB and the untreated control at week 8.

## DISCUSSION

Vinegar has been used for centuries for a variety of purposes and has well-documented antimicrobial properties (7). Although there are no standards of identity for vinegar, the U.S. Food and Drug Administration guidelines indicate that natural vinegars normally contain in excess of 4 g of acetic acid per 100 ml (31). The low pH of vinegar (2.0 to 3.0) is a limiting factor for its application in RTE meat and poultry products, as it can negatively affect physical and sensory characteristics. The advantages of buffering and drying the vinegar are threefold: it reduces the pungent vinegar flavor to a mild vinegar flavor, it has a less negative impact on the taste and flavor of the treated finished product,

and it can be used at lower application rates owing to a more concentrated acetic acid. This study demonstrated that BDV-SB at 0.6 and 0.8%, and BDV-PB at 0.7 and 0.9% controlled L. monocytogenes for 12 weeks in reduced-sodium RTE uncured turkey breast (approximately 76% moisture, pH 6.30, and 0.66% sodium) stored at 4°C. Lavieri et al. (15, 16) reported dried vinegar as a potential bacteriostatic ingredient for inhibiting the growth of L. monocytogenes inoculated into alternatively cured frankfurters and alternatively cured RTE ham. Their research showed that inclusion of 1% dried vinegar when formulating both of these meat products prevented the growth of L. monocytogenes for 14 weeks when stored at  $4 \pm 1$ °C. However, dried vinegar did not exhibit any bactericidal properties against L. monocytogenes in their studies. Porto-Fett et al. (22) showed no change in L. monocytogenes population in deli-style ham formulated with 1.5% buffered vinegar, with or without a stabilized solution of sodium chlorite, for up to 90 days of storage at 4°C, whereas 2.0 or 2.5% buffered vinegar reduced pathogen counts by 1.1 and 2.0 log CFU per slice, respectively. Roast beef formulated with 1.0 or 1.5% buffered vinegar showed an increase of 2.2 to 2.4 log CFU per slice, but they also found that roast beef formulated with 2.0 or 2.5% buffered vinegar decreased L. monocytogenes counts by 0.7 and 1.2 log CFU per slice, respectively, when stored for 90 days at 4°C. In another Listeria challenge study on uncured turkey breast formulated with 3.0% buffered vinegar and surface treated with or without a stabilized solution of sodium chlorite in vinegar, Porto-Fett et al. (23) observed counts decrease by approximately 0.7 to 1.3 log CFU per slice, respectively, when stored at 4°C for 90 days. However, when stored at 10°C, pathogen numbers increased by approximately 1.5 to 5.6 log CFU per slice after 48 days when formulated with 2.0 to 3.0% buffered vinegar and treated with or without 2% sodium chlorite in vinegar. McDonnell et al. (19) reported that 2.0% liquid buffered vinegar in sliced, uncured, deli-style turkey breast, alternatively cured boneless ham, and uncured roast beef delayed the growth of L. monocytogenes until 6, 6, and 12

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weeks of storage at 4°C, respectively. The authors speculated that the significant inhibition of pathogen growth in roast beef compared with the turkey breast and boneless ham could be because of differences in pH and moisture content of the products. Note that liquid buffered vinegar has a lower concentration of acetic acid when compared with dry vinegar; hence, higher application rates were used for liquid buffered vinegar.

In the current study, BDV-SB and BDV-PB showed similar antimicrobial efficacy against the two five-strain inocula of L. monocytogenes used for the challenge study. Variations were seen in pH and spoilage microflora among the three replications, and this could be because of differences in the raw material quality and processing conditions, such as slicing. Despite differences in the growth of spoilage microflora across the replications, the dry vinegar treatments showed consistent inhibition of L. monocytogenes, indicating their robustness in antimicrobial efficacy. To our knowledge, there is no published literature showing the antimicrobial efficacy of potassium-based dry vinegar; thus, this finding could be of significant importance for enhancing the safety of low-sodium or reduced-sodium RTE uncured turkey breast. Additional research must be conducted to determine the efficacy of these dry vinegarbased ingredients for controlling L. monocytogenes in a broader range of RTE products and also the impact of slightly to moderately higher storage temperatures, as the current data will not be sufficient for validating this technology in other RTE products.

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