Utilization of Buffered Vinegar to Inhibit the Growth of Listeria monocytogenes on Marinated and Cooked Chicken Breast

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Abstract: The objective of this experiment was to evaluate the efficacy of adding buffered vinegar to RTE broiler breast meat to inhibit Listeria monocytogenes growth on cooked broiler breast meat. Broiler breast fillets were vacuum-tumbled with a 15% solution (over green weight) for 30 min with a marinade consisting of different concentrations of dry vinegar (DV) or liquid vinegar (LV; 0%, 0.4, 0.6, and 0.8% DV, and 1.5% LV), 1.0% sodium chloride on a total raw product basis (RPB), 0.4% sodium tripolyphosphate on a RPB and water. After marinating, the chicken was cooked to an internal temperature of 77°C. The top and bottom of each piece of broiler breast meat (200 g) was surface inoculated with 1 mL of ~5 log CFU/g of a 3 strain mixture of L. monocytogenes such that there was a target concentration of 3 log CFU/g L. monocytogenes on each chicken breast. Each chicken breast was then placed into individual packages that had the headspace exchanged for a modified atmosphere (95% CO₂, 5% O₂) and stored at refrigeration temperature (2°C ± 2°C) for 0 to 60 d and sampled at 5 d intervals. No differences in L. monocytogenes counts existed among buffered vinegar treatments at any storage time. At storage times from 35 to 60 d, broiler breast meat that was treated with buffered vinegar had fewer L. monocytogenes counts (P < 0.05) than the untreated broiler breast meat and continued to control L. monocytogenes growth through 60 d of storage. Additionally, the chicken treated with 0.8% DV and 1.5% LV had 2.0 log CFU/g or less L. monocytogenes counts after 60 d of storage.

Keywords: acetic acid, broiler breast, buffered vinegar, Listeria monocytogenes, shelf life
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Introduction

Listeria monocytogenes is a Gram-positive, ubiquitous non-spore forming, rod shaped, facultative anaerobic bacterium that is able to endure high salt percentages (as high as 30%) and can grow at pHs ranging from approximately 4.3 to 9.4 under otherwise ideal conditions. L. monocytogenes is capable of growth at 0°C to 45°C, and can survive both freezing and water activity as low as 0.90 (Miller, 1992). L. monocytogenes is an opportunistic, psychotropic foodborne pathogen that causes listeriosis, which can cause septicemia and lead to an infection of the central nervous system with meningitis, encephalitis, or abscesses (Gandhi and Chikindas, 2007). Immunosuppressed individuals, children, elderly, and pregnant women are especially susceptible to listeriosis infections. The ability of L. monocytogenes to survive under numerous harsh conditions is a major concern in refrigerated, ready-to-eat (RTE) meat and poultry products, particularly when refrigeration is marginal (10°C or higher; Tompkin et al., 2001).

Ready-to-eat foods, including produce, fermented products, cooked and cured meat and poultry products have been associated with listeriosis-related outbreaks (Buchanan et al., 2017). Due to the increase in food-
bemote illness associated with *L. monocytogenes* in RTE meat and poultry products, the USDA-FSIS has issued a zero tolerance policy and HACCP regulations to address control measures in RTE products (Henning and Cutter, 2001; USDA-FSIS, 2003). *L. monocytogenes* contamination commonly results from exposing the finished product to the pathogen after thermal processing and prior to consumption. Cured or cooked RTE meat products, such as frankfurters and deli luncheon meat and poultry items are commonly consumed without additional heat treatment, which may lead to a serious health risk in the event of post-lethality contamination with *L. monocytogenes* (Samelis et al., 2001; Jiang and Xiong, 2015). An attempt to lower the frequency of microbial spoilage and recontamination, RTE food processors commonly use antimicrobials, including organic acids.

The most common organic acids that are used in meat products include acetic acid, propionic acid, and lactic acid. Organic acids are naturally occurring weak acids that are found in variety of fruit juices and fermented foods as the products of microbial metabolism that can be added to food products during processing (Alvarado and McKee, 2007). Most weak organic acids are generally recognized as safe (GRAS) and are among the most common chemical preservatives used in food. They are frequently added to RTE meat and poultry products to inhibit or delay bacterial spoilage, increase shelf life, and contribute to the flavor and sensory qualities of RTE meat and poultry products (Lück and Jager, 1997; Morey et al., 2014; Desai et al., 2014). Undissociated acids are more hydrophobic than disassociated acids. Organic acids, which are hydrophobic interact better with lipids in the microbial cell wall to disrupt microbial activity (Hirshfield et al., 2003; Ricke, 2003; Davidson et al., 2013). Even though organic acids are commonly used in foods, the optimal concentration for antimicrobial efficacy in certain foods still needs to be determined. Therefore, this study was conducted to determine the inhibitory effect of different concentrations of buffered vinegar in a marinade solutions on the growth of *L. monocytogenes* on cooked-RTE broiler breast meat.

**Materials and Methods**

**Sample preparation and marinating**

Broiler breast meat was obtained from 3.2 kg broiler chickens at a commercial chicken processing plant within 24 h postmortem. For each treatment within each of 3 replications, 30 breast pieces (6.8 kg of broiler breasts at approximately 230 g/breast) were marinated with a brine solution that was formulated for a target pick-up of 12 to 15% over initial weight. The brine solution contained water, 0.4% sodium tripolyphosphate (STPnew, ICL Performance Products, St Louis, MO) on a raw formulated product basis (RPB), 1.0% sodium chloride (NaCl) RPB (salt, Culinox 999, Morton Salt, NY), and 1 of 5 vinegar treatments which consisted of 0% dry vinegar (DV, eLm)inate V- Dry, Hawkins, Inc. Roseville, MN), 0.4, 0.6, 0.8% DV, or 1.5% liquid vinegar (LV, eLm)inate V, Hawkins, Inc. Roseville, MN) based on RPB. Each 8.8 kg of marinated broiler breast was placed in a BIRO Vacuum Tumbler (VTS-44, BIRO Manufacturing, Marblehead, OH; 825 mm in length and 393.7 mm width with twin 9.1kg drums) and vacuum tumbled (20 mm Hg) at 2 to 4°C for 30 min. After tumbling, the chicken breast from each treatments were weighed to determine marinade pick-up. Chicken breast from each treatment were then separately cooked in a Hobart Steam Oven (Troy, OH) at 177°C to a final internal temperature of 77°C. The cooked broiler breasts were then stored in vacuum packages (Model 75840157 Clarity Vacuum Pouches Koch Supplies Inc., Kansas City, MO) for 18 h at 2 to 4°C.

**Solution pick-up**

Solution pick-up was determined by calculating the difference in weight of the broiler breast prior to marinating and the weight of the broiler breast after marinating and vacuum tumbling. The solution pick-up was reported as a percentage and was calculated as follows:

\[
\% \text{ Solution pick-up} = \frac{\text{marinated weight} - \text{raw weight}}{\text{raw weight}} \times 100
\]

**Cooking loss**

Each treatment was weighed prior to cooking and then reweighed after cooking to a final internal temperature of 77°C. The temperature was measured by inserting a Taylor TruTemp thermometer (Oak Brook, IL) into the thickest portion of the broiler breast muscle. The cooked chicken was cooled to ambient temperature (20°C) and reweighed. Cooking loss was determined using the pre-cooked initial weight and final cooked weight and was reported as a percentage and calculated using the equation below.

\[
\% \text{ cook loss} = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100
\]
**pH measurement**

The pH of broiler breast samples were taken 24 h after cooking using an Accument pH meter (model Accument AP61, Fisher Scientific, Pittsburg, PA) with an attached meat penetration probe (penetration tip 05998–20, Cole Palmer, Vernon Hills, IL). The penetration probe was inserted 2.5 cm into the broiler breast muscle from top to bottom allowing the pH to stabilize. For each replication, 2 broiler breasts from each treatment (n = 6) were analyzed for pH.

**Proximate analysis**

Broiler breast meat samples (n = 3) within each treatment and replication were used to determine moisture, protein, and fat percentage using a near-infrared spectrometer (Food Scan Lab Analyzer model 78800, Foss Analytical, Eden Prairie, MN) according to AOAC Official Method 2007.04 (Horwitz and Latimer, 2007). Fresh samples were ground (Fisher Scientific Laboratory Homogenizer 500/08451666, Fisher Scientific LLC, Pittsburg, PA) through a 3-mm grinder plate and evaluated for proximate analysis immediately after grinding. Ground samples were packed tightly in a sample cup prior to analysis.

**L. monocytogenes inoculation**

Three verified strains of *L. monocytogenes* (ATCC 19115, ATCC 7644, ATCC 19144, American Type Culture Collection, Manassas, VA) were individually cultured in tryptic soy broth (Becton Dickinson) and cooked broiler breasts (approximately 200 g) were aseptically inoculated on both sides of the muscle with 1 mL of the *L. monocytogenes* culture was added at a volume of 0.1 mL and mixed with 1L of 0.1% peptone water (Oxoid Basingstoke, Hampshire, England) to form a *L. monocytogenes* inoculum of 5 log CFU/g. The marinated and cooked broiler breasts (approximately 200 g) were aseptically inoculated on both sides of the muscle with 1 mL of the *L. monocytogenes* mixture such that there was approximately 3.0 log CFU/g of *L. monocytogenes* cells inoculated on each chicken breast. The inoculum and chicken breasts were massaged for one min in 15.2 × 20.3 cm, 3-mil vacuum pouches (75001815, Rebel Butcher Supply Co. Inc., Flowood, MS) and then packaged (Turbovac 320-ST-S, Inject Star of the Americas Inc., Brookfield, CT) in modified atmosphere (95% CO₂, 5% O₂). The target inoculation level on each sample was 3 log CFU/g. The Modified Atmosphere Packaged (MAP) broiler breasts were stored at 2°C ± 2°C for up to 60 d and evaluated every 5 d for *L. monocytogenes*. MAP broiler breasts from replication 1 that were not inoculated were used to determine total plate counts (TPC) over storage time. The TPC was determined using tryptic soy agar (Becton Dickinson) and inverted TPC plates (3M Petrifilm Aerobic Count Plate, St. Paul, MN) that were incubated at 35°C for 48 h.

**Measurement of *L. monocytogenes* growth**

A 25-g portion of each breast (2 breasts per replication at each time point) was aseptically cut and placed into a stomaching bag and homogenized with 225 mL of 0.1% sterilized peptone water solution for 1 min in a Stomacher (Seward 400 Circulator, Seward Limited, Worthing, West Sussex, United Kingdom). Dilutions were made by serially placing 1 mL of the homogenate into dilution tubes with 9 mL of 0.1% sterilized peptone water solution. A 0.1-mL aliquot of the diluted samples was spread onto sterile plates of Difco Oxford Medium Base (BD Difco, Sparks, MD) that was supplemented with the antibiotic Polymyxin. The plates were incubated at 35°C for 48 h, and then examined for microbial growth. The *L. monocytogenes* colonies were manually counted and recorded as log colony forming units per gram (CFU/g). The *L. monocytogenes* limit of detection was 100 CFU/g (2 logs). When no colonies were detected, the values were below the limit of detection but were recorded as 1 log *L. monocytogenes* counts for the purpose of conducting statistical analysis.

**Acetic acid concentration**

A High Performance Liquid Chromatograph (HPLC) equipped with a reflective index detector (Agilent 1100, Agilent, Santa Clara, CA) was utilized to determine the acetic acid concentration in broiler breast meat from the 0, 0.4, 0.6, 0.8% DV, and 1.5% LV treatments on d 0 and d 45 of the experiment. A standard curve for acetic acid was produced by using HPLC measurements and concentrations of 0.05, 0.1, 0.2, and 0.4% HPLC-grade glacial acetic acid. One broiler breast from each of the 5 treatments was randomly selected from samples that were stored for d 0 and d 45 for each of the 3 replications to quantify acetic acid concentration. A 10-g sample of the randomly selected broiler breast was homogenized with 50 mL of 0.1 N sulfuric acid (H₂SO₄) and then centrifuged (Sovall Lynx 400, Thermo Scientific, Asheville, NC) for 15 min at 18,000 RPM at 4°C. One mL of the centrifuged samples was filtered through a 0.45 µm syringe filter and 25 ML was injected into an Ion Exclusion Column (300 mm × 7.8 Ion Aminex HPX– 87H Ion Exclusion Column, Bio-Rad, Hercules,
CA). The mobile phase was 0.01 N H₂SO₄ with the flow rate of 0.6 mL/min (Kim et al., 2009). The peak for acetic acid was analyzed using ChemStation software (Agilent Technologies, Waldbronn, Germany). The peak was identified, based on retention time of standard acetic acid and quantified using a standard curve.

**Statistical analysis**

A two-way factorial design within a randomized complete block design with 3 replications was used to determine if differences existed \((P < 0.05)\) among buffered vinegar treatments with respect to treatment, time, and treatment × time interaction (Verizon 9.4; SAS Inst. Inc., Cary, NC) for *L. monocytogenes* counts. A randomized complete block design was utilized to determine if differences existed \((P < 0.05)\) among vinegar treatments with respect to marinade pick-up, cooking loss, pH, proximate analysis, and acetic acid concentration. Tukey’s Honest Significant Difference (HSD) test was utilized to separate treatment means \((P < 0.05)\) when differences occurred among treatments (SAS Inst. Inc.).

**Results and Discussion**

**Solution pick-up, cooking loss, yields, proximate analysis, and acetic acid concentration**

No differences \((P > 0.05)\) existed between treatments with respect to marinade pick-up, yield based on green weight, pH, percentage moisture, and percentage fat (Table 1). This is similar to research reported by Badvela et al. (2016), which indicated that the use of buffered vinegar did not impact yields, pH, and proximate composition of uncurled deli turkey. In addition, Theron and Yues (2007) reported that buffered forms of organic acids have a functional advantage over pure forms since they do not significantly change the pH of the food. All else equal, if the pH of the chicken is not changed, it is likely that yields would be not affected either.

The acetic acid concentration found in the control treatment was less \((P < 0.05)\) than all other treatments which ranged from 2.1 to 4.2 mg/g. In addition, the acetic acid concentration in the 0.6 and 0.8% DV treatments and the 1.5% LV treatment had a greater acetic acid concentration \((P < 0.05)\) than the 0.4% DV treatment (Table 1). In theory, the 0.8% DV should have contained more acetic acid than the 0.6% DV and 1.5% LV treatments. Since muscle pH was not changed between treatments, there may have been greater concentrations of the undissociated form of acetic acid in treatments with higher concentrations of the buffered vinegar, even though minimal differences existed in acetic acid concentration among 0.6 and 0.8% DV and 1.5% LV treatments (Jiang and Xiong, 2015; Alvarado and McKee, 2007). The final concentrations of DV and LV in the cooked chicken were calculated as 0.23, 0.54, and 0.57% DV for the 0.4, 0.6, and 0.8% DV treatments and 1.13% LV for the 1.5% LV treatment since the buffered dry vinegar contains 74.15% acetic acid and the buffered liquid vinegar contains 31.75% acetic acid.

Chicken breasts from the control treatment (0%) and 0.6% DV treatment had less cooking loss \((P < 0.05)\) than chicken breasts from the 0.4% DV and 1.5% LV treatments. No other differences in cooking loss existed \((P > 0.05)\) among treatments (Table 1). Chicken breast from the 0.4% DV and 0.6% DV yield-

### Table 1. Marinade Pick-Up, Cooking Loss, Yields, pH, proximate composition, and Acetic Acid Concentration of marinated chicken breast [1.0% salt and 0.4% sodium tripolyphosphate (STP) that is vacuum tumbled with a 15% brine containing water, salt, STP and Dry vinegar (0, 0.4, 0.6, and 0.8% DV) or Liquid Vinegar (1.5% LV)]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Solution pick-up, %</th>
<th>Cooking loss, %</th>
<th>Yield based on green weight, %</th>
<th>pH</th>
<th>Moisture, %</th>
<th>Protein, %</th>
<th>Fat, %</th>
<th>Acetic acid concentration, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.7</td>
<td>29.4b</td>
<td>78.8</td>
<td>6.2</td>
<td>68</td>
<td>28.8b</td>
<td>3.1</td>
<td>ND2</td>
</tr>
<tr>
<td>0.4% DV</td>
<td>11.8</td>
<td>33.2a</td>
<td>74.9</td>
<td>6.2</td>
<td>66.7</td>
<td>31.3a</td>
<td>2.5</td>
<td>2.1b</td>
</tr>
<tr>
<td>0.6% DV</td>
<td>12.3</td>
<td>29.9b</td>
<td>78.8</td>
<td>6.25</td>
<td>68.2</td>
<td>30.9a</td>
<td>2.3</td>
<td>4.0a</td>
</tr>
<tr>
<td>0.8% DV</td>
<td>12.5</td>
<td>31.7ab</td>
<td>76.8</td>
<td>6.2</td>
<td>68.4</td>
<td>30.1ab</td>
<td>2.2</td>
<td>4.2a</td>
</tr>
<tr>
<td>1.5% LV2</td>
<td>12.2</td>
<td>32.6a</td>
<td>75.7</td>
<td>6.18</td>
<td>67.8</td>
<td>29.9ab</td>
<td>2.3</td>
<td>3.6a</td>
</tr>
<tr>
<td>P-value</td>
<td>0.154</td>
<td>0.014</td>
<td>0.151</td>
<td>0.744</td>
<td>0.54</td>
<td>0.007</td>
<td>0.51</td>
<td>0.001</td>
</tr>
<tr>
<td>SEM</td>
<td>0.78</td>
<td>0.8</td>
<td>1.5</td>
<td>0.048</td>
<td>1.03</td>
<td>0.44</td>
<td>0.51</td>
<td>0.66</td>
</tr>
</tbody>
</table>

a,bMeans within each column with different superscripts significantly differ \((P < 0.05)\) between treatments within each column.

1DV = Dry vinegar.

2LV = Liquid vinegar.

3ND = Not detected.
ed breast meat with a greater concentration of protein ($P < 0.05$) than broiler breast meat from the control treatment. This may be partially due to the control treatment having the highest numerical fat percentage and the lowest numerical cooking loss percentage as well as slight variability among raw chicken breast.

**Microbial analysis**

*L. monocytogenes* counts differed ($P < 0.05$) among storage time when averaged over buffered vinegar treatment and differed ($P < 0.05$) among buffered vinegar treatments when averaged over storage time. In addition, the buffered vinegar treatment × storage time interaction was significant ($P < 0.05$). The average initial concentration of *L. monocytogenes* inoculated chicken breasts was $2.5 \log \text{CFU/g}$ for all 5 treatments, and there were no differences among treatments at d 0 ($P > 0.05$; Fig. 1).

No differences ($P < 0.05$) existed in *L. monocytogenes* counts among chicken breasts that were formulated with different vinegar treatments from 5 to 30 d of storage, with approximately $2 \log \text{CFU/g}$ for all treatments. After 35 d of storage, all vinegar treatments exhibited a bacteriostatic effect, with approximately $2 \log \text{CFU/g}$ growth on chicken breasts treated with vinegar and no differences ($P > 0.05$) among treatments. However, chicken breasts subjected to the control treatment had $3.5 \log \text{CFU/g}$ growth, which was greater ($P < 0.05$) than all other treatments with the exception of the $0.6\%$ DV treatment. Similarly, Lavieri et al. (2014) demonstrated that buffered vinegar treatments demonstrate a bacteriostatic effect, but not a bactericidal effect, when applied to contami- nated cured frankfurters. *L. monocytogenes* counts among untreated chicken breast were $4.5 \log \text{CFU/g}$ after 40 d of storage, which was greater ($P < 0.05$) than the *L. monocytogenes* counts for all other vinegar treatments, which were approximately $2.0 \log \text{CFU/g}$. This trend continued from 40 to 60 d of storage. The *L. monocytogenes* counts for untreated chicken breasts were between 4.4 and 5.4 logs $\text{CFU/g}$, which was greater ($P < 0.05$) than the *L. monocytogenes* counts on chicken breasts with any of the vinegar treatments. These results are comparable to results reported by Porto-Fett et al. (2014), which indicated that buffered vinegar was more effective ($P < 0.05$) at inhibiting the growth of *L. monocytogenes* on the surface of uncured turkey breast when compared with samples that were not formulated with BV during extended storage. After 60 d of storage, there were no differences ($P > 0.05$) in counts among vinegar treatments. However, the $0.8\%$ DV and $1.5\%$ LV treatments yielded chicken breasts with

![Figure 1. Mean *Listeria monocytogenes* counts (log CFU/g) of cooked chicken breasts over storage time (0 to 60 d). $a,b$Means within each day with different superscripts differ ($P < 0.05$).](image-url)
2.0 log CFU/g or less L. monocytogenes, which indicates these treatments may be the best options for long-term prevention of L. monocytogenes growth. These results are similar to those of Badvela et al. (2016), who demonstrated that dry vinegar concentrations between 0.5 and 0.9% were bacteriostatic towards L. monocytogenes that was inoculated onto uncured turkey breast at 3 log CFU/g. However, in their study, the 0.4% dry vinegar treatment was not effective at controlling Listeria growth. These results are also in agreement with results reported by McDonnell et al. (2013) and Gonzalez-Fandos and Herrera (2014). These researchers reported that the antimicrobial effect of vinegar and other natural or clean-label antimicrobials is impacted by the concentration that is included in the product. Carpenter and Broadbent (2009) stated that the high levels of weak acid anions that accumulate in the cytoplasm impart an osmotic effect on the cell and on metabolic processes that occur within the cytoplasm. The accumulation of anions inside the cell may increase osmolarity and pressure that cause the cell to burst. Glutamate is a cellular anion that can lower the intracellular pH and inhibit cell function and lead to direct feedback inhibition of metabolic pathways due to the accumulation of anions (Roe et al., 1998).

The total plate count (TPC) for the control and treated broiler chicken breasts was less than 2.0 log CFU/g in replication 1, which indicates that the samples were not spoiled, and that inhibition of L. monocytogenes was likely due to the antimicrobial treatment, rather than to interference from competitive microflora. This is logical since Glass et al. (2013) reported that the population of microorganisms on cured deli-style turkey treated with organic acids and salts of organic acids were not significant enough to interfere with L. monocytogenes growth. In addition, Desai et al. (2014) found that the addition of buffered vinegar to chicken retail cuts increased the shelf life of the chicken by up to 16 d without negatively effecting quality and sensory properties. Since the TPC counts were low and there was no indication of spoilage throughout Rep 1, TPC was not evaluated in subsequent replications

Conclusions

All vinegar treatments (0.4, 0.6, 0.8, and 1.5% DV) inhibited L. monocytogenes growth on cooked broiler breast when compared to the control treatment (0%) when stored between 35 and 60 d at 2°C. Therefore, the addition of buffered vinegar (~5% acetic acid) to a marinade solution with salt and phosphate was effective at inhibiting L. monocytogenes growth for 60 d of refrigerated storage (2°C ± 2°C), which was approximately 30 d longer than the control treatment. After 50 d of storage, L. monocytogenes counts remained at 2 to 3 logs in the 0.4 and 0.6% DV treatments. In comparison, the 0.8% DV and 1.5% LV treatments remained stable at 2 logs of growth through 60 d of storage. In addition, there was no indication of microbial growth or spoilage. Future research could be conducted to determine if acetic acid concentration effects sensory attributes and consumer acceptability of marinated, cooked chicken breast.

Literature Cited


