PROCESSING, PRODUCTS, AND FOOD SAFETY

Microbiological and physical quality changes in vacuum loader cups associated with the use of various sanitizing compounds

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ABSTRACT Studies were conducted to determine the effects of various sanitizing compounds on the microbial and physical quality of shell egg processing vacuum loader cups. The sanitizing compounds used were as follows: sterile, distilled water; 200 μL/L of sodium hypochlorite; 200 μL/L of calcium hypochlorite; and 200 μL/L of peracetic acid. In the microbial inoculation study, cups were inoculated with Enterobacter cloacae because it was the most common isolate from a commercial study examining the flora found on vacuum loader cups. In all 3 replicates, aerobic plate counts and Enterobacteriaceae levels were similar for the clean control cups and the cups from the 2 chlorine treatments. Physical quality was measured via serial static compression testing using texture profile analysis. The serial compression mimicked the movement of the vacuum loader cups on the processing line. The strength of the vacuum loader cups was enhanced with exposure to any sanitizer treatment, including distilled water, compared with the controls throughout the 20 applications of the sanitizers. Durometer measurements were not consistent in monitoring vacuum loader cup quality and were determined to not be effective assessments for this application. The use of 200 μL/L of sodium hypochlorite or 200 μL/L of calcium hypochlorite successfully reduced microbial contaminants, had a positive effect on vacuum loader cup physical quality, and should be considered when developing sanitation programs for shell egg processing facilities.

Key words: sanitizer, shell egg, processing, equipment

INTRODUCTION An integral part of agricultural food processing is cleaning and sanitizing of processing equipment. In 2006, Bilgili discussed the cleaning standards for poultry processing equipment (Bilgili, 2006). Bilgili also presented the design standards for the American Meat Institute, which includes the ability for equipment to be cleaned to 1 cfu/mL when rinsed. Unfortunately, in the shell egg processing industry, all of the equipment used in processing is not clean-in-place compatible. Furthermore, processing lines can vary greatly in age. A survey conducted by Jones and Northcutt (2005) found that over 70% of respondents had shell egg processing equipment over 5 yr of age with the greatest percentage (50%) between 5 to 15 yr old. A challenge facing the egg industry is how to clean the equipment to ensure the wholesomeness of the process.

In 2002, our laboratory coordinated a review of shell egg processing plant sanitation programs in the south-eastern United States (Jones et al., 2003; Musgrove et al., 2004). During this study, it was determined that vacuum loader cups were reservoirs for bacterial growth (Jones et al., 2003). An additional study was conducted to determine the average level of select bacterial populations present on the vacuum loader cups in both an off-line and a mixed operation (Jones and Musgrove, 2008a). An off-line operation is a free-standing processing facility that requires nest run (unprocessed) eggs to be transported to the processing facility from outlying farms. A mixed operation processes eggs entering the facility via belts from production houses adjacent to the facility and also nest run eggs from outlying farms. It was determined that the average level of aerobic organisms present on a single vacuum loader cup rinsed with 20 mL of diluent was approximately 5.0 log cfu/mL. Enterobacteriaceae, a family of bacteria containing many of the common foodborne pathogens, levels averaged approximately 2.5 log cfu/mL. Furthermore, Listeria spp. were isolated from 72% of the vacuum loader cups tested.

Surveys have reported that in excess of 80% of shell egg processing facilities conduct daily cleaning and sanitation programs (Jones and Northcutt, 2005; Vinator et al., 2007). To develop an effective cleaning and
sanitation program in food processing, it is important to first understand the conditions in each processing facility and select the appropriate cleaning compounds. Cramer (2007) outlines 3 considerations for selecting cleaning compounds: 1) type of soil to be cleaned, 2) function of chemicals in the cleaning process, and 3) condition of plant water. Furthermore, Lee et al. (2007) noted that the ability of organisms to survive is affected by both type of organic matter present during cleaning and the washing temperature. Determining the optimum cleaning compound and procedure can be a difficult task.

The current study was undertaken to determine the effects of various sanitizers on the microbial and physical quality of vacuum loader cups. The findings of this research will assist in the development of more effective shell egg processing facility cleaning and sanitation programs.

**MATERIALS AND METHODS**

New vacuum loader cups (Diamond Systems, Farmington Hills, MI) were used for all experiments in the study. A stainless steel apparatus was used to hold 6 cups per treatment for all aspects of the study. The apparatuses were individually autoclaved for all replicates during the inoculation study to ensure sterility. Up to 6 apparatuses could be held on the spindle allowing for a complete replicate to be conducted together (Figure 1).

**Inoculation Study**

Vacuum loader cups were dipped in 70% ethanol for 30 s and allowed to dry overnight in sterile wire baskets wrapped with foil. A fresh inoculum of *Enterobacter cloacae* was prepared from cryovial storage by placing a bead in 10 mL of tryptic soy broth incubated at 37°C overnight. *Enterobacter cloacae* was chosen as the inoculum because it was the organism most frequently isolated from the commercial vacuum loader cup survey conducted in our laboratory (Jones and Musgrove, 2008b). The isolate was collected during the commercial study. The sterilized cups were inoculated in 1 x 10⁶ cfu/mL of *E. cloacae* in buffered peptone water for 1 min then placed on the sterile stainless steel apparatus to air dry for approximately 2 h.

Six treatments were used for the study: negative control (without inoculation or treatment), inoculated (no treatment), sterile distilled water, 200 μL/L of sodium hypochlorite (Commercial 6.15%, Clorox Co., Oakland, CA), 200 μL/L of calcium hypochlorite (DryTeq FG Briquettes, Arch Chemicals Inc., Norwalk, CT), and 200 μL/L of peracetic acid (15%, FMC Corp., Philadelphia, PA). All sanitizing solutions were prepared in 1.9-L pump sprayers (model 12P 1/2 gal Gilmour, BIC Supply, Brooklyn, NY). The concentration of chlorine and peracetic acid was monitored (SAM Chlorine 2, Chemetrics, Calverton, VA, and Peracetic Acid Test, EMD Chemicals Inc., Gibbstown, NJ, respectively). The cups for each chemical treatment were sprayed for 20 s with the corresponding sanitizer. The treated and control cups were allowed to dry at room temperature for 1 h. Afterward, each cup was aseptically placed in a sterile laboratory bag with 30 mL of sterile PBS and shaken for 1 min. The cups were aseptically removed and 0.1 mL of appropriate dilutions of rinsate were plated in duplicate on plate count agar spread plates and violet red bile glucose agar pour plates with overlay (Becton, Dickinson and Co., Sparks, MD). The plates were incubated for 48 h at 35°C and 24 h at 37°C, respectively. Three replicates were conducted.

**Physical Quality Study**

The next study monitored potential changes in the physical characteristics of new vacuum loader cups during 20 consecutive exposures to the selected sanitizers. Once again, 6 cups were exposed to each of the treatments: untreated controls, sterile distilled water, 200 μL/L of sodium hypochlorite, 200 μL/L of calcium hypochlorite, and 200 μL/L of peracetic acid. Cups were sprayed for 20 s and then allowed to air dry on the stainless steel apparatuses (Figure 1) for at least 3 h before quality testing.
A durometer (Shore A Durometer, no. 53-762-101, Fred V. Fowler Co. Inc., Newton, MA) was used to take 3 measurements per cup after each of the sanitizer applications. The readings were taken along the equator of the upper lobe of the vacuum loader cup. The durometer was selected because it is used by the rubber and plastic industries to monitor the stiffness of products.

Overall physical quality of the vacuum loader cups was measured utilizing a modified texture profile analysis (TPA; Texture Exponent Version 40.50, Texture Technologies Corp., Scarsdale, NY) on a TA.XT Plus Texture Analyzer, (Texture Technologies Corp.) fitted with a 750-g load cell, measurement platform (TA-90), aluminum disc (TA-30), and modified testing surface (Figure 2). The modified testing surface was molded on the air cell end of a large egg to allow for appropriate orientation of the cup during testing. The modified TPA was conducted with a test speed of 6 mm/s during compression and a 5 mm/s return speed. The first compression was 15 mm. There was a 5-s rest between compressions. The second compression was 25 mm. A 5-g trigger force and 2-mm trigger difference were used for the profile. During data analysis, the peak force of both

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**Table 1. Sanitizer and replicate effects on the Enterobacteriaceae counts of Enterobacter cloacae-inoculated vacuum loader cups (log cfu/mL)**

<table>
<thead>
<tr>
<th>Sanitizer</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated inoculated control</td>
<td>4.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.46&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.18&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sterile distilled water</td>
<td>3.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.87&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>200 µL/L of sodium hypochlorite</td>
<td>0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200 µL/L of calcium hypochlorite</td>
<td>0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200 µL/L of peracetic acid</td>
<td>1.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.27</td>
<td>0.13</td>
<td>0.13</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means within a column with different superscripts are significantly different (\( P < 0.05 \)).

<sup>b</sup>ND = none detected.
compressions was determined as well as the area under the curves of each compression (Figure 3). Each of the 6 cups per treatment was measured 4 times after each sanitizer application. Initial testing determined that the values derived from the first TPA measurement of a cup were not reproducible with the subsequent 3 measurements. Therefore, the initial measurement was not included in the data set and measurements 2 through 4 were used for analysis. A set of baseline readings was made for each vacuum loader cup for comparison of changes in physical quality due to sanitizer application. The study was conducted in 3 replicates.

**Data Analysis**

Data were analyzed by the GLM of SAS (SAS Institute, 2002). Plate counts resulting in no detectable colonies were entered into the statistical analysis as counts of 1.01 for logarithmic conversion. Plates with a colony count of 1 were converted to 1.2 for logarithmic conversion. Means were separated by the least squares method. Physical quality data were separated by replicates to determine the consistency of vacuum loader cup breakdown across treatments.

**RESULTS AND DISCUSSION**

**Inoculation Study**

The untreated controls, sodium hypochlorite, and calcium hypochlorite vacuum loader cups had similar overall *Enterobacteriaceae* counts (<0.20 log cfu/mL). The untreated, inoculated controls had the highest *Enterobacteriaceae* counts (4.02 log cfu/mL). The 200 µL/L of peracetic acid-treated cups had levels lower than the sterile distilled water-treated cups (1.22 and 2.82 log cfu/mL, respectively). A significant (P < 0.001) sanitizer × replicate interaction existed; therefore, the data were sorted by replicate and analyzed for differences in treatments (Table 1). The untreated controls and 2 chlorine treatments had similar levels of *Enterobacteriaceae* present in all 3 replicates. The 200 µL/L of peracetic acid-treated vacuum loader cups had the second lowest populations with the exception of the third replicate when they were similar to the untreated controls.

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**Table 2. Effect of replicate and 20 applications of sanitizer on durometer readings of vacuum loader cups**

<table>
<thead>
<tr>
<th>Sanitizer</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>32.7</td>
<td>23.0</td>
<td>30.9</td>
</tr>
<tr>
<td>Distilled water</td>
<td>25.9</td>
<td>26.0</td>
<td>27.8</td>
</tr>
<tr>
<td>200 µL/L of sodium hypochlorite</td>
<td>30.6</td>
<td>26.9</td>
<td>32.8</td>
</tr>
<tr>
<td>200 µL/L of calcium hypochlorite</td>
<td>27.9</td>
<td>29.5</td>
<td>29.5</td>
</tr>
<tr>
<td>200 µL/L of peracetic acid</td>
<td>27.2</td>
<td>28.1</td>
<td>28.6</td>
</tr>
<tr>
<td>SEM</td>
<td>0.6</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Means within a column with different superscripts are significantly different (P < 0.05).*

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Figure 4. Average maximum force detected during the second compression of vacuum loader cups after application of sanitizer as compared with the baseline measurement for each treatment.
treated controls and sodium hypochlorite- and calcium hypochlorite-treated cups. Aerobic plate count levels followed the same trends as *Enterobacteriaceae* levels and are therefore excluded from the discussion to provide brevity.

In the current study, sodium hypochlorite and calcium hypochlorite reduced the levels of aerobic organisms and *Enterobacteriaceae* on the surfaces of *E. cloacae*-inoculated vacuum loader cups to those comparable with the untreated controls. Although the presence of organic matter is known to reduce the effectiveness of chlorine as a sanitizer, the current study does give an indication that chlorine compounds have a potential as effective sanitizers on vacuum loader cups. It should be noted that the current inoculation was conducted with a pure culture in a buffered peptone water suspension and not an organic slurry as some poultry carcass decontamination studies have done. A recent study examining the efficacy of several sanitizers on fresh cut carrots determined that organic matter in processing water decreased the efficacy of chlorine but not peracetic acid; overall, 200 μL/L of chlorine was more effective than 40 μL/L of peracetic acid in reducing bacterial levels on the final product (Ruiz-Cruz et al., 2007). In the current study, the peracetic acid compound did not reduce bacterial levels as effectively as the 2 chlorine compounds. King et al. (2005) found 200 μL/L of peracetic acid not to be effective in reducing the number of organisms on chilled beef carcasses. Although, Bauermeister et al. (2008) have recently determined that a different peracetic acid compound could be an effective chiller treatment in poultry processing. Further investigation would be needed to determine if peracetic acid mixtures could be effective sanitizers for vacuum loader cups.

**Physical Quality Study**

There was a significant (*P* < 0.0001) sanitizer × replicate interaction for durometer readings. Table 2 shows the variability of the results. Although significant differences (*P* < 0.05) were seen among the sanitizers for each replicate, no clear trends were seen across the replicates for durometer readings. The inconsistency in readings for the control vacuum loader cups across the replicates indicates the weakness of durometer readings as a quality assessment tool for vacuum loader cups.

A set of baseline TPA measurements was made for each vacuum loader cup in the study. The results are presented as the average change from the baseline measurement after each sanitizer application. Significant (*P* < 0.05) sanitizer × replicate interactions existed for all measurements. No distinct differences were seen among the treatments during the first smaller compression of the vacuum loader cups. During the second larger compression (25 vs. 15 mm), a clear trend occurred in the changes in maximum detected force for the treatments compared with the baseline measurements. The control vacuum loader cups became much weaker after the baseline compression and continued to have the greatest negative change in overall strength through the subsequent measurements (Figure 4). All of the sanitizers (including distilled water) resulted in less physical quality deterioration, as determined by 2-stage compression testing. In many instances, the vacuum loader cups became stronger after subsequent applications of the sanitizer compounds. The changes from baseline measurements in area under the curve associated with the second compression are shown in Figure 5. The greater the negative change in area, the weaker the physical strength of the cup. The control vacuum loader cups
had the greatest negative change in area during the second compression and the distilled water-treated cups had the least. The 3 sanitizer compounds had similar reductions in overall structural quality as noted by the second larger compression. Two-stage compression testing was used to assess the physical quality of the vacuum loader cups because this most closely imitated the normal operational conditions for the cups. From the results in this study, it can be inferred that the application of distilled water or sanitizer compounds enhances the physical quality of the vacuum loader cups compared with untreated counterparts.

The use of 200 μL/L of sodium hypochlorite or 200 μL/L of calcium hypochlorite as a sanitizing treatment on vacuum loader cups effectively reduced the levels of E. cloacae on the cup surface to that of the untreated, clean control cups. Also, results indicated no detrimental effect of the compounds on the physical quality of the vacuum loader cups after 20 consecutive applications. There was an indication that the use of distilled water or sanitizing compounds enhances the structural properties of the vacuum loader cups. Additional research would be needed to determine the effectiveness of sodium hypochlorite and calcium hypochlorite in the commercial setting to reduce complex naturally occurring flora on the vacuum loader cups.

ACKNOWLEDGMENTS

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REFERENCES


