Research Note

Comparison of Ozone and Chlorine in Low Concentrations as Sanitizing Agents of Chicken Carcasses in the Water Immersion Chiller

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ABSTRACT

The aim of this study was to investigate the effects of the use of chlorine or ozone as sanitizing agents in the water of chicken immersion chilling, using the residual levels usually applied in Brazil (1.5 ppm), comparing the effects of these treatments on the microbiological, physicochemical, and sensory characteristics of carcasses. Chicken carcasses were chilled in water (4 °C) with similar residual levels of ozone and chlorine until reaching temperatures below 7 °C (around 45 min). The stability of carcasses was assessed during 15 days of storage at 2 ± 1 °C. Microbiological, surface color (L*, a*, b* parameters), pH value, lipid oxidation (thiobarbituric acid reactive substances index), and sensory evaluation (on a 9-point hedonic scale for odor and appearance) analyses were carried out. The presence of Salmonella was not detected, coagulase-positive staphylococci counts were below 10³ CFU/ml, and Escherichia coli and total coliform counts were below 10⁴ CFU/ml of rinse fluid, and Escherichia coli and total coliform counts were below 10⁴ CFU/ml of rinse fluid until the end of the storage period for both treatments. Psychrotrophic microorganism counts did not differ (P > 0.05) between chlorine and ozone treatments, and both values were near 10⁴ CFU/ml of rinse fluid after 15 days at 4 ± 1 °C. pH values did not differ between treatments (P > 0.05) or during the storage period (P > 0.05). In addition, neither chlorine nor ozone treatment showed differences (P > 0.05) in the lipid oxidation of carcasses; however, the thiobarbituric acid reactive substances index of both treatments increased (P ≤ 0.05) during the storage period, reaching values of approximately 0.68 mg of malonaldehyde per kg. Samples from both treatments did not differ (P > 0.05) in their acceptance scores for odor and overall appearance, but in the evaluation of color, ozone showed an acceptance score significantly higher (P ≤ 0.05) than that for the chlorine treatment. In general, under the conditions tested, ozone showed results similar to the results for chlorine in the disinfection of chicken carcasses in the immersion chilling, which may indicate its use as a substitute for chlorine in poultry slaughterhouses.

Major changes in the poultry market during the last 30 years have placed Brazil among the largest producers and suppliers of poultry products. Chicken slaughter in Brazil follows the norms established by the Technical Regulation for the Technological Inspection of Poultry Meat of the Ministry of Agriculture, Livestock and Supply (1). In Brazil, the chilling process is usually by water immersion, with water temperatures below 4 °C. According to regulations, at least 2.5 liters of water per carcass should be renewed during the immersion chilling process, and this water may have a chlorine content of up to 5 ppm.

It is known that the reaction of chlorine with organic material results in the formation of potentially carcinogenic organochlorine compounds (trihalomethanes) (3, 24). On the other hand, the need for a potent antimicrobial agent has increased in recent years due to contamination of food by emerging microorganisms. The food industry has researched disinfectants that are effective against these pathogens and safe for use. One candidate is ozone, which has been used as a sanitizer for water treatment in Europe since the early 20th century (8).

Previous studies (2, 8, 11–13, 16, 18) have shown that ozone can be used as a safe and effective antimicrobial agent in foods when compared with chlorine and other disinfectants. Ozone exhibits certain characteristics that make it attractive for use as a sanitizer in food processing, including high reactivity and spontaneous decomposition into nontoxic products. As it decomposes rapidly in water, its use is safe because it leaves almost no residue in food. It has been used successfully to inactivate the microbiota of meats, dairy, poultry, fish, fruits, and vegetables. The U.S. Food and Drug Administration (22) and the U.S. Department of Agriculture (21) have approved the use of ozone in aqueous or gaseous form for use as a sanitizer in food processing plants, when combined with good manufacturing practices.

According to Foley et al. (6), the sanitization of fresh products with ozonated water is an excellent tool to reduce the population of microorganisms and parasites from their...
surface. Its large-scale use may reduce the risk of diseases; however, it is necessary to evaluate the tolerance of foods to particular doses and exposure times for ozonated water.

Two mechanisms have been identified for the inactivation of microorganisms. One of them is that ozone oxidizes the sulfhydryl groups and amino acids of enzymes, peptides, and proteins. The oxidation of sulfhydryl groups, which are plentiful in microbial enzymes, may explain the rapid inactivation of microorganisms by this sanitizer. Another mechanism is that ozone oxidizes polysaturated fatty acids into peroxy acids. The degradation of unsaturated lipids in the cell membrane by ozone results in cell rupture and, hence, in the leakage of cellular content. The double bonds of unsaturated lipids are particularly vulnerable to ozone attack. In gram-negative bacteria, the layers of lipoproteins and lipopolysaccharides are the first site of destruction, resulting in increased cell permeability and, eventually, cell breakage (7). Rowan et al. (14) suggest a greater susceptibility of gram-negative bacteria to the action of ozone. According to these authors, they are more susceptible to the lethal effect of ozone due to the lower resistance of cell membranes of this group, which is a hypothesis confirmed by optical microscopy images showing changes caused by the action of ozone on different bacteria.

According to Sheldon and Brown (16), the use of ozone in the water of an immersion chiller for chicken at concentrations between 3 and 4.5 ppm, keeping the carcass immersed for 45 min, resulted in a microbial reduction of about 2 log cycles. Yang and Chen (25) performed the same test with concentrations of 3.88 ppm of ozone dissolved in water for 20 min and also treated chicken necks at concentrations of 2.48 ppm with immersion times of 5 and 9 min, obtaining microbial reductions of about 1, 0.6, and 3 log cycles, respectively.

The aim of this study was to compare ozone and chlorine as antimicrobial agents at low residual levels (1.5 ppm) in the water of chicken immersion chilling by evaluation of the microbiological, physicochemical, and sensory stability of carcasses under refrigerated storage.

**MATERIALS AND METHODS**

**Water immersion chilling of chicken carcasses.** Freshly slaughtered chickens were obtained from a commercial slaughterhouse immediately after evisceration but before chilling (hot carcasses). Carcasses were packaged in polyethylene bags that were transported inside Styrofoam boxes filled with ice to the laboratory where immersion chilling was performed. The time elapsed between obtaining the hot carcasses and water immersion chilling was about 60 min. Chilling was performed in two separate chillers, one for each treatment, and at the same time. Each chiller consisted of a stainless steel tank measuring approximately 1 m³. Both chillers were filled with 400 liters of a mixture of water and ice at a temperature of 4°C. When carcasses were added to the tanks, more ice was added in order to maintain the temperature at 4°C. The chiller for ozone treatment was connected to a recirculation and ozonation system. The ozone generation system (by corona effect, with multiple coaxial tubes) consisted of two ozonators (model BRO3, BrasilOzônio, São Paulo, Brazil) attached to two oxygen concentrators. The time of water immersion chilling of carcasses in both treatments (chlorine and ozone) was around 45 min until the carcasses reached a temperature below 7°C, which is the temperature requirement established by Brazilian legislation (1).

During the entire process, the carcasses were constantly agitated inside the tanks with a plastic stick in order to maintain a constant temperature and consistent concentration of the sanitizing agents. The ozone concentration in the chiller water was measured every 10 min after the placement of carcasses with a test kit using the DPD colorimetric method and maintained at a mean value of 1.5 ppm (ranging from 1.3 to 1.6 ppm) throughout the period of immersion of the carcasses. In the treatment of carcasses with chlorinated water, sodium hypochlorite was added at equal intervals, maintaining a residual chlorine level similar to that of ozone (mean chlorine value of 1.5 ppm, ranging from 1.1 to 2.0) throughout the chilling period. The chlorine content in water was determined by an ortho-tolidine colorimetric method test kit. The chlorine residual level of 1.5 ppm was used in order to comply with Brazilian requirements (1), which permit up to 5 ppm of chlorine in the renewal water of the immersion chiller. In this sense, the addition of chlorine was determined not only in order not to exceed 5 ppm but also to maintain the same level as was achieved for residual ozone by the ozonation system. After chilling, the carcasses were packed in polyethylene bags and stored in a cold storage chamber at 2 ± 1°C for 15 days.

**Evaluation of stability during refrigerated storage.** Analyses of the microbiological and physicochemical stability of chicken carcasses during refrigerated storage were performed on days 1 (day of slaughter), 5, 8, 12, and 15. The sensory evaluations were carried out only on days 1, 5, and 12 following slaughter and were not carried out after 15 days because carcasses had already shown alterations (unpleasant odor) caused by microbiological deterioration. Microbiological evaluation of the carcasses was also carried out just before the chilling stage on hot carcasses (not treated carcasses, i.e., carcasses which had been packaged in plastic bags at the slaughterhouse and transported with ice, about 1 h after the evisceration process) in order to compare the microbial counts before and after sanitizing with chlorine or ozone. All analyses were performed in three replications of the experiment (20 carcasses per treatment per replication) at different periods, except for the microbiological comparison among hot carcasses and carcasses just after the chilling process, which were performed in two replications.

**Microbiological analyses.** Analyses were conducted to determine *Escherichia coli* and total coliform counts according to the Petrifilm method (3M, Microbiology Department, St. Paul, MN), coagulase-positive staphylococci and aerobic psychrotrophic bacterium counts according to Silva et al. (17), and *Salmonella* according to the methodology described by Kushida (9). The surface washing technique was used for the preparation of the dilutions. Chickens were rinsed in polyethylene bags with 300 ml of sterile buffered peptone water for 5 minutes. For the analyses of *Salmonella*, enrichment was carried out by incubating the liquid medium overnight at 35 ± 2°C. The results of microbiological counts were expressed as CFU per milliliter of rinse.

**TBARS index of lipid oxidation.** The direct extraction method proposed by Vyncke (23) was used for the thiobarbituric acid reactive substances (TBARS) index. Previously ground skin-on and deboned cuts (breast, thigh, and drumstick) of carcasses from each treatment were analyzed. The results were expressed as milligrams of malonaldehyde per gram of sample.

**pH values.** The same material as that used for the TBARS index analysis was used (ground skin-on cuts). Ten grams of sample was homogenized with 40 ml of distilled water. pH measurements
Colorimetric analysis. Skin color was determined using a portable MiniScan XE colorimeter (HunterLab, Reston, VA) with D65 light source, 10° observation angle, and 30-mm cell opening, using the CIELab system L*, a*, b* scale. The device was calibrated prior to sample reading with one white and another black standard. Three measures were taken on the breast skin and another three measures on the leg skin of each treatment.

Sensory analysis. For sensory analysis, a consumer test was conducted to determine the differences, if any, between chicken carcasses treated with chlorine or ozone as perceived by consumers. Students, faculty, and staff from Faculty of Animal Science and Food Engineering/Pirassununga who regularly buy and prepare fresh poultry were recruited to assess whether at the time of purchasing or handling they would differentiate and purchase the products treated with chlorine or ozone. Sixty consumers participated in the test, 72% women and 28% men between 19 and 48 years old. At each storage interval (days 1, 5, and 12), the consumers evaluated the color, odor, and overall appearance of carcasses by using a 9-point hedonic scale (1 = disliked extremely and 9 = liked extremely). Samples were stored in isothermal boxes (two carcasses from each treatment in each box) with ice and a cover, which was opened only during the evaluation of each treatment by each consumer. The isothermal boxes were coded with three-digit random numbers and presented in a monadic way to consumers according to a randomized complete block design.

Statistical analysis. Microbiological, physicochemical, colorimetric, and sensory data were analyzed by analysis of variance (ANOVA) (\( P \leq 0.05 \)), considering the effects of treatment and storage time and the treatment \( \times \) storage times interaction in the statistical model. In order to evaluate the differences in acceptance score between treatments, paired comparisons of means were carried out using the Tukey test (\( P \leq 0.05 \)). The statistical data analysis was carried out using SAS and Statistica software (Statsoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Regarding the microbiological stability of chicken carcasses treated with chlorine and ozone during refrigerated storage, Salmonella was not detected, and coagulase-positive staphylococci counts were below \( 10^2 \) CFU/ml of rinse fluid at all intervals evaluated (days 1, 5, 8, 12, and 15). With respect to counts of aerobic psychrotrophic bacteria, \( E. coli \), and total coliforms, the ANOVA results showed no significant differences between treatments (\( P > 0.05 \)) nor any effect of the treatment \( \times \) time interaction (\( P > 0.05 \)). However, the refrigerated storage period significantly (\( P \leq 0.05 \)) influenced the counts of the three types of microorganisms. In the case of psychrotrophic microorganisms, chlorine and ozone treatments showed the same trend of increasing counts over time (Fig. 1). After 12 days of storage at 2°C following both treatments, the carcasses had average counts of around 8 log CFU/ml of rinse fluid, and on day 15 of storage, they already had average counts of around 9 log CFU/ml of rinse fluid, with pronounced changes in odor as a result of microbial deterioration. These results are consistent with the results reported by Leitão (10) and Russell (15) that the onset of deterioration can be detected when the microbial count reaches \( 10^6 \) CFU/cm\(^2\), that changes with respect to odor can be in evidence from values of \( 10^7 \) CFU/cm\(^2\) and changes in flavor from \( 10^8 \) CFU/cm\(^2\), and that surface slime can be observed with a microbial count of \( 10^9 \) CFU/cm\(^2\).

Regarding \( E. coli \), Figure 2 shows that the counts on carcasses treated with either chlorine or ozone decreased linearly over the 15 days of storage, reaching counts of around 0 on day 15. Stivarius et al. (19) also observed reductions in the \( E. coli \) counts during 7 days of storage at 4°C on beef trimmings treated with either 1% ozonated water or 200 ppm of chlorine dioxide. In addition, in the case of total coliform counts, a similar behavior following both treatments was observed over time. The results in Figure 3 show that the average counts of total coliform bacteria decreased linearly over the 15 days of storage.

The total counts of psychrotrophic bacteria showed large increases over the refrigerated storage period (from values of around \( 10^3 \) to above \( 10^9 \) CFU/ml of rinse fluid in 15 days), unlike the counts of coliform bacteria, which remained below \( 10^5 \) CFU/ml of rinse fluid throughout the
storage period. A possible explanation for this behavior is that since coliform bacteria grow better at room temperature, the low storage temperatures associated with competition by psychrotrophic bacteria led to this reduction.

The ANOVA results for pH values showed no significant effect with respect to treatment ($P > 0.05$) or storage time ($P > 0.05$) or the treatment $\times$ time interaction ($P > 0.05$). This means that in both treatments, the average pH of carcasses remained stable throughout the storage time, showing an overall average of 6.1 during the storage period. Similar results were found by Manousaridis et al. (11), who assessed the effect of ozone on the stability of shucked mussels and found that pH was not useful as a quality index, since it remained constant throughout the storage period.

With regard to lipid oxidation (TBARS index), ANOVA showed that carcasses treated with chlorine or ozone were not significantly different ($P > 0.05$), and no effect of a treatment $\times$ time interaction was observed ($P > 0.05$). However, there was a significant influence from the storage time ($P \leq 0.05$). Figure 4 shows that both treatments presented a slight increase in the TBARS index during storage at $2\pm1^\circ$C, with mean values near 0 on the first day of storage and reaching an average value of 0.68 mg of malonaldehyde per kg of sample in 15 days. Similarly, upon storage of cooked chicken patties, Erickson (5) found a significant increase in the TBARS index after 3 days of refrigerated storage at $2^\circ$C.

Due to the strong oxidizing power of chlorine and ozone and the lipid profile rich in polyunsaturated fatty acids that is typical of chicken, problems related to high levels of lipid oxidation during storage could be expected. However, considering that thiobarbituric acid values of up to 1.59 mg of malonaldehyde per kg of sample are too low to be perceived by sensory analysis or harmful to health (20), it could be concluded that samples from both treatments remained appropriate with respect to lipid oxidation, even at 15 days of refrigerated storage.

The ANOVA results for the $L^*$ and $b^*$ parameters of objective skin color showed no significant difference between treatments ($P > 0.05$) or storage times ($P > 0.05$) and there was no effect of a treatment $\times$ time interaction ($P > 0.05$) on the skin color of chickens. The $L^*$ values showed averages of 68.0 and 68.5 for chlorine and ozone treatments, respectively. The $b^*$ values had means of 4.7 and 6.1 for chlorine and ozone, respectively. The ANOVA results for the $a^*$ values showed that carcasses subjected to either of the two treatments did not differ ($P \leq 0.05$) and that there was no effect of a treatment $\times$ time interaction ($P > 0.05$); however, there was significant influence from the storage time ($P \leq 0.05$). In both treatments, a slight decrease of $a^*$ values over time was observed, showing averages around 0 on the first day of storage until they reached an average of around $-2.0$ in 15 days. However, this variation does not represent practical effects since it was not perceived by consumers in the sensory evaluation.

Regarding sensory analysis, samples from both treatments did not differ ($P > 0.05$) in the acceptance score of odor and overall appearance (Table 1), maintaining averages around 6.0 (liked slightly) throughout the storage period. However, in the color attribute evaluation (Table 1), ozone treatment presented an acceptance score (6.0 = liked slightly) significantly higher ($P \leq .05$) than that of chlorine treatment (5.0 = neither liked nor disliked). The storage time influenced only the odor acceptance ($P \leq 0.05$), with both treatments presenting significant decreases in the acceptance score of this attribute throughout the evaluation period (Table 1).

Table 1 presents the means and standard deviations of the attributes evaluated for each storage time and disinfection method. For both methods, it could be observed that for up to 12 days of storage, color and appearance maintained their mean acceptance scores; however, odor presented a slight decrease. From 12 days of storage, the odor acceptance scores were close to indifference (5.0 = neither liked nor disliked). The data in Table 1 show that odor was the attribute with the greatest loss of acceptance in comparison with color and overall appearance during the storage period. Similarly, sensory evaluation of odor characteristics of ground beef treated with either 1% ozonated...
TABLE 1. Means and standard deviations for color, odor, and overall appearance scores for each disinfection method and storage daya

<table>
<thead>
<tr>
<th>Treatment or dayb</th>
<th>Color Mean</th>
<th>Color SD</th>
<th>Odor Mean</th>
<th>Odor SD</th>
<th>Overall appearance Mean</th>
<th>Overall appearance SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone</td>
<td>5.7 ± 1.70</td>
<td>6.0 ± 1.57</td>
<td>5.9 ± 1.51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>5.3 ± 1.74</td>
<td>5.8 ± 1.58</td>
<td>5.6 ± 1.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>5.4 ± 1.87</td>
<td>6.1 ± 1.63</td>
<td>5.9 ± 1.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>5.5 ± 1.84</td>
<td>6.3 ± 1.52</td>
<td>5.9 ± 1.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 12</td>
<td>5.6 ± 1.46</td>
<td>5.3 ± 1.43</td>
<td>5.6 ± 1.37</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values in the same column with same letter are not significantly different (Tukey test at 5% significance; n = 55 consumers).
b Day of storage at 2 ± 1°C.

water or 200 ppm of chlorine dioxide for 7 min or 15 min showed no effect among different treatments, but an increase in off-odor was found after 7 days of refrigerated storage (19).

When evaluating the effects on sensory characteristics achieved by dipping chicken legs (15 min at 18 ± 1°C) into solutions (wt/vol) of 12% trisodium phosphate, 1,200 ppm of acidified sodium chloride, 2% citric acid, 220 ppm of peroxyacids (Inspexx 100, Ecolab, St. Paul, MN), and water, del Rio et al. (4) also found similar average scores (overall acceptability in a 9-point hedonic scale) for all treatments on day 0 and day 1 of storage at 3°C. However, the overall acceptability scores for all treatments decreased at different levels during refrigerated storage, from scores of around 8 at the beginning to scores of around 6 (trisodium phosphate, acidified sodium chloride, and citric acid) or less after 5 days at 3°C.

In conclusion, under the conditions evaluated in this study, carcasses treated with ozone showed microbiological, physicochemical, and most sensory results similar to the results for carcasses treated with chlorine during refrigerated storage (2 ± 1°C). The results obtained in this study, together with previous knowledge of the possible generation of unwanted byproducts (chloramines) with the use of chlorine, permit recommending the use of ozone as a possible substitute for chlorine for industrial use in the disinfection of chicken carcasses during water immersion chilling.

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