Evaluation of ozonated water as a microbiological risk mitigation option in pork production

Quessy S.¹
Larivière-Gauthier G.¹*, Fravalo P.², Fournaise S.², Letellier A.¹

¹Veterinary Medicine Faculty, Research Chair for Meat safety, Montreal University, St-Hyacinthe, Canada. ²Olymel Sec, Boucherville, Canada

Chaire de Recherche en Soutabilité des Viandes, Faculté de Médecine Vétérinaire, Université de Montréal, 3200 Sicotte, C.P. 5000, J2S 7C6, St-Hyacinthe, Québec, Canada

email: guillaume.lariviere-gauthier@umontreal.ca

Abstract
Ozone is an oxidative molecule with a bactericidal effect. This molecule can be solubilized in water and was proposed as an alternative disinfecting solution to be used in food production plants. Ozone molecule possesses many industrially relevant characteristics, such as the absence of residues following its application, usability at meat industries’ room temperature, and applicability during production activities. The objective of this study was to evaluate the benefits of an ozonated water rinse on the wrapping of meat logs at the entrance of a slicing plant. From a single batch, the surface of ten units (meat logs : ML) of cylindrical shape of approximately 7000 cm² were entirely swabbed before treatment and compared with 3 groups of 10 units that were passed through a curtain of either chlorinated water (20 ppm), ozonated water (3.5 ppm) or tap water only. As part of the bacteriological analysis, total aerobic counts were measured, Salmonella and Listeria monocytogenes detection were individually conducted on each units, and enumeration of E.coli and coliforms were completed. The results obtained from 4 different batches showed a very low aerobic contamination at the entrance of the plant before treatment (2.49 log cfu/ML). The chlorinated water and the ozonated water treatment reduced significantly the bacterial contamination (respective diminution of 0.83 log cfu/ML and 0.63 log cfu/ML), while reduction from the tap water treatment was not significant (0.21 log cfu/ML). All samples were free of the researched pathogens, and coliforms counts were below the technical threshold for numeration. These results show that an ozonated water treatment is an effective tool in reducing aerobic flora contamination before the meat slicing process. It also indicates that ozonated water could be an alternative to chlorinated water treatments as it represents an effective method to control product wrapping contamination prior to its entrance at the slicing plant.

Introduction
Ready-to-eat production and transformation plants need to take important bacterial control measures due to the nature of their products, which are to be consumed without further treatments by consumers. Plant operators must prevent the introduction and proliferation of Listeria monocytogenes in the plant. This bacteria can produce biofilms that are hard to eliminate and multiplies in the product at a low temperature (4°C) (1). It has already been documented that potentially pathogenic bacteria such as Listeria monocytogenes can enter the plants with the products and become established in the processing environment (2). These strains can then contaminate the equipment and the meat during the cutting or slicing operations (3). This reinforces the importance of efficient control measures at the entrance of the product in the plant.

Ozone is an oxidative molecule that has a bactericidal effect. This molecule can be solubilized in water, and its use as a disinfecting solution has been proposed in food production plants. This product possesses many industrially relevant characteristics such as the absence of residues following its application, usability at meat industries room temperature, and applicability during activities (3).

In this study, ozonated water was assessed as a new tool to control product contamination at the entrance of a cold cuts slicing plant.

Material and Methods
Samples : In an industrial cold-cuts slicing and wrapping plant, the entire exterior surface (7000 cm²) of the wrappings of ten randomly chosen units of cold-cut meat logs (ML) were individually sampled with a cotton swab for four different lots.
(units of same product and shipped in the same box) to evaluate the initial level of contamination of the products shipped at the plant.

**Treatment:**
Three groups of ten units from the same lot were passed through a curtain of chlorinated water (20 ppm), ozonated water (3,5 ppm) or tap water at a temperature of 4°C. Each unit passed through the curtain in 30 seconds on a conveyor belt and after removal of water surplus was sampled as explained previously.

**Bacteriological procedure:**
Right after sampling, each swab was placed in 4 ml of neutralizing buffer at the plant. Total aerobic counts were measured on petrifilm (3M). Salmonella and Listeria monocytogenes detection were individually conducted on each of the swabs using modified government of Canada methods (MFLP-75 and MFHPB-30) and enumeration of E.coli and coliforms on petrifilm (3M) completed the bacteriological analysis.

**Statistics:**
The effectiveness of the three treatments was determined by comparing the mean aerobic **B** **o** **ro** and coliform contamination (in log cfu / units) before and after the treatments using Student t test (SPSS software license U Montreal). Differences in proportion of Salmonella and Listeria monocytogenes positive samples depending on treatment were considered.

**Results**
Table no. 1 : Aerobic **B** **o** **ro** contamination before and after treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (log cfu/units)</th>
<th>Standard deviation</th>
<th>Difference Control/Treated</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.49</td>
<td>0.69</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>Ozone</td>
<td>1.86</td>
<td>0.34</td>
<td>0.63</td>
<td>30</td>
</tr>
<tr>
<td>H2O</td>
<td>2.28</td>
<td>0.46</td>
<td>0.21</td>
<td>20</td>
</tr>
<tr>
<td>Chlorine</td>
<td>1.65</td>
<td>0.13</td>
<td>0.83</td>
<td>30</td>
</tr>
</tbody>
</table>

Significant aerobic **B** **o** **ro** reduction for the ozonated and chlorinated water treatments (t-test p < 0.05)
No significant reduction for the tap water treatments (t-test p > 0.05)

**Pathogens detection:**
Salmonella: 0 / 120 ML
Listeria monocytogenes: 0 / 120 ML
Coliforms and E.coli: 120 ML under detection limit (40 cfu / ML)

**Discussion**
The samples have been collected during four different visits of a plant that receives cold-cut meat in the form a log wrapped in a plastic envelope for the slicing and packaging operations. The aerobic contamination of the wrapping of four different types of meat products has been evaluated. The results show that the initial aerobic contamination of the meat logs wrapping is very low even before treatment with a mean aerobic contamination of 2.49 log cfu / ML. The sampled units were also free of the two researched pathogens (Listeria monocytogenes, Salmonella spp.) and the coliform and E. coli contamination was under the detection limit (40 cfu / ML). These low values demonstrate a good control of the risks of contamination at the end of the transformation procedures and during transportation between the transformation plant and the slicing plant.

After the application of the chlorinated or the ozonated water treatments on the wrapping of the meat logs, the mean aerobic contamination was significantly reduced when compared with the control samples with a mean reduction of 0.83 and 0.63 log cfu / ML respectively. However, the reduction for the chlorinated water treatment could be underestimated here as many of the samples were under the detection limit and were estimated at 39 cfu / ML, which is a conservative value, for the mean calculation. For the tap water treatment the reduction was 0.21 log / units and was not significant. The lack of significant reduction for this treatment demonstrates that the observed reduction in the two other tested methods...
is not caused by a physical removal of the bacteria by the water during the application but by a real bactericidal effect of the active molecules present in the water (ozone and chlorine). This reduction of the bacterial presence on the wrappings confirms that ozonated water has a bactericidal effect and that the chlorinated water treatment, which is currently used in the plant, is very effective.

The effectiveness of these treatments to reduce the presence of the pathogens could not be evaluated as all the samples before and after treatment did not contain them. However events that could lead to the contamination of the product by these microorganisms during the transport or the preparation can’t be excluded. The inactivation of different species of Salmonella, Listeria monocytogenes and E. Coli by ozonated water has already been described in laboratory experiments with mean reduction of more than 0.5 log for each of these microorganism (4). These reduction are relevant in the present industrial context were the presence of microorganisms is low as we can assume that in the case of a contamination by pathogenic bacteria it would also be very low. Based on the results of these previous studies and the results obtained on the reduction of the presence of the aerobic flora, ozonated water treatment could be an effective way to mitigate the microbiological risk on the product wrapping. Hence, using total aerobic counts as indicators, we can assume that this treatment could be an effective way of preventing the introduction of potentially pathogen bacteria in the plant with the product, reducing the risks of contamination of the environment by Listeria and of the final product during the subsequent steps such as the peeling of the wrapping.

**Conclusion**

The results obtained in this study show that the meat logs entering this plant have a very low microbial charge on their wrapping and are free from Listeria and Salmonella. The study also confirms the effectiveness of the chlorinated water treatment, which is currently used at this plant, to reduce to a minimum the product aerobic flora wrapping contamination. Furthermore, we showed that ozonated water is effective and can be considered as an interesting alternative to the chlorinated water treatment to control bacterial introduction in the plant by the product.

**References**


