Microbiological and chemical analyses of ice collected from a commercial poultry processing establishment

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ABSTRACT A study was conducted to evaluate the microbiological and chemical characteristics of ice collected from a commercial poultry further processing facility. During each of 3 visits, the following ice samples were collected: 1) freshly prepared, unused ice; 2) product-contact ice from ice-packed poultry parts; 3) product-contact ice from ice-packed poultry that had been visibly inspected and condemned as not for reuse; and 4) product-contact ice from ice-packed poultry that had passed visible inspection and had been prepared for reuse by washing (rinse with potable water and drain).

The overall pattern for lowest to highest numbers of total aerobic microorganisms, coliforms, Escherichia coli, and Enterobacteriaceae was as follows: unused ice < washed ice < product-contact ice < condemned ice. Mean levels of total aerobic microorganisms; coliforms, and Enterobacteriaceae in the unused ice were 0.3, 0.41, and 0.4 log10 cfu/mL, respectively. No E. coli was detected in the unused or washed ice, and levels were 0.5 and 1.5 log10 cfu/mL in the product-contact and condemned ice samples, respectively. Mean levels of bacteria enumerated in condemned ice were 0.8, 1.0, and 0.6 log10 cfu/mL higher than the levels of bacteria found in product-contact ice for coliforms, E. coli, and Enterobacteriaceae, respectively. Washing and draining the product-contact ice decreased counts by 0.9, 0.7, 0.5, and 1.7 log10 cfu/mL for total aerobic microorganisms, coliforms, E. coli, and Enterobacteriaceae, respectively.

All of the ice samples had similar pH values (pH 6.1 to 6.4). Unused and washed ice were not significantly different for total solids, total suspended solids, total Kjeldahl nitrogen, and chemical oxygen demand. Condemned ice contained the highest concentration of total solids, total suspended solids, total Kjeldahl nitrogen, and chemical oxygen demand, with levels more than 3 times that found in product contact ice. Data from the present study demonstrate that visible contamination in ice corresponds with increased microbiological and chemical contamination. Product-contact ice may be washed and the washing procedure can reduce the bacterial, solids, nitrogen, and organic loads.

Key words: poultry processing ice, water reuse, ice microbiology, ice chemistry

INTRODUCTION

Ice may be used to cool food during commercial processing, product distribution, or retail sales (Pawsey and Howard, 2001). Ice reduces product temperature and retards the growth of microorganisms. However, previous research has demonstrated that ice may also be a vehicle for transmission of pathogenic bacteria and viruses to foods (Moore et al., 1953; Tjoa et al., 1977; Alvarez-Seoane, 1980; Tsuno et al., 1984; Talbot et al., 1987; Felix, 1989; Moyer et al., 1993; Schmidt and Rodrick, 1998; Pawsey and Howard, 2001; Falcão et al., 2002; Kim and Harrison, 2008). Nichols et al. (2000) evaluated 4,346 samples of ice from retail and catering establishments and reported recovering levels of coliforms (317 samples), Escherichia coli (35 samples), and enterococci (35 samples) that were greater than 10^2 cfu/100 mL in ice used to cool drinks. A similar study was conducted on commercially bagged ice prepared from chlorinated or well water and collected from 6 different retail establishments (Falcão et al., 2002, 2004). These researchers reported finding 50 E. coli strains belonging to 33 different serotypes isolated from 23 of the 60 ice samples analyzed. This ice was designated for human consumption or refrigerated display of seafood (Falcão et al., 2002, 2004). It has been suggested that newly prepared ice may become contaminated from poor quality water or a severe lack of hygiene during production (Moyer et al., 1993). Kim and Harrison (2008) examined the transfer of E. coli O157:H7 from ice to romaine lettuce or from...
Norwalk virus was the suspected cause of an outbreak at the manufacturing plant. In another outbreak, Pennsylvania that was traced back to flood water contamination involving macaroni salad and gelatin prepared with contaminated ice (Felix, 1989).

The US Food and Drug Administration addresses the use of ice as food and as a cooling medium in the Food Code stating that unpackaged foods may not be in direct contact with ice or water if these components can enter the product (US Food and Drug Administration, 2005). Raw poultry and raw fish are considered exceptions if received immersed in ice in shipping containers. Under these conditions, raw poultry and raw fish may remain submersed in ice in shipping containers "while in storage awaiting preparation, display, service or sale" (US Food and Drug Administration, 2005). Ice and water are the primary cooling media used during poultry processing. In the United States, poultry processing establishments have been estimated to use approximately 227 billion liters of water each year at a cost of more than $240 million (Carawan et al., 1999; Merka, 2001; Northcutt and Jones, 2004). Because of the limited availability of fresh water, increasing water-saver costs, and wastewater discharge restrictions, poultry processing establishments have implemented water conservation and reuse programs, including programs for reusing ice. In 1999, the USDA published a regulation addressing the reuse of processing solutions (USDA, 1999). According to the regulation, processing solutions (water, ice, brines, liquid smoke, or propylene glycol) recovered from chilled or ready-to-eat poultry products may be reused for the same purposes provided they are free from pathogenic bacteria and fecal coliforms and provided that no physical, chemical, and microbiological hazard(s) are present (USDA, 1999). For ice reuse, the USDA Sanitation Performance Compliance Guidelines (USDA; 1999) suggests that facilities develop procedures for collecting, draining, washing (rinse with potable water), and visibly inspecting ice for large debris (poultry meat and fat) before reuse. Because ice reuse is gaining in popularity in the poultry industry, a study was conducted to examine the microbiological and chemical characteristics of freshly prepared ice in comparison to ice recovered from ice-packed poultry and either condemned because of large debris or washed for reuse.

**Sample Collection**

Ice was collected from a commercial poultry processing establishment (further processor) on 3 separate days of production, 1 day a week for 3 consecutive weeks. All of the ice samples were collected from totes used by employees to transport and distribute product and ice. During each visit, the following samples were collected: 1) freshly prepared, unused ice; 2) product-contact ice from ice-packed poultry; 3) product-contact ice from ice-packed poultry that had been visibly inspected and condemned as not for reuse; and 4) product-contact ice from ice-packed poultry that had passed the visible inspection and had been prepared for reuse by washing and draining. Ice that had been in contact with product was sampled after the product had been removed. The employee that removed the product then inspected the ice for visible contamination (meat, fat, skin, bone, or red discoloration). After the visible inspection, this same employee then transported the totes of ice to another location where another individual washed the ice with a high-pressure hose for >1 min in the same tote. If visible contamination was noted at any time, the ice was discarded from further use.

During each of the 3 visits to the processing plant, 6 samples of each treatment were collected from different totes of ice. All of the samples were collected from a single processing plant. Three samples from each treatment were analyzed for numbers of bacteria, and 3 other samples from each treatment were analyzed for chemical characteristics.

**Microbiological Analyses**

Ice samples were collected in sterile specimen cups that were sealed in resealable zip storage bags and transported to the laboratory for analysis. Time between collection and analysis was approximately 2 h to allow time for the ice to melt before plating samples. Serial dilutions of the ice were prepared using 0.1% peptone. Total aerobic microorganisms were determined on ice samples by plating 0.1 mL of the serial dilutions on plate count agar (Becton Dickinson, Sparks, MD). Petri dishes containing plate count agar and samples were incubated at 35°C for 48 h. After incubation, visible colonies were counted and reported as total aerobic microorganisms. Coliform and E. coli counts were determined on the serial dilutions of the ice by plating 1 mL onto duplicate E. coli Petrifilm plates (3M Health Care, St. Paul, MN). Petrifilm plates were incubated at 35°C for 48 h and colonies indicative of coliforms and E. coli were counted. Enterobacteriaceae were enumerated using duplicate pour plates of violet red bile glucose agar (BD Diagnostic Systems, Sparks, MD) containing 1 mL from a serial dilution. All violet red bile glucose plates were overlaid with approximately 5 mL and then incubated at 35°C for 48 h. Plates with the typical presumptive Enterobacteriaceae colonies were counted and reported.

**Chemical Analyses**

Ice samples were evaluated for pH, total solids (TS), total suspended solids (TSS), total Kjeldahl nitrogen (TKN), and chemical oxygen demand (COD). The
pH was measured directly on melted ice using a pH meter (Fisher Scientific Accuorn Model 10 pH meter, Pittsburgh, PA). Total solids content was determined using 100 mL of water that was dried at 180°C using 1-h cycles until a constant, cool weight was obtained (APHA, 1998). Total suspended solids content was determined by filtering 200 mL of melted ice through glass fiber filters. The filters were then dried at 104°C for 1 h, allowed to cool overnight, and then weighed to yield a residue that was reported as milligrams per liter of TSS (APHA, 1998). Total Kjeldahl nitrogen was determined using the micro-Kjeldahl method (APHA, 1998). Total Kjeldahl nitrogen was reported as milligrams per liter. For the COD determinations, melted ice was acidified with concentrated sulfuric acid (1 mL added to 9 mL of sample) and COD was determined in milligrams per liter as recommended by APHA (1998).

**Statistical Analyses**

Statistical analyses of the number of bacteria were performed after logarithmic transformation (log_{10} cfu/mL). All data were analyzed using the GLM procedure of SAS (SAS, 1999). The main effects of the model were treatment (ice samples), replication, and interaction effects. Means were separated using the least squares means option and reported along with the SE (SAS, 1999).

**RESULTS AND DISCUSSION**

Table 1 shows the mean numbers of microorganisms recovered from unused, product-contact, product-contact and condemned, and product-contact and washed ice collected at a commercial further poultry processing establishment. Mean counts and prevalence of total aerobic microorganisms (0.3 log_{10} cfu/mL; 6 of 9 positive), coliforms (0.4 log_{10} cfu/mL; 3 of 9 positive), and *Enterobacteriaceae* (0.4 log_{10} cfu/mL; 2 of 9 positive) in the unused ice were significantly lower than counts and prevalence of microorganisms recovered from the 3 other ice sample treatments. No *E. coli* was detected in the unused or washed ice.

With the exception of total aerobic microorganisms, the highest levels of bacteria were recovered from condemned ice (ice that failed visible inspection). Levels of bacteria enumerated in condemned ice were 0.8, 1.0, and 0.6 log_{10} cfu/mL higher than the levels of bacteria found in product-contact ice for coliforms, *E. coli*, and *Enterobacteriaceae*, respectively. The presence of visible meat, fat, bone, skin, and other debris in the product-contact and condemned ice carry associated bacteria. When inspection-passed, product-contact ice was washed (washed) and drained, numbers of bacteria decreased by 0.9, 0.7, 0.5, and 1.7 log_{10} cfu/mL for total aerobic microorganisms, coliforms, *E. coli*, and *Enterobacteriaceae*, respectively. Although prevalence of coliforms in product-contact ice was reduced with washing (3 of 9 positive vs. 9 of 9 positive), the numbers of coliforms in the product-contact and washed ice were not significantly different (2.0 and 1.3 log_{10} cfu/mL, respectively).

Large amounts of reused ice could present a hazard if not handled correctly and if allowed to come into contact with product. Kim and Harrison (2008) found that inoculated (10^7 cfu/mL of *E. coli* 0157:H7) and frozen water could transfer 3.5 log_{10} cfu/cm^2 of *E. coli* 0157:H7 to previously uncontaminated lettuce. Moreover, contaminated lettuce (5.5 log_{10} cfu/mL of *E. coli* 0157:H7) could transfer 3.3 log_{10} cfu/mL of *E. coli* 0157:H7 to previously uncontaminated melted ice (Kim and Harrison, 2008). Melted ice that became contaminated by contacting lettuce was found to transfer approximately 1.8 log_{10} cfu/mL of that contamination to adjacent lettuce leaves that were previously uncontaminated (Kim and Harrison, 2008). Freezing and thawing ice has been found to injure bacteria, but some bacteria have been found to become viable after thawing (Dickens et al.,

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Unused ice</th>
<th>Product-contact ice</th>
<th>Condensed ice</th>
<th>Washed ice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total aerobic microorganisms</strong></td>
<td>0.3 ± 0.16 (6/9)</td>
<td>3.5 ± 0.09 (9/9)</td>
<td>3.8 ± 0.12 (9/9)</td>
<td>2.8 ± 0.10 (9/9)</td>
</tr>
<tr>
<td><strong>Coliforms</strong></td>
<td>0.4 ± 0.19 (3/9)</td>
<td>2.0 ± 0.07 (9/9)</td>
<td>2.8 ± 0.04 (9/9)</td>
<td>1.3 ± 0.11 (6/9)</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>ND (0/9)</td>
<td>0.5 ± 0.11 (9/9)</td>
<td>1.5 ± 0.09 (9/9)</td>
<td>ND (0/9)</td>
</tr>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>0.4 ± 0.22 (2/9)</td>
<td>3.0 ± 0.22 (9/9)</td>
<td>3.6 ± 0.08 (9/9)</td>
<td>1.3 ± 0.10 (9/9)</td>
</tr>
</tbody>
</table>

**Note:** Numbers of bacteria in a row without a common superscript are significantly different (P < 0.05).

**Means ± SE for log_{10} colony-forming units per milliliter of ice.

**Unused ice refers to freshly prepared ice that has never contacted product.

**Product-contact refers to ice that has been in contact with ice-packed poultry.

**Condensed refers to product-contact ice that did not pass visible inspection and was condemned as not for reuse.

**Washed refers to product-contact ice that did pass visible inspection and was rinsed with potable water to prepare it for reuse.

**ND = not detected.
Northcutt and Smith (2008) reported that recycled poultry chiller water contained 42 to 1,23 mg/L (Northcutt et al., 2005, 2008). In another study, found or egg processing water may range from 80 to 3,800 mg/L (Northcutt et al., 2008). Total Kjeldahl nitrogen values can be used to determine the grams of protein in a sample, whereas COD is related to the level of organic material. Previous reports have shown that TKN values of discharged poultry or egg processing water may range from 80 to 3,800 mg/L (Northcutt et al., 2005, 2008). In another study, Northcutt et al. (2008) reported that recycled poultry chiller water contained 42 to 123 mg/L of TKN and 428 to 1,23 mg/L of COD. The TKN and COD levels found in condemned ice are similar to those found in recycled poultry chiller water (Northcutt et al., 2008).

Table 2. Mean pH, total solids (TS), total suspended solids (TSS), total Kjeldahl nitrogen (TKN), and chemical oxygen demand (COD) for ice samples (unused, product-contact, condemned, and washed) collected from a commercial further poultry processing establishment.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Unused ice²</th>
<th>Product-contact ice³</th>
<th>Condemned ice⁴</th>
<th>Washed ice⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.36 ± 0.05</td>
<td>6.28 ± 0.05</td>
<td>6.13 ± 0.19</td>
<td>6.10 ± 0.14</td>
</tr>
<tr>
<td>TS (mg/L)</td>
<td>108.2 ± 25.0</td>
<td>184.2 ± 15.6</td>
<td>528.5 ± 71.0</td>
<td>924.4 ± 11.3</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>3.27 ± 0.15</td>
<td>20.4 ± 1.70</td>
<td>71.8 ± 12.0</td>
<td>6.13 ± 0.94</td>
</tr>
<tr>
<td>TKN (mg/L)</td>
<td>0.44 ± 0.17</td>
<td>15.8 ± 0.73</td>
<td>65.2 ± 0.9</td>
<td>2.94 ± 20.0</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>9.8 ± 1.2</td>
<td>137.3 ± 8.10</td>
<td>528.2 ± 76.4</td>
<td>26.5 ± 3.45</td>
</tr>
</tbody>
</table>

¹Mean values for analyses in a row without a common superscript are significantly different (P < 0.05). No difference was found for pH values.
²Means ± SE.
³Unused ice refers to freshly prepared ice that has not contacted product.
⁴Product-contact refers to ice that has been in contact with ice-packed poultry.
⁵Condemned refers to product-contact ice that did not pass visual inspection and was condemned as not for reuse.
⁶Washed refers to product-contact ice that did pass visible inspection and was rinsed with potable water to prepare it for reuse.

REFERENCES


Moreover, it is important to consider that unlike poultry meat, which is consumed after it is fully cooked, products like lettuce are typically eaten without additional preparation steps. Cross-contamination is undesirable in any food product, but may be more serious in foods consumed raw.

Melted ice samples were also chemically analyzed to determine the amount of solids and organic material. There was no significant difference in the pH values measured for the ice samples (Table 2). Unused ice and washed ice were not significantly different for TS, TSS, TKN, and COD. Condemned ice contained the highest concentration of TS, TSS, TKN, and COD, with levels more than 3 times that found in product-contact ice. The TKN measurements correspond to the amount of protein that is present in a sample, whereas COD is related to the level of organic material. Previous reports have shown that TKN values of discharged poultry or egg processing water may range from 80 to 3,800 mg/L (Northcutt et al., 2005, 2008). In another study, Northcutt et al. (2008) reported that recycled poultry chiller water contained 42 to 123 mg/L of TKN and 428 to 1,23 mg/L of COD. The TKN and COD levels found in condemned ice are similar to those found in recycled poultry chiller water (Northcutt et al., 2008). Total Kjeldahl nitrogen values can be used to determine the grams of protein in a sample (16% nitrogen in each protein molecule). The reported TKN values for product-contact and condemned ice were equivalent to approximately 10 mg of poultry protein/L of ice. Because this ice was condemned, these values represent lost product.

Data from the present study demonstrate that visible contamination in ice corresponds with increased microbiological and chemical contamination. Product-contact ice may be washed and the washing procedure can reduce the bacterial, solids, nitrogen, and organic loads. Unused ice and product-contact ice that has been washed may be chemically indistinguishable when comparing pH, TS, TSS, TKN, and COD.