Effect of selected modified atmosphere packaging on *Campylobacter* survival in raw poultry

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**ABSTRACT** Most current research on *Campylobacter* has focused on preharvest or processing plant cross-contamination. Little is known about the effect of storage environment on the survival of *Campylobacter* on raw poultry. We evaluated the effects of modified storage atmosphere and freezing on the survival of naturally occurring *Campylobacter* on raw poultry. Broiler carcasses (*n* = 560) were collected as they exited the chiller in 2 commercial processing plants and were sampled for the detection of *Campylobacter*, *Escherichia coli*, psychrophiles, and total aerobes at 0 and 14 d of refrigerated (2°C) storage. Gases evaluated were air, 100% O₂, 100% CO₂, and a standard poultry modified atmosphere packaging mixture (5% O₂ + 10% CO₂ + 85% N). Freezing was included as a control group. All carcasses were sampled by the whole-carcass rinse method. The rinse fluid was recovered and pooled from 5 individual rinses, and serial dilutions were made for examination of *Campylobacter* (42°C, 48 h), *E. coli* (37°C, 24 h), psychrophiles (plate count agar, 4°C, 7 d), and total aerobic bacterial populations (plate count agar, 37°C, 24 h). *Campylobacter* counts for all treatments were reduced during the 14-d storage period but the 100% O₂ treatment caused a significantly (*P* < 0.05) greater reduction than the other gas treatments. For the psychrophiles, storage in air resulted in the greatest growth after 14 d, with reduced psychophilic growth allowed by either O₂ or the modified atmosphere packaging mixture (not different from each other). Of the treatments evaluated, CO₂ allowed the least growth of psychrophiles. Proliferation of *E. coli* and aerobes was the greatest when packaged in air after 14 d, whereas CO₂ packaging resulted in the least growth. These data suggest that storage under O₂ may reduce *Campylobacter* recovery and slow psychophile and aerobe recovery following storage.

**Key words:** modified atmosphere, packaging, *Campylobacter*

INTRODUCTION

*Campylobacter jejuni* is considered to be one of the leading causes of human foodborne illness in the United States (USDA-FSIS, 1997; CDC, 2009). *Campylobacter jejuni* caused between 1 and 7 million cases of gastroenteritis in 1995 (Buzby and Roberts, 1995). Relatively little is known about the effect of the processing environment and packaging on the survival of *Campylobacter* on raw poultry. Large numbers of poultry carcasses exiting chill immersion systems are contaminated with *Campylobacter*. *Campylobacter* has been found at 87% prevalence on the surface of broiler breast fillets and at 20% prevalence in the deep tissue (Luber and Bartelt, 2007). However, little is known about the effect of the choice of packaging on the survival of *Campylobacter* and, consequently, its presence upon arrival of the package at the retail level. Cold storage, freezing, and exposure to air have all been reported to reduce *Campylobacter* survival. However, little information exists as to the relative effects and lethality of the ice pack, chill pack (tray pack), freezing, vacuum packaging, or modified atmosphere packaging (MAP) methods. Such information is critical in assessing the risk associated with these packaging methods. Therefore, the objective of the present study was to evaluate the effect of different packaging types and storage conditions on *Campylobacter* and spoilage organisms.
Of 0.6 g of H2O/square inch per 24 h at 38°C; 1 inch = 24 h at 0°C RH with moisture vapor transmission rate

Wolfertschwenden, Germany). AGW vacuum packaging unit (Sepp Haggemüller KG, casses were vacuum sealed to 100 kPa using a Multivac

tical analysis. The use strategy of rinsate pools for each
carcasses in the box because the entire box of carcasses
was combined and analyzed as an experimental
box was considered a single experimental unit in the statis-
was further characterized serologically
phase contrast (100×). The morphologically confirmed
on Campy Cefex plates, and incubated as described
al., 1997). Following enrichment, samples were streaked
Campylobacter colonies were confirmed as a member of the genus by microscopical examina-
tion of morphology and motility on a wet mount under phase contrast (100×). The morphologically confirmed
Campylobacter was further characterized serologically using a latex agglutination kit specific for Campy-

Other Bacteria. Duplicate serial dilutions (1–4) of pooled carcass rinsates (100 μL/plate) were spread on the appropriate selective or nonselective agar and incubated for the specific organisms as follows: total aerobes on plate count agar, incubated for 24 h at 37°C; Escherichia coli on m-Endo plate agar, incubated for 24 h at 37°C; Salmonella on brilliant green agar, incubated for 24 h at 37°C; and psychrotrophic bacteria on plate count agar, incubated for 10 d at 4°C. After the ap-

Microbiological Analyses

Campylobacter. A 10-mL sample of each pooled carcass rinse sample was transferred to 10 mL of a double-strength modified Bolton’s broth and incubated for 4 h at 37°C, followed by 20 h at 42°C in a microaerobic environment (5%, O2, 10% CO2, 85% N2; Musgrove et al., 1997). Following enrichment, samples were streaked for isolation on Campy Cefex plates and incubated for 24 to 48 h at 42°C in the microaerobic environment (Stern et al., 1992). An additional sample was taken from each pooled carcass rinse sample, serially diluted (1–4), enumerated (100 μL/plate) by plating directly on Campy Cefex plates, and incubated as described above. Suspect Campylobacter colonies were confirmed as a member of the genus by microscopical examina-

Materials and Methods

Samples

Broiler carcasses obtained from a commercial pro-
cessor were collected after chill immersion, placed in corrugated boxes with liners, and packaged in a modi-

Table 1. Bacteria (log$_{10}$ cfu) recovered from carcass rinses of pooled broiler carcasses stored at 2°C for 14 d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Campylobacter $^1$</th>
<th>Salmonella</th>
<th>Escherichia coli</th>
<th>Psychrophiles</th>
<th>Aerobes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0 d)</td>
<td>1.36 ± 1.18$^A$ (16/25)</td>
<td>0</td>
<td>2.36 ± 0.83$^B$</td>
<td>2.58 ± 0.24$^D$</td>
<td>4.19 ± 0.51$^{AB}$</td>
</tr>
<tr>
<td>100% CO$_2$</td>
<td>1.00 ± 1.07$^{AB}$ (7/25)</td>
<td>0</td>
<td>2.03 ± 0.64$^B$</td>
<td>4.21 ± 0.69$^C$</td>
<td>3.74 ± 0.62$^B$</td>
</tr>
<tr>
<td>5% O$_2$ + 10% CO$_2$ + 85% N$_2$</td>
<td>0.55 ± 0.83$^{AB}$ (9/25)</td>
<td>0</td>
<td>2.71 ± 0.72$^{AB}$</td>
<td>6.37 ± 0.22$^B$</td>
<td>4.39 ± 1.34$^{AB}$</td>
</tr>
<tr>
<td>100% O$_2$</td>
<td>0.15 ± 0.41$^B$ (3/25)</td>
<td>0</td>
<td>2.47 ± 0.90$^B$</td>
<td>6.35 ± 0.42$^B$</td>
<td>4.26 ± 1.47$^{AB}$</td>
</tr>
<tr>
<td>Air stored</td>
<td>0.93 ± 0.17$^{AB}$ (12/25)</td>
<td>0</td>
<td>3.27 ± 0.72$^A$</td>
<td>7.05 ± 0.48$^A$</td>
<td>5.02 ± 1.56$^A$</td>
</tr>
</tbody>
</table>

$^A$–$^D$Means among bacteria with no common superscript differ significantly ($P < 0.05$).
$^1$Values in parentheses are positive samples/total samples evaluated.


appropriate incubation period, all bacteria were inspected and the individual bacteria were enumerated.

**Statistical Analysis**

Bacterial counts from direct serial dilution plating were transformed to $\log_{10}$ colony-forming units per milliliter of rinse recovered. Data were measured using factorial ANOVA and main effects were deemed significant at $P \leq 0.05$. Significant differences were further separated using Duncan's multiple range tests using DUNCAN of SAS (Luginbuke, 1987). Differences among groups in the incidence of *Campylobacter* contamination were analyzed by chi-squared analysis. All analyses were conducted using SAS.

**RESULTS**

**Experiment 1**

*Campylobacter* counts for all treatments were reduced during the 14-d storage period, but only 100% O$_2$ (0.15 log$_{10}$) treatment caused a significantly ($P \leq 0.05$) greater reduction than the other gas treatments when compared with the controls (1.36 log$_{10}$; Table 1). *Salmonella* was not detected in the study. For psychrophiles and *E. coli*, storage in ambient air resulted in the greatest growth after 14 d, with reduced psychrophilic growth allowed by either O$_2$ or MAP. Of the treatments evaluated, CO$_2$ allowed the least growth of psychrophiles. For aerobes, storage in ambient air (5.02 log$_{10}$) resulted in the greatest growth and CO$_2$ (3.4 log$_{10}$) produced the least growth, although no significant changes were found when compared with the controls (4.19 log$_{10}$).

**Experiment 2**

Different packaging methods were compared with MAP at 2 and 14 d of storage (Table 2). Using the d 0 control (1.50 log$_{10}$), *Campylobacter* was significantly higher only in the carcasses stored for 2 d in a whole-carcass ice pack (2.63 log$_{10}$). Frozen carcasses (0.63 log$_{10}$) stored for 14 d resulted in the lowest number of *Campylobacter* of all carcasses stored. Carcasses stored in the ice pack (1.49 log$_{10}$) or frozen (0.63 log$_{10}$) for 14 d had significant reductions in *Campylobacter* when compared with the d 2 control (2.63 and 1.74 log$_{10}$, respectively).

Broiler carcasses stored for 2 d in an ice pack (5.25 log$_{10}$) or O$_2$ MAP (5.07 log$_{10}$) had significantly higher numbers of aerobes when compared with the d 0 control (4.23 log$_{10}$). Furthermore, frozen carcasses (3.29 log$_{10}$) stored for 14 d had a significant reduction in aerobes when compared with the d 0 control. Similarly, carcasses stored for 14 d in ice pack, O$_2$ MAP, mixed-gas MAP, and frozen all had significant reductions of aerobes when compared with the d 2 controls.
Initial levels of psychrophiles recovered from broiler carcasses were 2.95 log_{10} cfu on d 0. Broiler carcasses stored for 14 d in any of the reported packaging methods resulted in significant increases in psychrophiles when compared with the d 0 control or each d 2 control with the exception of the frozen carcasses.

**DISCUSSION**

Food spoilage can be caused by a wide variety of factors such as bacterial growth, metabolites, and pH changes (Huis in’t Veld, 1996). The use of vacuum packaging systems has dramatically increased the shelf life of food products. The increased shelf life has also led the investigations of the use of MAP systems. Within these systems, a flush of different gases may be used to maintain flavor and quality of food for a long period of time. Many of these MAP or vacuum samples were flushed with mixed gases or a gas mixture (5% O_2 + 20% CO_2 + 75% N_2) used to isolate *Campylobacter*. Therefore, the present study evaluated different MAP mixtures for the recoverability of *Campylobacter*, other pathogens (*Salmonella* and *E. coli*), and spoilage bacteria (psychrophiles and total aerobes).

The temperatures ranged from immediately frozen to 3°C and storage lasted up to 14 d. More than half (56.6%) of the carcasses or parts were contaminated with *Campylobacter*, which was slightly higher than the observed values reported by Musgrove et al. (2003), with 48% of weep and carcass rinse samples positive for *Campylobacter*. Furthermore, numeric reductions of *Campylobacter* were seen in every treatment in this experiment except for whole-carcass chill pack after 14 d of storage compared with the d 2 controls. Only whole-carcass ice packs and frozen carcasses had significant reductions in *Campylobacter* when compared with their corresponding d 2 controls. The overall incidence of *Campylobacter* decreased in all MAP treatment with the exception of air-stored samples, which is in agreement with Blankenship and Craven (1982), who found that *Campylobacter jejuni* decreased 1 to 2 log cfu at 4°C in air and CO_2 MAP after 31 d of storage. Pure O_2 had no effect on any of the bacteria evaluated. Although not evaluated in this study, vacuum packaging has been shown to allow the growth of pseudomonads, lactobacilli, and *Enterobacteriaceae* (Jiménez et al., 1997). Freeze-chilling products after thaw may actually increase pathogen growth during chilling phases (Patsias et al., 2008). Furthermore, additional freezing does not eliminate all the bacteria and in some instances may increase numbers (Forsythe and Hayes, 1998).

The results of the present study using MAP to control *Campylobacter* could be enhanced further if combined with other intervention strategies such as acetic acid treatment (Jiménez et al., 1999). Jiménez et al. (1999) found that chicken breast incubated in 70% CO_2, 30% N, and acidic acid decontamination had decreased total viable counts, pseudomonads, lactobacilli, and *Enterobacteriaceae*. Alternatively, because Safe2O (Mionix Corporation, Port Aransas, TX) has previously been shown to decrease total aerobes, pseudomonads, *Listeria*, *Staphylococcus*, and psychrophils (Dickens et al., 2004), this product might provide further activity in combination with MAP under O_2. del Río et al. (2007) reported that 12% trisodium phosphate was effective in controlling *Staphylococcus*, *Bacillus*, *Salmonella Enteritidis*, *E. coli*, and *Yersinia*; this is another approach that might show additive or synergistic activity in combination with MAP O_2 packaging. Similarly, 1,200 mg/kg of acidified sodium chlorite reduced *Staphylococcus*, *Bacillus*, *E. coli*, and *Yersinia* after 5 d of storage at 3°C; % citric acid reduced *Staphylococcus*, *Salmonella Enteritidis*, and *E. coli*; and 220 ppm of peroxyacids reduced *Listeria* and *Salmonella* after 5 d of storage, providing further possibilities for investigation of possible combination effects (del Río et al., 2007).

The present data suggest that O_2 may be a preferred environment for MAP of fresh poultry as concerns regarding *Campylobacter* contamination of carcasses grow. This study showed that MAP under O_2 actually reduced *Campylobacter* recovery and slowed psychophile and aerobe recovery following storage. Further studies regarding the effects of MAP under O_2 and the effects on sensory evaluation and shelf life are needed before widespread adoption of this practice.

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**REFERENCES**


