Package Systems and Storage Times Serve as Postlethality Controls for *Listeria monocytogenes* on Whole-Muscle Beef Jerky and Pork and Beef Smoked Sausage Sticks†

APRIL SHAYNE S. LOBATON-SULABO,1 TYLER J. AXMAN,1 KELLY J. K. GETTY,1,2* ELIZABETH A. E. BOYLE,1,2 NIGEL M. HARPER,1 RAMALDEEP K. UPPAL,1,3 BRUCE BARRY,3 AND JAMES J. HIGGINS4

1Food Science Institute, 2Department of Animal Sciences and Industry and 4Department of Statistics, Kansas State University, Manhattan, Kansas 66506; and 3Oberto Sausage Company, 7060 South 238th Street, Kent, Washington 98032, USA

MS 10-172: Received 19 April 2010/Accepted 9 October 2010

**ABSTRACT**

To validate how packaging and storage reduces *Listeria monocytogenes* on whole-muscle beef jerky and smoked pork and beef sausage sticks, four packaging systems (heat sealed [HS] without vacuum, heat sealed with oxygen scavenger, nitrogen flushed with oxygen scavenger [NFOS], and vacuum) and four ambient temperature storage times were evaluated. Commercially available whole-muscle beef jerky and smoked pork and beef sausage sticks were inoculated with a five-strain *L. monocytogenes* cocktail, packaged, and then stored at 25.5°C until enumerated for *L. monocytogenes* at 0, 24, 48, and 72 h and 30 days after packaging. The interaction of packaging and storage time affected *L. monocytogenes* reduction on jerky, but not on sausage sticks. A >2-log CFU/cm² reduction was achieved on sausage sticks after 24 h of storage, regardless of package type, while jerky had <2-log reductions for all packaging types. At 48 h, log reductions were similar (P > 0.05) for all types of jerky packaging, ranging from 1.26 to 1.72 log CFU/cm²; however, at 72 h, mean *L. monocytogenes* reductions were >2 log CFU/cm², except for NFOS (1.22-log CFU/cm² reduction). Processors could package beef jerky in HS packages with oxygen scavenger or vacuum in conjunction with a 24-h holding time as an antimicrobial process to ensure a >1-log CFU/cm² *L. monocytogenes* reduction or use a 48-h holding time for HS- or NFOS-packaged beef jerky. A >3-log CFU/cm² mean reduction was observed for all beef jerky and sausage stick packaging systems after 30 days of 25.5°C storage.

*L. monocytogenes* is a concern for many ready-to-eat (RTE) meat and poultry products because of the ability of *L. monocytogenes* to survive in processing environments and the potential for postprocessing contamination. The U.S. Department of Agriculture’s Food Safety and Inspection Service (FSIS) has applied a zero-tolerance policy to *L. monocytogenes* in RTE meat and poultry products because of the severity of listeriosis and the ability of *L. monocytogenes* to grow at refrigeration temperatures (4). In 2002, the Centers for Disease Control and Prevention reported that there are about 2,500 cases annually from listeriosis, including 500 deaths and nearly $200 million in monetary losses in the United States (3). Between 1990 and 1999, Levine et al. (12) reported *L. monocytogenes* prevalence to be 0.52% in jerky and 3.56% in small-diameter cooked sausage. Beef jerky and snack sticks have been recalled due to *L. monocytogenes* contamination detected through routine sampling conducted by processors or regulators (1, 14–18). *L. monocytogenes* contamination of cured and uncured RTE meat is a major safety concern (17) because RTE meats have a long shelf life and are consumed without further heating, and because of the emergence of multiple resistance in *Listeria* spp. because acquisition of a replicon from staphylococci (10). In efforts to prevent *L. monocytogenes* contamination in RTE products, the FSIS mandated that establishments employ one of three alternatives to control *L. monocytogenes* on postlethality exposed product (17).

*L. monocytogenes* contamination of RTE meat or poultry products occurs postprocessing because *L. monocytogenes* is easily killed by heating or cooking (19). Thus, antilisterial intervention should not only exhibit inhibitory activity on *L. monocytogenes*, but should also exhibit bactericidal activity against *L. monocytogenes* postlethality and throughout the product shelf life. The FSIS defines a “postlethality treatment” as an antimicrobial process that reduces *L. monocytogenes* by >1.0 log (17). Hurdles frequently applied in RTE meat products are low pH; low water activity (aw); preservatives such as nitrite, sorbate, and sulfite; in-package thermal processing; refrigerated storage; and vacuum packaging (19). An “antimicrobial agent” is defined as a process that can inhibit *L. monocytogenes* from growing by >2.0 log (17).

Research has shown that vacuum packaging of jerky can generate a 1-log *L. monocytogenes* reduction after 1 or more weeks of storage at ambient temperature, and that *L. monocytogenes* is not able to survive on a jerky product during its shelf life (6, 7, 17). There is a lack of information to evaluate effectiveness of a postlethality treatment allowing products to be held for a shorter time. Combining...
modified atmosphere packaging with short-term storage prior to distribution could be an effective antimicrobial process that would also act as a postlethality treatment. In order for a lethality treatment to be used, the FSIS requires that it must be scientifically validated to provide evidence that will show the effectiveness of the antimicrobial process. Therefore, the objective of our study was to evaluate the effect of packaging methods and storage time on reducing *L. monocytogenes* in commercially prepared, whole-muscle beef jerky and smoked pork and beef sausage sticks.

**MATERIALS AND METHODS**

**Experimental design.** This study consisted of four packaging treatments, heat sealed without vacuum (HS), heat sealed with oxygen scavenger (HSOS), nitrogen flushed with oxygen scavenger (NFOS), and vacuum (VAC), and four storage times (24, 48, 72 and 30 days) at 25.5 °C. For each packaging treatment and storage time, two samples of inoculated jerky were evaluated for *L. monocytogenes* populations. A replication consisted of a single lot of commercially processed beef jerky and smoked pork and beef sausage sticks. Three replications were completed for each product.

**Product description.** Three different production lots of whole-muscle beef jerky and smoked pork and beef sausage sticks were commercially obtained from a single manufacturer. Each lot was assigned to one of the three replications. Commercial, whole-muscle beef jerk sticks were packaged in a nitrogen-flushed, O₂-impermeable, seizable, 5-mil-thick, low-linear-density polyethylene clear pouch (19 by 36 cm; TPG Co., Ltd., Gyeonggi-do, South Korea) that contained an O₂ absorber (40 by 38 ± 2 mm, absorption capability of 36 ml in a 24-h water displacement test, deoxygenation time at room temperature of 1 to 2 days, below 0.01% O₂; O₂-Zero, BJ100, TPG Co., Ltd., Ltd.). Beef jerk ingredients included beef, corn syrup, dextrose, hydrolyzed corn syrup, soy protein, salt, natural smoke flavor, flavorings, water, vinegar, sugar, molasses, sodium erythorbate, caramel color, sodium nitrite, and citric acid. According to the manufacturer’s data, the jerky contained 2.1% fat, 28.1% moisture, 39.6% protein, a 0.71 moisture-to-protein ratio (MPR), 4.8% salt, and a pH of 5.4. Total plate count, coliforms, and *Escherichia coli* populations were <1 log CFU/cm², based on the manufacturer’s data. Received sausage sticks were packaged in nitrogen-flushed, O₂-impermeable packaging containing an oxygen absorber, similar to whole-muscle beef jerky. Oxygen concentration inside each package was <0.1%. Ingredients in the smoked sausage sticks were pork heart, beef, pork fat, salt, dextrose, wheat flour, natural flavorings, paprika, potassium nitrate, lactic acid starter culture, sodium nitrite, sodium nitrate, and butylated hydroxyanisole, and sticks had been treated with a solution of potassium sorbate to control *Listeria monocyctogenes*. This resulted in an initial inoculation level of 4.84 log CFU/cm².

**Inoculation, packaging, and enumeration.** Beef jerky was aseptically removed from pouches, cut into square pieces 4 by 4 cm, dipped into inoculum, and held for 1 min. Pieces were removed from the inoculum and allowed to air dry at 23 °C for approximately 1 to 2 h, to an a₀ level of approximately 0.80 (because the dipping procedure increased a₀ slightly). This resulted in an initial inoculation level of 4.33 log CFU/cm². Four smoked sausage sticks (length of 14.0 cm, diameter of 1.0 cm) were aseptically transferred into a nonfiltered stomacher bag (Fisherbrand, Fisher Scientific, Pittsburgh, PA) containing 40 ml of *L. monocytogenes* cocktail. Sausage sticks were gently hand massaged for 2 min and then held for another 2 min. Next, sausage sticks were removed from the bag containing the cocktail and hung with sterile binder clips to dry for 1 h at 25.5 °C, resulting in an initial inoculation level of 4.33 log CFU/cm².

*L. monocytogenes* was immediately enumerated on two samples of each product (two jerky pieces or two sausage sticks) to determine initial attachment. For both products, two samples were evaluated for each packaging treatment and time combination for a total of 34 samples, including time 0 (initial *L. monocytogenes* attachment, per replicate). Jerky and sausage sticks were then assigned to one of the four packaging treatments: HS, HSOS, NFOS, or VAC. All samples were placed in a 5-mil-thick, clear, low-linear-density polyethylene pouch (O₂ transmission rate: 17 ml/100 in² [~645 cm²] in 24 h, moisture vapor transmission rate of 0.084 g/100 in² [~645 cm²] in 24 h) sized 19 by 15 cm. Samples assigned to NFOS were flushed for 10 s with food-grade 100% N₂ by using a vacuum packager (Multivac C100, Multivac Sepp Haggenmüller GmbH and Co.KG, Wolfertschwenden, Germany). The VAC samples were packaged by using the vacuum packager with a vacuum level of 600 mm Hg (~80 kPa). The HS and HSOS samples were packaged with an impulse sealer (model H-1029, ULINE, Chicago, IL) at ambient temperature. An O₂ absorber was added to each sample packaged in NFOS or HSOS prior to heat sealing or N₂ flushing. Each of the four packaging treatments was subsequently held at 25.5 °C for 24, 48, and 72 h and for 30 days. For all times including time 0 (initial *L. monocytogenes* level), two packages of each product were used for enumeration. After each holding period, a sample was placed into a filtered stomacher bag (Fisher Scientific). For jerky, 34 ml of 0.1% peptone water (Difco, BD) was added to the bag, while 50 ml was used for sausage sticks. The sample and diluent were then pummeled in a stomacher (Stomacher Mix 1 Lab Blender; Microbiology International, Frederick, MD) for 1 min. Serial dilutions were prepared. *L. monocytogenes* populations were enumerated by spread plating 0.1 ml of diluent onto modified oxford agar plates (Difco, BD) in duplicate and incubating plates for 48 ± 2 h at 35 ± 2 °C.

**TABLE 1. Strains of microorganisms used for Listeria monocytogenes inoculum**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 7644</td>
<td>Human</td>
</tr>
<tr>
<td>ATCC 19115</td>
<td>Human</td>
</tr>
<tr>
<td>ATCC 19118</td>
<td>Chicken (England)</td>
</tr>
<tr>
<td>ATCC 19112</td>
<td>Human spinal fluid (Scotland)</td>
</tr>
<tr>
<td>SLR 2249, Cornell University</td>
<td>Human</td>
</tr>
</tbody>
</table>
TABLE 2. Chemical analyses of beef jerky packaged in HSOS, and package O₂ concentration in three packaging environments and stored at 25.5°C

<table>
<thead>
<tr>
<th>Time</th>
<th>a₀ w°</th>
<th>MPR°</th>
<th>HSOS°</th>
<th>NFOS°</th>
<th>HS°</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h‡</td>
<td>0.78 A b</td>
<td>0.72 A</td>
<td>19.0 B</td>
<td>0.2 A</td>
<td>18.9 A</td>
</tr>
<tr>
<td>24 h</td>
<td>0.79 A</td>
<td>0.69 A</td>
<td>0.1 A</td>
<td>0.2 A</td>
<td>18.9 A</td>
</tr>
<tr>
<td>48 h</td>
<td>0.79 A</td>
<td>0.74 A</td>
<td>0.1 A</td>
<td>0.1 A</td>
<td>19.0 A</td>
</tr>
<tr>
<td>72 h</td>
<td>0.78 A</td>
<td>0.68 A</td>
<td>0.1 A</td>
<td>0.1 A</td>
<td>18.8 A</td>
</tr>
<tr>
<td>30 days</td>
<td>0.78 A</td>
<td>0.70 A</td>
<td>0.1 A</td>
<td>0.2 A</td>
<td>18.9 A</td>
</tr>
</tbody>
</table>

° Water activity.
A Moisture-to-protein ratio.
C O₂ concentration of vacuum-packaged treatment was not determined.
D Heat sealed with oxygen absorber.
E Nitrogen flushed with oxygen absorber.
F Heat sealed.
G Initial a₀ prior to packaging.
H Within the same column, means with different letters are significantly different (P < 0.05).

a₀, pH, MPR, and O₂ determination. a₀ was measured to determine if changes in a₀ resulted because of the dipping procedure or the hand massaging procedure. Jerky pieces and sausage sticks dipped into sterile TSB were used to measure a₀. Two samples from the HSOS treatment were measured before dipping, immediately after drying, and after 24, 48, and 72 h and 30 days at 25.5°C.

a₀ was determined with an a₀ meter (AQUALAB CX2 series 3TE, Decagon, Pullman, WA), calibrated with a 0.760 NaCl verification standard (6.0 M in water) and distilled water at 25.5°C. A beef jerky piece was cut into a hexagonal shape (6 cm in diameter and 3.2 cm) and placed into a sample container; sausage sticks were minced and placed in a sample container. For each sample, duplicate readings were taken at 25.5°C.

Oxygen concentration of the HS, HSOS, and NFOS packages was measured at 0, 24, 48, and 72 h and 30 days at 25.5°C, with an O₂ analyzer (Checkpoint-O₂, PBI Dansensor, DK-400 Kongsted, Denmark). Oxygen concentration was measured prior to L. monocytogenes enumeration, by piercing the package with the O₂ detector needle, inserted at a 45° angle, 1.0 cm away from the seal.

For sausage sticks only, pH was determined throughout storage. A slurry was prepared by using 10 g of sample with 90 ml of distilled water in a double-chamber filter bag (Fisherbrand, Fisher Scientific), stomached for 1 min, and then filtered with Whatman no. 1 filter paper (The Lab Depot, Inc., Dawsonville, GA). The filtrate pH was measured with a calibrated pH meter (Acumet 925, Fisher Scientific) fitted with a flat-surface combination electrode (no. 476550, Corning Inc., Corning, NY).

Statistical analysis. The experiment was a completely randomized 4 x 4 factorial design, with beef jerky or sausage sticks as the experimental unit. The model included the main effects of packaging treatment and storage time, and the interaction of packaging treatment and storage time. Analysis of variance was performed with the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Least-squares means were calculated for each independent variable. Statistical significance was set at P < 0.05.

RESULTS AND DISCUSSION

The initial mean a₀ of whole-muscle beef jerky was 0.73 prior to inoculation. The chemical analysis and package O₂ concentration for noninoculated beef jerky dipped in sterile TSB media are shown in Table 2. The a₀ remained constant at 0.78 to 0.79, while the MPR ranged from 0.68 to 0.74. Across all storage times, HSOS and NFOS had an average O₂ concentration of less than 0.2%, and HS averaged 18.9% O₂.

An interaction occurred between packaging and storage time on the reduction of L. monocytogenes on whole-muscle beef jerky. After 24 h of 25.5°C storage, >1.0-log CFU/cm² reductions were observed for VAC and HSOS, whereas reductions were only 0.6 and 0.9 log CFU/cm² for NFOS and HS, respectively (Fig. 1). After 48 h, log reductions for L. monocytogenes populations were >1.2 log CFU/cm², regardless of packaging treatment (P > 0.05). For the 72-h holding time, log reductions for L. monocytogenes populations were >2.2 log CFU/cm² for all packaging treatments, except NFOS, which had a 1.2-log CFU/cm² reduction (P < 0.05). After 30 days, L. monocytogenes reductions were similar (P > 0.05) for all packing treatments, and >3.5-log CFU/cm² reductions were observed.

The jerky a₀ of <0.79 was sufficient to prevent growth of L. monocytogenes, which has a minimum a₀ for growth of 0.92 (7). Other studies investigating longer storage times have shown reduction of L. monocytogenes in RTE meat products (7, 8). L. monocytogenes populations decreased 2.4 log CFU after 1 week in 21°C storage on beef jerky vacuum packaged having an a₀ of 0.75, and after 4 weeks of storage, no surviving cells were recovered (7). L. monocytogenes showed greater reduction on vacuum-packaged beef jerky products (a₀ ranging from 0.47 to 0.87), declining by 0.6 and 2.3 log CFU after 21°C storage for 1 and 4 week(s), respectively (7). In our study, beef jerky packaged in HSOS...
TABLE 3. Chemical analyses of pork and beef smoked sausage sticks packaged in HSOS, and package O$_2$ concentration of smoked sausage sticks packaged in three packaging environments and stored at 25.5°C.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>$a_w$</th>
<th>pH</th>
<th>MPR</th>
<th>HSOS</th>
<th>NFOS</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.82</td>
<td>4.99</td>
<td>0.94</td>
<td>19.0</td>
<td>0.1</td>
<td>18.9</td>
</tr>
<tr>
<td>24</td>
<td>0.82</td>
<td>5.01</td>
<td>0.93</td>
<td>0.1</td>
<td>0.1</td>
<td>18.9</td>
</tr>
<tr>
<td>48</td>
<td>0.82</td>
<td>5.10</td>
<td>0.88</td>
<td>0.1</td>
<td>0.1</td>
<td>19.0</td>
</tr>
<tr>
<td>72</td>
<td>0.82</td>
<td>5.10</td>
<td>0.86</td>
<td>0.1</td>
<td>0.1</td>
<td>18.8</td>
</tr>
<tr>
<td>30 days</td>
<td>0.82</td>
<td>5.00</td>
<td>0.87</td>
<td>0.1</td>
<td>0.1</td>
<td>18.7</td>
</tr>
</tbody>
</table>

- Water activity.
- Moisture-to-protein ratio.
- O$_2$ concentration of vacuum-packaged treatment was not determined.
- Heat sealed with oxygen absorber.
- Nitrogen flushed with oxygen absorber.
- Heat sealed.
- Within the same column, means with different letters are significantly different ($P < 0.05$).

or VAC in conjunction with a 24-h storage time at 25.5°C experienced a $>1.0$-log CFU/cm$^2$ reduction in $L$. monocytogenes. Beef jerky packaged in HS, HSOS, NFOS, and VAC in conjunction with a 48-h storage time at 25.5°C reduced $L$. monocytogenes populations by at least 1.0 log CFU/cm$^2$. Large and small jerky processing facilities could use any of these packaging methods in conjunction with a storage time of at least 48 h at 25.5°C as a $L$. monocytogenes postlethality control treatment.

Pork and beef smoked sausage sticks with beef-collagen casings had an initial MPR of 0.89, pH of 5.1, and an $a_w$ of 0.82, based on chemical analysis conducted after receiving the commercial samples. The chemical analysis and package O$_2$ concentration for noninoculated smoked sausage sticks dipped in sterile TSB media are shown in Table 3. The $a_w$ remained constant at 0.82 throughout storage, and pH ranged from 4.99 to 5.10. The MPR of smoked sausage sticks ranged from 0.86 to 0.94 during 30 days of storage. It remained constant from 0 to 24 h, and then decreased ($P < 0.05$) after 48 h at 25.5°C storage.

Immediately after packaging, HSOS, NFOS, and HS contained 19.0, 0.1, and 18.9% O$_2$, respectively (Table 3). The O$_2$ concentration in NFOS and HS remained constant throughout storage at 25.5°C. On the other hand, the O$_2$ concentration in HSOS decreased dramatically from 19.0 to 0.1% after 24 h of storage at 25.5°C, and then remained constant ($P > 0.05$) up to 30 days. The addition of an O$_2$ scavenger in HSOS was effective in reducing the O$_2$ concentration after 24 h of ambient temperature storage.

There was no interaction ($P > 0.05$) between packaging treatments and storage times, indicating that packaging and storage times acted independently on $L$. monocytogenes reduction on smoked sausage. $L$. monocytogenes log reductions were 2.47 to 3.01 ($P > 0.05$) on smoked sausage sticks packaged in HS, VAC, and HSOS (Fig. 2). NFOS had the lowest log reduction at $<2$ log CFU/cm$^2$.

As a facultative anaerobic organism, $L$. monocytogenes utilizes O$_2$ for ATP production via aerobic respiration but can easily shift to fermentation when O$_2$ is absent. The high concentration of O$_2$ in HS did not enhance the survival of $L$. monocytogenes. Even VAC or HSOS, which provided anaerobic or low-oxygen conditions, did not enhance $L$. monocytogenes survival. These results do not support the findings of Rutherford et al. (13), who found air and vacuum packaging allowed $L$. monocytogenes proliferation in RTE shrimp.

Buchanan and Klawitter (2) found $L$. monocytogenes grew at intermediate temperatures (19 and 28°C), under aerobic conditions at pH 4.5 in tryptose phosphate broth. On the other hand, $L$. monocytogenes recovered and survived for extended periods at 37°C under anaerobic conditions. Buchanan and Klawitter (2) found that O$_2$ restriction also enhanced $L$. monocytogenes growth at 19°C. In our study, NFOS was less effective in reducing $L$. monocytogenes on smoked sausage sticks. Francis and O’Beirne (5) found that an atmosphere of 100% N$_2$ permitted survival of pure cultures of $L$. monocytogenes, but populations did not significantly change ($P > 0.05$) during storage.

The mean $L$. monocytogenes log reduction (CFU per square centimeter) on smoked sausage sticks after storage at 25.5°C up to 30 days is shown in Figure 3. Increasing storage time from 24 to 72 h or 30 days significantly increased $L$. monocytogenes reduction in smoked sausage sticks. A $>2.0$-log CFU/cm$^2$ $L$. monocytogenes reduction was achieved after 24 h. After 30 days of storage at 25.5°C, $L$. monocytogenes was reduced by 3.25 log CFU/cm$^2$, regardless of the packaging system. Ingham et al. (7) reported a mean $L$. monocytogenes log reduction of 1.4 log CFU per sample on vacuum-packaged beef snack sticks that were stored for
L. monocytogenes

5 weeks at 5°C. They used a storage temperature much cooler than the ambient temperature used in this study.

Regardless of storage time, packaging smoked sausage sticks in HS, HSOS, or VAC reduced *L. monocytogenes* populations by >2 log CFU/cm². Processors could also use a 24-h holding period for all packaging systems to achieve >2-log CFU/cm² *L. monocytogenes* reductions as a postlethality treatment for smoked sausage sticks.

**ACKNOWLEDGMENTS**

Support for this study was provided by the U.S. Department of Agriculture’s Cooperative State Research, Education, and Extension Service under agreement 2003-34211-12998, and the Kansas Department of Commerce and Housing, Agriculture Marketing Division.

**REFERENCES**


