A Research Note

Fate of *Listeria monocytogenes* on Modified-Atmosphere Packaged Turkey Roll Slices

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ABSTRACT

The growth of *Listeria monocytogenes* on turkey roll slices stored at 4 and 10°C under a variety of different modified-atmospheres (MAs) was examined. While increasing in numbers on turkey roll slices stored in air, or in environments containing CO₂ levels of 30 or 50% (remainder N₂), *L. monocytogenes* was inhibited by a MAs containing 70% CO₂, 30% N₂. In all cases, *Listeria* did not grow as well as any of the MAs as compared to air. In addition, for all MAs tested, pseudomonads were inhibited to an equal or greater extent than *L. monocytogenes*. It is recommended that any MA-packaged turkey sandwiches with a shelf-life approaching 30 days, should be stored in at least 70% CO₂ to guard against the potential growth of *L. monocytogenes*.

Key Words: Modified atmosphere packaging, turkey roll, *Listeria monocytogenes*, Pseudomonas, lactic acid bacteria, gas atmosphere.

A modified-atmosphere packaged (MAP) food is one which is packaged in something other than air. Although this technology originated in the 1930s, it became popular only after Marks and Spencer in the United Kingdom introduced a wide range of products packaged in different atmospheres in the early 1980s. While many of the technical problems related to MAP foods have been addressed, research into the microbiological safety has been lacking (2). *Listeria monocytogenes* is now a well recognized foodborne pathogen which can grow at refrigeration temperatures (3). In a recent study that evaluated the microbiological quality of various MAP sandwiches *L. monocytogenes* was present in 5 of 58 lots of product examined, with two of these lots being MAP turkey sandwiches (4). To assess the safety of these latter type products, the present study was conducted to follow the growth and/or survival of *L. monocytogenes* on MAP turkey roll slices stored under different atmospheres and at various temperatures.

MATERIALS AND METHODS

Organisms.

*Listeria monocytogenes* strain Scott A (serotype 4b) was used for inoculation experiments. Stock cultures were maintained at 4°C in a semisolid medium consisting of meat extract (5.0-g), peptone (10.0-g), NaCl (3.0-g), Na₂HPO₄ x 12H₂O (2.0-g) and agar (10.0-g) dissolved in 1 L of distilled water, final medium pH 7.4 (Institut Pasteur, Paris). Organisms were inoculated onto tryptose agar (Difco Laboratories, Inc., Detroit, MI), incubated at 30°C for 48 h and then were regrown at 30°C for 24 h in 5ml of tryptic soy broth (TSB; Difco) containing 0.6% yeast extract. For inoculation studies, appropriate dilutions of the culture were made in 0.1% (wt/vol) peptone water.

Sample analysis.

Whole turkey roll slices (pre-sliced) were purchased from a large local supermarket. Turkey roll slices (25.0 g) from each experiment were prestored for the pretreatment of the presence of *L. monocytogenes* using methodology previously described (5). The turkey roll slices (25.0 g) were inoculated with *L. monocytogenes* strain Scott A to give a final concentration of approximately 10⁸ cells/g. For inoculation, 200 μl of inoculum was placed on the surface of the turkey roll and then evenly spread with a sterile plastic disposable spreader. The slices were placed in high barrier bags (OTR 12cc/m²/24 h at 22°C) of dimensions 8" x 8". The multivac proportional gas mixer (Model KM 100-3M) was used to give the desired proportions of CO₂ and N₂ in the package headspace as follows: MA=p (30% CO₂, 70% N₂), MA=g (50% CO₂, 50% N₂) and MA= (70% CO₂, 30% N₂). Samples were then stored at 4°C and 10°C for time periods of up to 1 month.

At various intervals, samples were stomached (Colworth Stomacher 400 Lab Blender, Seward, U.K.) in 0.1% (wt/vol) peptone water and appropriate dilutions spread plated in duplicate onto *Listeria* Selective Medium (LPM) and Oxford media for *Listeria*, MRS agar (Oxoid Ltd., Nepean, Ontario) for the lactic bacilli, and Cetrimide agar (Oxoid) for the pseudomonads. During the enumeration phase, two presumptive *Listeria* spp. colonies per plate were picked and confirmed as *L. monocytogenes*, following methodology previously described (5). At each sampling time, gas atmospheres within the bags were analyzed with a Varian gas chromatograph (Model 3300, Varian Canada, Inc., Toronto, Ontario) fitted with a thermal conductivity detector, and using porapak Q (80-100 mesh) and molecular sieve 5A (80-100 mesh) columns in series. Helium was used as carrier gas at a flow rate of 30 ml/min. The column oven was initially set at 50°C for 3 min., and then was temperature programmed to increase by 50°C/min to 150°C. The injector and detector were set at 160 and 200°C, respectively. Gas samples were withdrawn using a 1.0 ml gas-tight Pressure-
RESULTS AND DISCUSSION

Initial gas atmospheres within the bags containing turkey roll slices generally varied by only ±5% (results not shown). During 4 weeks of storage at 4°C, *L. monocytogenes* populations increased an average of 2.32, 2.12, 1.58 and -0.58 logs on turkey roll slices packaged in air, MA30, MA50 and MA70, respectively, while at 10°C, average log increases of 3.78, 2.63, 1.28 and -0.57 were observed over the 30 d storage period (Figs. 1 and 2). Thus, although *L. monocytogenes* was able to increase to similar numbers as the air samples in MA30 and MA50, CO2 levels of 70% (MA70) were sufficient to inhibit the growth of *L. monocytogenes* at both 4 and 10°C (Figs. 1 and 2). The average initial levels of lactic acid bacteria and pseudomonads for all samples on day 0 was 4.4 x 10^3 and 4.2 x 10^5 CFU/g, respectively. Counts of lactic acid bacteria on MRS increased to ca.10^7 to 10^8 CFU/g and did not appear to be affected by the presence of *Listeria* or by the MA (Figs. 1 and 2). Pseudomonad counts, on average, increased to a greater extent than *Listeria* while growing on turkey roll in air. However, under MA70, MA50 or MA30, the pseudomonads did not grow as well as in air, and generally did not increase in numbers as well as did *Listeria* or the lactobacilli (Figs. 1 and 2). Thus, early warning signs of spoilage that are usually caused by the growth of the pseudomonads to high numbers, would not be observed. Turkey rolls appeared organoleptically (appearance, smell) acceptable during MAP storage at 4 or 10°C for 1 month. By the 3rd week of storage, however, turkey roll stored in air at either temperature was organoleptically unacceptable.

Hart et al. (4) examined the growth of *L. monocytogenes* on skinless chicken breast meat stored at 1, 6 and 15°C under various gas atmospheres. At 1°C, the organism failed to grow under any of the gas atmospheres, including air. At 6°C storage, the organism grew slowly in an atmosphere of 30% CO2 remainder N2, increasing approximately 1 log in number. Although in the presence of 30% CO2, the organism did not increase in number over the 15-day storage period, pseudomonad counts increased about 2 to 3 logs in number, regardless of the storage atmosphere.

Wimpfheimer et al. (8) found that under a MA of 75% CO2, 25% N2 both *L. monocytogenes* as well as other aerobic bacteria failed to grow on raw chicken stored at either 4, 10 or 27°C. However, in a MA containing some O2 (72.5:22.5:5; CO2:N2:O2), *L. monocytogenes* increased in number by almost 6 logs at 4°C over the 21 d storage period, while the aerobic colony count decreased more than 4 logs, as compared to samples packaged in air at the same temperature.

Marshall et al. (6,7) did a series of experiments examining the influence of different MAs on the competitive growth of both *L. monocytogenes* and *Pseudomonas fluorescens* on precooked chicken nuggets stored at 3, 7 or 11°C. The atmospheres used were either air, 76% CO2:13.3% N2; 10.7% O2 (MA1) or 80% CO2:20% N2 (MA2). Both MAs apparently were effective in either increasing the lag phase or reducing the growth rate of *L. monocytogenes*. Similar to the results of Wimpfheimer et al. (8), Ms con-
taining no O₂ was more restrictive to the growth of the organism. In both MAs, growth of P. fluorescens was generally inhibited to a greater extent than L. monocytogenes (6). In a companion paper, Marshall et al. (7) also found that at 3°C (but not at higher temperatures), P. fluorescens could stimulate the growth of L. monocytogenes on precooked chicken packaged in both air and MA. Conversely, L. monocytogenes inhibited the growth of P. fluorescens during the late stages of incubation in a MA at 11°C, but not at 3 or 7°C.

In our study, L. monocytogenes grew on turkey roll slices in MAs containing CO₂ levels as high as 50%, although growth was poorer in all MAs tested as compared to growth in air. In all instances, Listeria never grew as well in any of the MAs as compared to air. In all three MAs, the pseudomonads were generally inhibited to an equal or greater extent as compared to L. monocytogenes. These results are in agreement with those obtained by other investigators (6).

However, our study has shown that L. monocytogenes strain Scott A was unable to grow on turkey roll slices stored under an atmosphere of 70% CO₂:30% N₂, either at 4° or 10°C. This is in contrast to the work of Marshall et al. (6) who found the organism capable of growing on prewashed MAP chicken nuggets at 3, 7 and 11°C under both MA1 and MA2, but is similar to the work of Wimpfheimer et al. (8), who found that L. monocytogenes could not grow on raw chicken stored under an anaerobic MA consisting of 75% CO₂, 25% N₂. In high CO₂ environments background microorganisms and/or inhibitory chemicals might act synergistically or additively to inhibit the growth of L. monocytogenes. Both the pH (average 6.2±0.05) and u_w (0.98±0.02) values of the MAP turkey roll (results not presented) would not have presented any barriers to the growth of L. monocytogenes. Background levels of bacteria would have been substantially less in number on the precooked chicken nuggets as compared to raw chicken. The turkey roll used in this study contained unknown spices, salt, as well as a high background level of lactic acid bacteria, all of which, in combination with the high CO₂ environment, could have contributed to the inhibition of the organism. Modified-atmosphere packaged (MAP) sandwiches containing turkey can have a refrigerated shelf-life as long as 30 days. Some of the sandwiches currently on the market are stored under an atmosphere of 30% CO₂:70% N₂ or 5% CO₂:95% N₂ (1). In these situations, one could expect at least a 2-log increase in numbers on turkey at 4°C and under a temperature abuse situation (10°C), close to a 3-log increase could be expected. Thus, it is recommended that a minimum of 70% CO₂ be included in the atmosphere surrounding any MAP turkey containing sandwiches having a long shelf-life.

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REFERENCES