Comparative Growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on Precooked Chicken Nuggets Stored under Modified Atmospheres

DOUGLAS L. MARSHALL*1, PATTI L. WIESE-LEHIGH, JOHN HENRY WELLS2, AND A. JAMES FARR3

1Departments of Food Science, 2Agricultural Engineering and 3Poultry Science, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803

(Received for Publication May 20, 1991)

**ABSTRACT**

The purpose of this study was to determine the effects of modified atmosphere packaging (MAP) on growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked dark-meat chicken nuggets during refrigerated storage. The two organisms were separately inoculated on nuggets and stored under modified atmospheres (MA1 or MA2) at 3, 7, or 11°C. The results show that the growth of *P. fluorescens* was inhibited by MAP to a greater extent than was the growth of *L. monocytogenes*. Even though growth of *L. monocytogenes* was inhibited by MAP, the organism was still capable of growth at all three temperatures. The effectiveness of MAP decreased with increasing temperature. Little difference in inhibition of growth was observed for either organism with MA1 or MA2.

Considerable interest in modified atmosphere packaging (MAP) of muscle foods has occurred in the United States over the past several years (22). The demand for refrigerated convenience foods with extended shelf lives has driven this technology. Reduced oxygen and increased carbon dioxide concentrations in food packages can increase shelf life via the inhibition of aerobic spoilage bacteria. However, the growth of psychrotrophic pathogenic bacteria under such environments may not be inhibited (10). Since spoilage may not be evident in MAP foods, consumers could judge such products as wholesome even though pathogens may be abundant. The microbiological safety of MAP foods continues to be questioned and is the subject of intense research (10).

Numerous studies have been conducted to assess the effects of MAP on raw poultry products (1-4, 11, 13, 16, 17, 19). However, little work has been done using precooked convenience items such as poultry nuggets. These products provide an especially hazardous risk to consumers since they often require little reheating prior to consumption. *Listeria monocytogenes* is a foodborne pathogenic bacterium that can grow at refrigerator temperatures (14, 20).

*Pseudomonas fluorescens* causes bacterial spoilage of refrigerated foods (7). Although both species are readily destroyed by proper cooking, they are common as postprocessing contaminants and could be expected on precooked poultry nuggets. Therefore, the objective of this study was to determine the effects of MAP on growth of *L. monocytogenes* and *P. fluorescens* on precooked dark-meat chicken nuggets during refrigerated storage.

**MATERIALS AND METHODS**

**Preparation of nuggets**

Twenty pounds of hand deboned boiler thigh meat was ground through a 70-mm diameter plate with 3.2-mm holes. One-half percent NaCl by weight was added to the ground product. The product was tumbled in a Globus Inject Star MC massager using a 20-L drum with 80% vacuum, rotating at low speed for 15 min. Massaged meat was tempered to -1.7°C with dry ice before passing through a Hollymatic Super former with a nugget plate sized for 20-g portions. Formed nuggets were frozen in a carbon dioxide cabinet freezer and stored at -10°C until used. Thawed, raw nuggets were placed individually in 2-oz plastic jars and autoclaved at 120°C for 15 min. Following cooling at room temperature, the fat was removed by aseptic decanting, leaving a sterile cooked nugget weighing approximately 10 g. The nuggets were stored overnight at 5°C and inoculated the following day.

**Preparation of inocula**

*L. monocytogenes* strain Scott A and *P. fluorescens* (ATCC 13525) were maintained as stock cultures through monthly transfers on trypticase soy-0.6% yeast extract agar slants (TSYECA) (BBL Microbiology Systems, Cockeysville, MD) and stored at 4°C.

Cultures from TSYEA were subcultured overnight in trypticase soy-0.6% yeast extract broth by quiescent incubation at 25°C to obtain working cultures for each experiment. Serial dilutions of the working cultures were made in sterile peptone water (0.1%) to obtain the desired inocula. One ml of a diluted culture was transferred into a sterile 4-L beaker containing 2 L sterile peptone water and a stir bar to obtain an inoculum of approximately 10⁴ CFU/ml. One beaker was used for inoculating *L. monocytogenes*, one for *P. fluorescens*, and one for a negative control. Negative controls had 1 ml sterile peptone water added and were used as unincoculated controls to test for sterility. For inoculation, 50 sterilized nuggets were removed aseptically from the plastic jars and placed on a 1 ft² piece of sterile cheese cloth and dipped into...
an inoculating solution with constant agitation for 5 min. After inoculation the nuggets were placed on sterilized paper towels to drain for 10 min and then transferred aseptically to pairs in sterile 100 x 15-mm plastic petri dishes (Scientific Products Division, Baxter, McFar Park, IL). The entire inoculation procedure was performed in a laminar flow hood (Labconco, Kansas City, MO) to prevent environmental contamination. The inoculation schedule for each temperature and storage atmosphere treatment was 50 nuggets inoculated with L. monocytogenes, 50 with P. fluorescens, and 10 without organisms.

Packaging of nuggets

Petri dishes containing two nuggets each were packaged in 7" x 8" plastic barrier bags (Koch Model 014609, Kansas City, MO). The barrier bags have O2 transmission rates of 9 cc/m2 in 24 h. Bags containing samples for air storage (A) were heat sealed without evacuation or gas flush. Bags containing samples for modified atmosphere storage were evacuated to -950 mbar, backflushed with a commercial gas mix (either MA, or MA2) to +200 mbar, and heat sealed using a Multivac Model A300/22 gas packaging machine (Kansas City, MO). The commercial gas mixes used contained 76% CO2:13.3% N2:10.7% O2 [identified as modified atmosphere 1 (MA1)] or 80% CO2:20% N2 [identified as modified atmosphere 2 (MA2)].

Enumeration of bacteria

Bacteria were enumerated using standard microbial count methods (15). At specified time periods, two nuggets per treatment were analyzed for the number of each bacterium. Each nugget was aseptically removed from the petri plates and placed individually into a tared stomacher bag to which a sufficient volume of sterile peptone water was added to achieve an initial dilution of 1:10. The diluted nugget was homogenized for 2 min using a Stomacher (Tekmar Model STO 400, Cincinnati, OH) and additional decimal dilutions were performed as needed using peptone water. Duplicate surface platings were performed by removing 0.1 ml from the diluted samples. L. monocytogenes was counted on Baird-Parker agar (Difco, Detroit, MI) spread plates and P. fluorescens was counted on violet red bile agar (Difco) spread plates. Inoculated plates were incubated at 27°C for 48 h prior to counting. The performance of Baird-Parker and violet red bile for the enumeration of the bacteria was compared with TSYEA and no significant differences were found for the recovery of either bacterium (data not shown).

Experimental protocol

The experimental design used in this study was a 3 x 3 factorial with two replicate experiments. Each replicate experiment consisted of three temperatures (3, 7, and 11°C) and three atmospheres (A, MA1, and MA2). At each sampling point, two nuggets were used to calculate microbial populations for each replicate. Mean values were reported as the average of duplicate platings of each of four nuggets per sampling point. The number of bacteria present on the nuggets was determined for each sampling and expressed as log10 CFU per g.

RESULTS AND DISCUSSION


growth of L. monocytogenes

The growth of L. monocytogenes at 3, 7, and 11°C is shown in Fig. 2A, 2B, and 2C, respectively. At 3°C, a lag period of approximately 3 d was observed when the organism was grown in air but was extended to 9 d in the two modified atmosphere treatments. Both modified atmospheres substantially reduced growth throughout the duration of the experiment. At 7°C the lag period for P. fluorescens in air was less than 1 d (Fig. 2B). When grown in the two modified atmospheres, the lag periods were substantially increased to 6 d. Once growth was initiated, the organism grew at the same rate in both MAP treatments. Fig. 2C

Figure 1A, 1B, and 1C. Growth of L. monocytogenes at 3, 7, and 11°C, respectively, on chicken nuggets packaged in air (A), modified atmosphere 1 (MA1), or modified atmosphere 2 (MA2).
Figure 2A, 2B, and 2C. Growth of *P. fluorescens* at 3, 7, and 11°C, respectively, on chicken nuggets packaged in air (A), modified atmosphere 1 (MA1), or modified atmosphere 2 (MA2).

shows data for the growth of *P. fluorescens* at 11°C. All three treatments had lag periods of less than 1 d. However, growth was greatly reduced in the two MAP environments compared with growth in air.

*L. monocytogenes* was able to initiate growth sooner than *P. fluorescens* at 3°C but not at higher temperatures. *L. monocytogenes* reached concentrations in the vicinity of 10 million per g by day 18 in all three atmospheres at 3°C, while *P. fluorescens* was only able to attain this population size when grown in air. Thus, during low temperature storage, the population of *L. monocytogenes* would be consistently high compared with *P. fluorescens* regardless of the storage atmosphere, assuming similar likelihoods of initial inocula. This raises a potential safety concern because the product would not be obviously spoiled but still have substantial numbers of pathogenic *L. monocytogenes* present. At elevated temperatures, *P. fluorescens* was able to attain higher population levels than *L. monocytogenes* only when grown in an air environment. Conversely, under modified atmospheres, *L. monocytogenes* again was able to grow to high numbers before *P. fluorescens* could reach estimated spoilage levels of 10^7/g. The time difference to reach 10^7/g between *L. monocytogenes* and *P. fluorescens* was in excess of 7 d regardless of the temperature or modified atmosphere used.

It is well documented that in muscle foods stored under MAP growth of gram-negative organisms is inhibited more than gram-positive organisms (5,6,8,9,12,18). Under air storage conditions, the spoilage of meat products is primarily due to the growth of gram-negative bacteria. By inhibiting the growth of the latter with MAP, a significant increase in the shelf life of products can be achieved.

However, during storage of these products, the growth of gram-positive organisms will predominate. The results of the present study substantiate previous research in modified atmosphere packaging. *P. fluorescens*, a gram-negative bacterium, was inhibited to a greater extent by MAP than was *L. monocytogenes*, a gram-positive bacterium.

Consumers judge the wholesomeness of meat products primarily upon appearance and odor. Gram-negative organisms produce characteristic changes to a product that are indicative of spoilage during prolonged storage. Conversely, *L. monocytogenes* does not produce evidence of spoilage, even when present in high numbers. If precooked ready to eat products such as chicken nuggets are stored under MAP, *L. monocytogenes* could grow to large population sizes during refrigerated storage before spoilage would be evident. Thus, consumers could unknowingly consume a potentially toxic product.

**SUMMARY AND CONCLUSIONS**

Listeria monocytogenes was moderately inhibited by MAP using two low oxygen atmospheres compared with storage in air. However, growth was not completely retarded nor was the organism killed by these storage environments. The effectiveness of MAP decreased as the storage temperature increased. This implies that proper control of temperature is essential for slowing the growth of the pathogen even under modified atmospheres. It should also be noted that *L. monocytogenes* was capable of growth at the lowest temperature used in the study (3°C). Like *L. monocytogenes*, *P. fluorescens* was inhibited by MAP, although to a greater extent. *P. fluorescens* also was capable of growth at the lowest temperature. Similarly, the effectiveness of MAP decreased with increasing temperature.

Foodservice and retail consumers who are demanding products with increased convenience, availability, and value may be attracted to MAP poultry products. The results of this study demonstrate that potentially dangerous situations can occur in MAP food products, including precooked poultry. Since modified atmospheres selectively inhibit spoilage bacteria such as *P. fluorescens* over pathogens like *L. monocytogenes*, obvious signs of spoilage may not be present even though pathogenic organisms can be in dangerously high concentrations. Processors should be aware of this fact and measures in addition to MAP must be taken to control *L. monocytogenes* in cooked products. Such steps include vigorous plant sanitation, proper cooking temperatures, proper packaging methods, and strict temperature control during refrigerated storage.

**ACKNOWLEDGMENTS**

The authors thank S. C. Ingham for securing financial support provided under Project #478 by the Southeastern Poultry & Egg Association. The authors thank the following individuals for technical assistance: L. S. Andrews, H. K. Salmon, and S. Donnelly.

**REFERENCES**


*JOURNAL OF FOOD PROTECTION, VOL. 54, NOVEMBER 1991*

cont. on p. 851
difficult, and the NPV is helpful in this consideration. A NPV of 47% means that there is only a 47% chance that the herd with a negative test result is truly negative. As mentioned above, this poor NPV can possibly be improved by taking repeated samples from the same herd on consecutive days (10,15).

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of USDA:APHIS:VS, the veterinarians of Ohio’s Department of Agriculture, and the participating dairy producers.

REFERENCES