

# Fate of *Listeria monocytogenes* on Vacuum-packaged Pepperoni Stored at 4, 12, or 25°C

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## ABSTRACT

*Listeria monocytogenes* should be destroyed during processing of pepperoni, but it may be reintroduced during slicing or packaging of the product. In this study we evaluated the fate of *L. monocytogenes* on the surface of inoculated product during storage and potential differences in survival of three types of inocula cultured under different environmental conditions. The inocula were composed of 10 strains of *L. monocytogenes* which were cultured separately in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE), with or without 1% glucose (acid-adapted and non-habituated inoculum, respectively), or grown in TSBYE (30°C, 24 h) and then habituated in pepperoni extract (10% w/w in distilled water) at 7°C for 72 h (extract-habituated inoculum). Commercially available pepperoni (two replicates, three samples per treatment) was sliced, and inoculated to a level of contamination of 3-4 log CFU/cm<sup>2</sup>, before it was vacuum-packaged and stored for up to 180 days at 4, 12, or 25°C. The samples were analyzed periodically for surviving populations (PALCAM) and total bacterial counts (tryptic soy agar with 0.6% yeast extract). Overall, the levels of the pathogen and total counts decreased continuously during storage, regardless of inoculum type. The pH of the product was relatively stable (4.64-4.82) throughout the storage. When the product was stored at 4°C the levels of acid-adapted inoculum dropped below the detection limit (-0.48 log CFU/cm<sup>2</sup>) at 32 days, whereas the other two types of inocula survived for up to 47 days of storage. In product stored at 12°C the non-habituated inoculum survived longer than other inocula. At 25°C, the acid-adapted inoculum died faster than the non-habituated inoculum. Thus, the rate of death varied with temperature of storage and type of inoculum. The results of this study may be useful in risk assessment studies and in helping processors select proper storage conditions for pepperoni.

## INTRODUCTION

*Listeria monocytogenes* is a ubiquitous foodborne pathogen that can persist in food processing environments. The pathogen can survive at low pH and water activity and shows an increased resistance to low pH after exposure to sub-lethal acidic conditions. Processing of fermented sausages may provide ample opportunity for the pathogen to acquire such resistance or cross-protection. Similarly, persistence of *L. monocytogenes* in the processing environment and under unfavorable conditions may alter its resistance to subsequent hurdle interventions. Pepperoni is a dry fermented sausage that can be consumed without reheating. Typical drying and fermentation steps involved in the processing of pepperoni reduce levels of *L. monocytogenes*, but do not eliminate the pathogen. In addition to potential survivors, the pathogen may be introduced during peeling, slicing or packaging of the product. The microbiological testing program conducted by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) revealed that between 1990-1999 the cumulative prevalence of *L. monocytogenes* in dry and semi-dry fermented sausages was 3.25% (Levine et al., 2001). The USDA-FSIS employs a "zero-tolerance" rule for *L. monocytogenes* in ready-to-eat (RTE) meats and requires food processors to control the pathogen in products that are exposed to the processing environment after the lethality step by application of one out of three alternatives (FSIS, 2006). Alternative 1 requires application of both a post-lethality treatment, which must reduce at least 1 log of the pathogen, and an antimicrobial agent that suppresses its growth. Alternative 2 requires application of either a post-lethality treatment or antimicrobial agent that suppresses growth of the pathogen plus microbiological testing of the processing environment and FSIS verification. The third alternative relies on sanitation measures, environmental testing and the verification procedures that are more frequent than procedures required by the second alternative. Intrinsic factors of pepperoni, such as low pH and a<sub>w</sub> usually do not allow growth of the pathogen and therefore processors may comply with the second alternative of the requirement if the product does not allow more than 1-log increase of the pathogen throughout the shelf life. To comply with the first alternative of the rule, which does not require frequent environmental testing, processors must also apply a post-lethality treatment to the product that does not support growth of the pathogen. Microbial populations on the surface of dry fermented sausages may be reduced by hurdles such as low pH and a<sub>w</sub> during storage of the product. However, the effectiveness of the hurdles may depend on the storage temperature of the contaminated product and may depend on physiological history of the pathogen.

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## OBJECTIVE

The objective of this study was to determine the fate of *L. monocytogenes* on sliced pepperoni that was vacuum-packaged and stored at 4, 12 and 25°C and to evaluate potential differences in behavior of *L. monocytogenes* inoculum that was cultured under various conditions.

## MATERIALS AND METHODS

### Bacterial strains.

•Each *L. monocytogenes* inoculum consisted of 10 strains of food, environmental or clinical origin: N1-225<sup>1</sup>, N1-227<sup>1</sup>, R2-500<sup>1</sup>, R2-501<sup>1</sup>, R2-763<sup>1</sup>, R2-764<sup>1</sup> and R2-765<sup>1</sup> (serotype 4b), 558 (serotype 1/2), NA-1 (serotype 3b), N-7150 (serotype 3a).  
<sup>1</sup>kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY; Fugett et al., 2006)

•Extract-habituated and acid-adapted inocula were prepared by activating strains in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE, 30°C, 24 h).

•Non-habituated inoculum was prepared by strain activation in TSBYE w/o glucose (TSBYE - G).

•Activated strains were subcultured in TSBYE - G (non-habituated inoculum), TSBYE - G supplemented with 1% glucose (acid-adapted inoculum) or TSBYE (extract-habituated inoculum).

•Before use, non-habituated strains were combined in a sterile centrifuge tube; the same procedure was done to the acid-adapted cells.

•Cells were harvested by centrifugation and resuspended in sterile PBS. Extract-habituated inoculum was prepared by centrifugation washing of TSBYE-subcultured cells, resuspended in sterile pepperoni extract (10% w/w in distilled water) and habituated for 72 h at 7°C (Lianou et al., 2007). The target inoculum level was 3-4 log CFU/cm<sup>2</sup>.

### Inoculation.

•Pepperoni was sliced, surface-inoculated (25 µl) and kept at 4°C, (15 min) to allow for cell attachment.

•Product was vacuum-packaged and stored at 4, 12 or 25°C for 180 days.

### Microbiological analysis.

•At each sampling interval, 3 slices from each package were transferred into sterile bags and 25 ml of maximum recovery diluent (MRD; 0.85% of NaCl and 0.1% buffered peptone) was added to each bag. Samples were manually shaken 30 times for 30 s to detach cells.

•Ten-fold serial dilutions of the rinse were prepared and surface-plated onto PALCAM agar (Difco) or tryptic soy agar with 0.6% yeast extract (TSAYE) for enumeration of the *L. monocytogenes* and total microbial populations, respectively.

•Inoculated PALCAM agar plates and TSAYE plates were incubated at 30°C for 48 h and 25°C for 72 h, respectively.

•When levels of *L. monocytogenes* decreased below the detection limit (-0.48 log CFU/cm<sup>2</sup>) by direct plating, samples were enriched to determine presence of the pathogen.

### Other analyses.

•Water activity (a<sub>w</sub>) values were measured only on day-zero.

•The pH of pepperoni was measured after microbiological analysis and punneling.

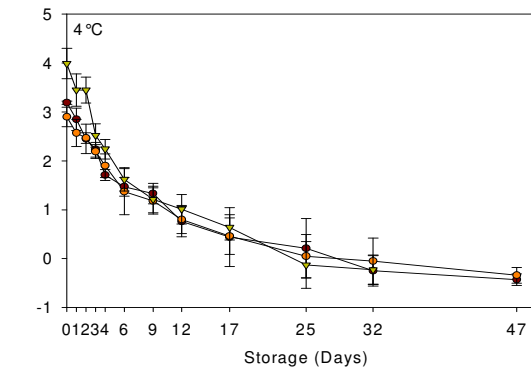
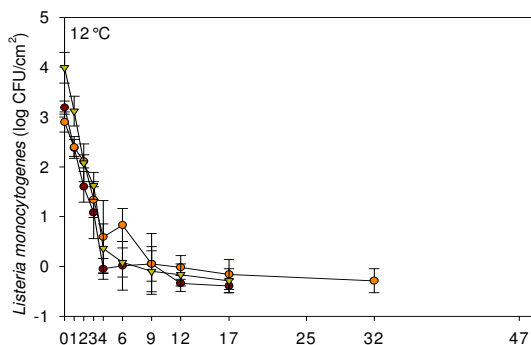
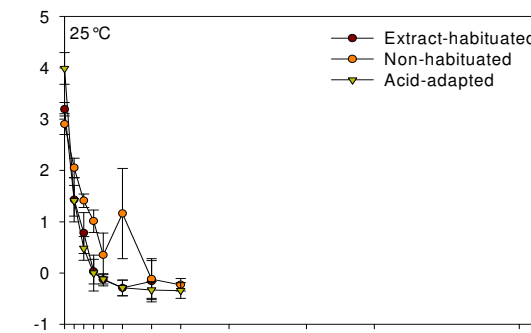
### Statistical analysis.

•Two repetitions of the experiment, each with three samples per treatment at each sampling time (n = 6).

•Randomized complete block design with factorial arrangement of the treatments.

•Death rates were determined as the slopes of the lines fitted to the linear part of the curve by simple linear regression.

## RESULTS



## Death rates of *L. monocytogenes* on the surface of sliced pepperoni that was vacuum-packaged and stored at 4, 12 or 25°C<sup>a</sup>

Storage temperature	Inoculum origin	Death rate (log CFU/cm <sup>2</sup> /day)	r <sup>2</sup>
25°C	Extract-habituated	0.801 - 0.808	0.939 - 0.993
	Non-habituated	0.596 - 0.606	0.918 - 0.990
	Acid-adapted	0.875 - 1.047	0.826 - 0.947
12°C	Extract-habituated	0.729 - 0.827	0.689 - 0.996
	Non-habituated	0.536 - 0.596	0.939 - 0.954
	Acid-adapted	0.862 - 0.886	0.768 - 0.836
4°C	Extract-habituated	0.327 - 0.385	0.897 - 0.978
	Non-habituated	0.187 - 0.299	0.935 - 0.986
	Acid-adapted	0.341 - 0.569	0.963 - 0.986

<sup>a</sup>Values for death rate are means (n = 3) as determined from *L. monocytogenes* counts on PALCAM agar  
<sup>b</sup>Coefficient of determination for the linear regression

- The pH of the pepperoni was 4.67 ± 0.01 and was stable throughout the storage period. The initial a<sub>w</sub> was 0.827 ± 0.01.
- Numbers of *L. monocytogenes* on pepperoni decreased gradually during storage.
- After 60 days of storage at each temperature the pathogen was not detected by enrichment.
- Survivor curves did not exhibit a shoulder period, the time interval where levels of the pathogen remained at the level of inoculation. Pathogen counts declined linearly for all types of inocula when the product was stored at 12 or 25°C. At 4°C the linear decline was observed during the first 4 days of storage regardless of inoculum type. After 4 days of storage at 4°C the decline in pathogen numbers was less dramatic; the pathogen persisted for 47 days.
- At 4°C all three inocula died slower than at 12 or 25°C.
- At 12 and 25°C the death rates were similar.
- The non-habituated inoculum died faster at all three temperatures compared to the acid-adapted and extract-habituated inocula.
- Similar death rates were obtained for acid-adapted and extract-habituated inocula at all three temperatures.

## CONCLUSIONS

Storage of pepperoni before distribution or storage before retail sale may virtually eliminate *L. monocytogenes* from the product made under good manufacturing practices. It may be advisable for food processors to store sliced vacuum-packaged pepperoni at ambient temperature prior to retail distribution in order to reduce or eliminate *L. monocytogenes* from the product.

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