Report #4 Survival of *Listeria monocytogenes* on head cheese

Validation of Head Cheese Formulation as an Antimicrobial Agent Against *Listeria monocytogenes*

INTRODUCTION

The formulation of head cheese involves addition of large amounts of vinegar to pieces of meat. The finished product typically has a pH below 4.6 and, in some cases, the pH may be less than the 4.4 level which is cited as the minimum pH allowing growth of *Listeria monocytogenes* (2). At the request of a head cheese processor, the present study was conducted to validate a traditional head cheese manufacturing formulation as an effective antimicrobial agent against *L. monocytogenes*. Such a validation would allow the processor to operate under Alternative 2 of the USDA interim final rule addressing the control of *Listeria monocytogenes* on ready-to-eat meat and poultry products (1).

MATERIALS AND METHODS

Overview Head cheese from three different lots was inoculated with a multi-strain cocktail of *Listeria monocytogenes*. After samples were inoculated they were vacuum packed, and stored at 41°F (5°C). Three inoculated samples and one uninoculated sample were analyzed at day 0, day 8, and day 31 to determine the number of *L. monocytogenes* cells present.

Head Cheese Three loaves of head cheese representing three separate lots were shipped overnight to the laboratory in cooler boxes with ice packs and stored at 41°F 5°C until used. The pH values of the three lots were 4.4, 4.2, and 4.4. For this study, each loaf of head cheese was divided into 10 slices with dimensions of 4.25 inches x 2.25 inches x 0.75 inches (10.8 cm x 5.7 cm x 1.9 cm).

Preparation of Inoculum Table 1 shows the strains used in this study. Frozen stock cultures were maintained in Brain Heart Infusion (BHIB; Difco, Becton Dickinson, Sparks, MD) with 10% (v/v) added glycerol (Fisher Scientific, Itasca, IL). Working cultures were prepared by growing each strain for two passages in BHIB and then streaking on Brain Heart Infusion Agar (BHIA; Difco). Following growth on BHIA for 24 h at 95°F (35°C), the working cultures were stored at 41°F (5°C). A colony of each strain was streaked on BHIA and grown for 24 h at 95°F (35°C), following which a colony of each culture was grown separately in 9 ml of BHIB for 24 h at 95°F (35°C). All cultures were combined, and then centrifuged at 8,000 x g for 10 minutes. Each resulting pellet was then re-suspended to original volume in Butterfield's Phosphate Diluent (BPD, Nelson-Jameson, Marshfield, WI).

Table 1. Organisms used in this study.

Microorganism	Strain Designation	Original Source
Listeria monocytogenes	Scott A[a]	Human isolate- MA epidemic
Listeria monocytogenes	LM 101[a]	Hard salami
Listeria monocytogenes	LM 108[a]	Hard salami
Listeria monocytogenes	LM 310[a]	Goat cheese
Listeria monocytogenes	V7[a]	Raw milk

[a] Obtained from the laboratory of Eric Johnson, Food Research Institute, University of Wisconsin-Madison

Inoculation of Head Cheese For inoculation, slices of head cheese were placed on aluminum foil that had previously been treated with 70% (v/v) ethanol in a laminar flow bio-safety hood. Each slice was inoculated with 0.01 oz (0.3 ml) of a 1:10 dilution (BPD) of the cocktail. The inoculum was spread evenly over the surface of the piece of head cheese using a sterile bent plastic rod (Daigger, Inc., Vernon Hills, IL). After inoculation, the pieces were allowed to dry for 15 min., flipped, and the procedure was repeated on the other side. Then, slices were aseptically transferred to a vacuum-packaging bag (FoodSaver, Tilia, Inc., San Francisco, CA), vacuum-packaged, and stored at 41°F (5°C). Uninoculated control slices of head cheese were packaged and stored in the same way.

Enumeration of Inoculum Organisms After inoculation, enumeration for three inoculated samples was done. For enumeration, each vacuum sealed bag was first treated with 70% ethanol, then opened using scissors that had been flame sterilized. Next, a 1 inch x 1 inch x 0.25 inch section was aseptically excised from one randomly chosen side of the slice and placed into a whirl pack bag. Then, 3.3 oz (99 ml) of BPD was added to the sample bag and the contents were stomached for 2 minutes on medium speed using a Stomacher 400 lab blender (Fisher Scientific, Itasca, IL). Subsequent dilutions were made in BPD and spread-plated on LSA agar (Oxoid, Inc., Ogdensburg, NY) with added Listeria Selective Supplements (Oxford formulation; Oxoid) for enumeration of *L. monocytogenes*. LSA plates were incubated at 95°F (35°C) for 48 h, typical colonies (brown to black colonies with a zone of black precipitate) were counted, and the logarithm of Colony-Forming Units (log CFU) was calculated for each sample. In addition to the enumeration described above, day 8 samples were also diluted and plated on BHIA, incubated 48 h at 95°F (35°C). This analysis provided an estimate of all aerobic bacteria on the product surface, including injured (but viable) and uninjured *L. monocytogenes*. To confirm that colonies counted on LSA were *L. monocytogenes* , one typical colony per plating per lot of head cheese was transferred to BHIA and incubated for 24 h at 95°F (35°C). Presumptive *L. monocytogenes* colonies from each BHIA plate were tested for Gram reaction, cell morphology, oxidase reaction, and biochemical characteristics (API Listeria; bioMerieux, Inc., Hazelwood, MO). Throughout the study, all presumptive colonies were confirmed as the *L. monocytogenes*.

RESULTS AND DISCUSSION

The results (Table 2) clearly show that *L. monocytogenes* rapidly died off on the surface of the head cheese. Population decreases after 8 days averaged about 2.8 logs as determined using LSA and 2.5 logs as determined with BHIA plating. All colonies observed on BHIA after the day 8 plating had typical *L. monocytogenes* morphology. Counts on BHIA after 8 days of refrigeration were 0– 0.4 logs higher than on LSA, indicating the presence of some injured, but viable, *L. monocytogenes* cells on the head cheese. However, by 31 days no survivors were detected on LSA. In order to claim that an antimicrobial agent is effective in preventing growth of *L. monocytogenes* on a ready-to-eat meat or poultry product, the agent must not allow more than a 1 log increase in *L. monocytogenes* populations (2). In the head cheese, the *L. monocytogenes* population decreased, so the processor may consider the product formulation to be an effective antimicrobial agent. Other head cheese processors should determine the typical pH of their product. If the pH is within the 4.2 – 4.4 range described in the present study, the processor may consider the product formulation to be an effective antimicrobial agent, allowing operation under Alternative 2 of the USDA regulations (1).

Table 2. Log CFU/g (standard deviation in parentheses) of *Listeria monocytogenes*.

	A	B	C
Day 0	5.9 (0.1)	5.7 (0.1)	5.8 (0.1)
Day 8	3.2 (0.3)	2.0[a] (0)	3.1 (0.4)
Day 8 (BHIA)	3.6 (0.3)	2.2 (0.4)	3.1 (0.1)
Day 31	0.9[b] (0.0)	0.9[b] (0.0)	0.9[b] (0.0)

a- no LM detected on 1:100 dilution, assigned value of 1.9 log CFU/g
b- no LM detected on 1:10 dilution, assigned value of 0.9 log CFU/g

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REFERENCES

1. 1. United States Department of Agriculture, Food Safety & Inspection Service. 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; final rule. Federal Register: June 6, 2003 (Vol. 68, No. 109, pp. 34207-34254).
2. United States Department of Agriculture, Food Safety & Inspection Service. Compliance guidelines to control *Listeria monocytogenes* in post-lethality exposed ready-to-eat meat and poultry products. http://www.fsis.usda.gov/OPPDE/rdad?FRPubs/97-013F/CompGuidelines.doc. Accessed 19 April 2004.