CHANGES IN *LISTERIA MONOCYTOGENES* POPULATIONS IN RAW AND COOKED BEEF, PORK, LAMB AND CHICKEN MEAT

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Background

A major concern, when considering the phenotypical characteristics of *Listeria monocytogenes,* is its potential for psychrotrophic growth. There are studies that have shown growth of *L. monocytogenes* in beef at several temperatures (Gill and Reichel, 1989; Grau and Vanderlinde, 1988), while others have reported no growth of this bacterium when raw ground beef was stored at either 4°C or 25°C (Johnson *et al.*, 1988; Shelef, 1989). Lack of growth was also reported in untreated and radiation-sterilized beef stored at 5°C (Buchanan and Klawitter, 1991).

Objectives

This study was designed to determine survival, growth, or decline of *L. monocytogenes* inocula, prepared at 4°C and 37°C, in raw and cooked vacuum-packaged ground meat from four animal species stored at 4°C.

Methods

Chicken breasts and lamb shoulder meat were purchased from a retail store. Pork Boston butts and beef rounds were obtained from the Department of Animal Sciences of Colorado State University. Meat from each species was ground (UNIVEX.NSF Testing Laboratory, Ann Arbor, MI) with a 0.95 cm plate, vacuum packaged (-0.85 bar) and frozen (-23°C) for 4-5 days. The ground meat was then thawed at 3°C for 48 hr and formed into 50-g patties (20% fat) which were inoculated, individually vacuum packaged (-0.85 bar) in pouches (15 cm x 22 cm) (Koch) and stored at 4°C. Additional ground meat patties, prepared as described above, were placed in sterile (100 mm x 15 mm) Falcon petri plates (Becton Dickinson and Company, Lincoln Park, NJ) and microwaved for 30 sec in an Amana® Commercial Radarange® RC/20Se microwave oven (2450 MHz, 208/230v, 4000 watt), at full power before inoculation.

Ten strains of *L. monocytogenes* were grown, individually, in trypticase soy broth (TSB) (Difco Laboratories, Detroit, MI) with 0.6% yeast extract (Difco). One inoculum of each of the 10 strains was grown at 37°C for 24 hr, and another at 4°C for 10 days. The inoculum of each strain from each incubation temperature was diluted appropriately with sterile phosphate buffer solution (Sigma Chemical Co., St. Louis, MO) to obtain a cell concentration of approximately 10^6 CFU/ml for those grown at 4°C, and 10^5 CFU/ml for those grown at 37°C. Aliquots of all strains from each temperature were then mixed to make the composite inocula.

One-third of the raw or cooked ground meat patties from each meat type were surface inoculated with 0.5 ml of the inoculum made with cells grown at 4°C. Another one-third of the patties were surface inoculated with 0.5 ml of the inoculum made with cells grown at 37°C. The same amount of sterile phosphate buffer solution was added to the last one-third of the samples to serve as controls. The microwaved patties, however, were inoculated only with the culture prepared at 37°C for 24 hr and not with the culture prepared at 4°C for 10 days. All packaged patties were stored at 4°C. Duplicate samples of all treatments were analyzed immediately after inoculation and during storage by surface plating on trypticase soy agar (TSA) (Difco) and on Lithium Chloride Phenylethanol Moxalactam agar (LPM) (Difco) to determine total bacterial populations and *L. monocytogenes* populations, respectively. Plates were incubated at 37°C for 48 hr. The pH was also determined, at each sampling time, in the blended samples using a Corning® pH meter 125 (Corning Incorporated, Big Flats, NY).

Results and Discussion

The temperature (4°C or 37°C) at which the inocula were prepared did not result in any major differences or changes in *L. monocytogenes* populations in inoculated raw beef, pork and lamb meat (Table 1). In raw chicken meat, however, population numbers of the inoculum prepared at 37°C increased by approximately 2 log CFU/g, while those of the inoculum prepared at 4°C increased by approximately 1 log CFU/g, in the first seven days of storage at 4°C. Thereafter (up to 49 days), populations remained relatively constant (Table 1). Numbers of *L. monocytogenes* decreased slowly, by approximately 1.7 log, in raw ground beef, and by approximately 0.5 log in ground pork and lamb, over the storage (4°C) period of seven weeks (Table 1). In uninoculated products, total bacterial populations increased by 4.5 log CFU/g in raw chicken patties, 3.9 log CFU/g in raw pork patties and 3.4 log CFU/g in raw lamb patties, while, in uninoculated raw beef patties, total plate counts remained relatively constant (approximately 5.5 log CFU/g) during the 49-day storage (4°C) period (data not shown).

Numbers of *L. monocytogenes* increased by 1.4-1.8 log CFU/g in cooked ground meats (Table 2). These increases might have been higher if the initial inoculum levels had been lower. However, we used these higher inoculum levels because the objective was to determine any changes, including decreases, in microbial counts that might occur in meat from different species. In contrast to results for the raw beef patties, 14 days of storage at 4°C of cooked beef patties caused the populations of *L. monocytogenes* to increase by 1.5 log CFU/g. Total bacterial population remained constant (2.5-2.7 log CFU/g) in all uninoculated cooked samples. Obviously, the organisms that survived cooking were not psychrotrophic, and therefore, did not multiply at 4°C.

Values of pH decreased from 5.59, to 4.95, in raw beef over a 21-day storage period, and increased to 5.80 by the end of the study (Table 3). A similar trend was observed in pH values of raw pork, which decreased from 6.17, to 5.76, over a 21-day storage period, and increased to 6.74 by the end of the 49-day storage period. In raw chicken, pH values increased from 6.09, to 6.33, and in raw lamb, they decreased from 5.94, to 5.38, over a 35-day storage period (Table 3). The decrease in pH could have had some effect on the lack of growth of *L. monocytogenes*. However, the initial pH of raw pork was higher than the initial pH of raw chicken, and there was growth in raw chicken but not in raw pork. The lowest pH reached was 4.95, which is not low enough to be inhibitory for the growth of *L. monocytogenes*. Therefore, the results suggest that in addition to pH, there may be other factors that affect the growth of *L. monocytogenes* in raw meat and that those factors may be different for meats of different species origin and depending on the physical state of the meat (cooked vs. raw). There were no changes in the pH of the cooked beef and chicken over time. However, pH values of cooked pork decreased from 6.84, to 6.25, from day 0 to day 14 of storage, and pH values for lamb increased from 6.23, to 6.74, over the 14-day storage period (Table 3).

Conclusions

The temperature at which the inoculum was prepared had no major effect on the behavior of the pathogen in raw beef, pork and lamb incubated at 4°C; however, in raw chicken, *L. monocytogenes* cells from inoculum prepared at 37°C multiplied more than did cells from the inoculum prepared at 4°C. Overall, the potential for the psychrotrophic pathogen *L. monocytogenes* to grow, in meats stored at 4°C, will depend at least on species and state (raw vs. cooked) of the meat, pH, microbial competition and temperature of inoculum preparation.

References

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Table 1. *Listeria monocytogenes* populations (log CFU/g; mean ± s.d.) in vacuum-packaged raw patties of ground beef, pork, lamb and chicken inoculated with cells grown at 4 or 37°C, during storage at 4°C.

Type of meat	Inoculum preparation temperature (°C)	Storage Time (Days) 0	7	14	21	28	35	49
Beef	4	5.3±0.1	4.2±0.1	4.3±0.3	3.9±7.0	3.7±0.1	3.6±0.0	3.6±0.2
	37	4.1±0.1	3.6±0.4	3.5±0.2	3.6±0.6	3.0±0.1	2.9±0.2	3.1±0.2
Pork	4	5.5±0.1	5.3±0.1	4.9±0.1	5.2±0.0	5.0±0.1	5.0±0.2	5.0±0.2
	37	4.2±0.0	4.6±0.1	3.9±0.6	4.4±0.0	4.1±0.3	4.3±0.0	4.2±0.1
Lamb	4	5.6±0.1	5.9±0.1	5.2±0.4	5.6±0.2	5.4±0.0	5.6±0.3	5.0±0.2
	37	4.1±0.1	4.6±0.1	3.9±0.3	4.4±0.8	4.1±0.1	3.4±0.5	3.6±0.0
Chicken	4	6.6±0.1	7.7±0.0	7.6±0.1	7.4±0.0	7.6±0.1	7.4±0.1	7.3±0.3
	37	4.2±0.2	6.0±0.5	6.7±0.0	6.5±0.2	6.8±0.1	6.7±0.2	6.8±0.5

Table 2. *Listeria monocytogenes* populations (log CFU/g; mean ± s.d.) in cooked, inoculated (inoculum preparation temperature: 37°C), vacuum-packaged patties of ground beef, pork, lamb and chicken during vacuum storage at 4°C.

Type of meat	Storage Time (Days) 0	3	7	14
Beef	6.3±0.1	6.7±0.1	7.2±0.1	7.8±0.4
Pork	6.9±0.2	7.2±0.4	8.2±0.1	8.3±0.1
Lamb	6.9±0.1	7.1±0.1	8.1±0.0	8.4±0.0
Chicken	6.5±0.2	7.2±0.0	8.0±0.6	8.3±0.0

Table 3. Values of pH of raw and cooked patties of ground beef, pork, lamb and chicken during storage at 4°C.

Type of meat	State of Meat	Time (Days) 0	3	7	14	21	28	35	49
Beef	Raw	5.59±0.00	----[a]	5.19±0.01	5.12±0.04	4.95±0.02	4.97±0.03	4.95±0.02	5.80±0.01
	Cooked	5.84±0.00	5.85±0.01	5.85±0.01	5.80±0.01	----	----	----	----
Pork	Raw	6.17±0.00	----	6.08±0.01	6.01±0.12	5.76±0.04	5.88±0.20	5.80±0.02	6.74±0.00
	Cooked	6.84±0.04	6.84±0.00	6.81±0.00	6.25±0.08	----	----	----	----
Lamb	Raw	5.94±0.00	----	5.54±0.02	5.48±0.02	5.40±0.11	5.35±0.04	5.38±0.02	6.25±0.08
	Cooked	6.23±0.04	6.21±0.03	6.36±0.00	6.74±0.08	----	----	----	----
Chicken	Raw	6.09±0.00	----	6.37±0.02	6.20±0.04	6.17±0.04	6.19±0.04	6.33±0.16	6.24±0.10
	Cooked	6.25±0.02	6.24±0.03	6.23±0.01	6.24±0.10	----	----	----	----

[a]Not done.