THERMAL DESTRUCTION OF LISTERIA MONOCYTOGENES IN MEAT PRODUCTS

J.N. SOFOS, G.R. SCHMIDT, N. BOONMASIRI AND L. YEN

Departments of Animal Sciences and Food Science and Human Nutrition, Colorado State University, Ft. Collins, CO 80523 USA

SUMMARY

Various experiments were designed to examine the extent of thermal destruction of *Listeria monocytogenes* in uncured and cured ground pork of variable fat (5-31%) and moisture (25-50% water added) contents cooked to different temperatures (50-67°C). Although variable fat and moisture levels affected heating rates, cooking to constant final temperatures resulted in similar extent of thermal destruction in low fat as well as high moisture formulations. Curing ingredients reduced extent of thermal destruction in lean as well as fat- and water-added formulations cooked to 62°C, but when the ground pork products were cooked to 67°C, destruction of *L. monocytogenes* exceeded 6 logCFU/g in uncured as well as cured treatments.

INTRODUCTION

Listeria monocytogenes is a pathogen of increasing concern for the meat industry because it has caused some recent foodborne infection outbreaks fatal to sensitive individuals. There are at least two documented cases of listeriosis from consumption of contaminated poultry products, one being materno-fetal and the other affecting an immunocompromised cancer patient (Johnson et al., 1990; Miller et al., 1990). Studies from throughout the world have demonstrated that 0-90% of fresh and processed muscle food samples examined were contaminated with *L. monocytogenes*. Epidemiological anad microbiological surveys conducted by the United States Center for Disease Control (Schuchat et al., 1992; Pinner et al., 1992) implicated meat products, such as undercooked chicken and delicatessen products, as causes of sporadic listeriosis.

Because the organism is ubiquitous and can cause fatal foodborne infections, there is obviously a need for its control in meat products. Concerns over *Listeria* in meat products are even greater if we consider that as a psychrotrophe, it can multiply at temperatures as low as 0°C; that it is resistant to sodium chloride; and that it is more heat resistant than other nonspore-forming pathogens such as *Salmonella* (Mackey and Bratchell, 1989; Mackey et al., 1990; Boyle et al., 1990; Farber and Peterkin, 1991; Ryser and Marth, 1991; Yen et al., 1991). Its heat resistance increases with increasing temperatures of culture propagation; presence of curing ingredients; and heat shocking/tempering (48°C) or slow heating (Mackey et al., 1990; Bhaduri et al., 1991; Yen et al., 1991, 1992; Farber and Brown, 1990; Quintavalla and Campanini, 1991). These effects may be of particular importance in slowly cooked meat products, such as sous vide items. Because of these factors, and since low-fat, high-moisture meat products are preferred by health-conscious consumers in recent years, the objective of this study was to evaluate the effect of variable fat, water and cure contents, and temperature of cooking on thermal destruction of *L. monocytogenes* in ground pork.

MATERIALS AND METHODS

Strains of *L. monocytogenes* used in this study as a mixed culture included Scott A (human isolate), Brie-1 (Brie cheese isolate), F5027 (raw milk isolate), F5069 (milk isolate), LM-103M (sausage isolate), V7 (raw milk isolate), and V37 CE (raw milk isolate). The original cultures were provided by Drs. Catherine W. Donnelly, Michael Doyle, Jeffrey Farber and Irene V. Wesley. Initial inoculum was prepared from each culture in trypticase soy broth (TSB, Difco Laboratories, Detroit, MI) at 30°C for 24 hr. Equal volumes of all 24-hr cultures were then mixed and the initial population was estimated with a spectronic-20 colorimeter at 525 nm and confirmed by plating on trypticase soy agar (TSA, Difco).

Pork meat and fat of each experiment were ground separately, first through a 0.8 cm plate, and then through a 0.2 cm plate. Appropriate amounts of lean and fat were mixed as necessary to formulate batches for each treatment. Additional treatments were formulated by adding water (25-50%) to the meat, or a meat curing mixture consisting of 2.5% sodium chloride, 1% dextrose, 0.055% sodium erythorbate and 0.015% sodium nitrite. Before being inoculated, all treatments were subjected to pH determination and moisture and fat analysis.

After the temperature reached approximately 4°C, the meat of each treatment was inoculated with the mixed culture. Treatments

of various experiments were inoculated with 10^4-10^8 cells/g. Uninoculated controls were also included to check natural microflora and its destruction by heat. Each treatment consisted of 1485 g of meat and 15 ml of cell suspension, mixed mechanically (Kitchen Aid® mixer, model K45SS, Hobart Corp., Troy, OH) for 3 min. Inoculated samples and uninoculated controls were then stuffed (140 g) into baby food jars (6 cm inside diameter, 7 cm inside height). All sample jars were kept in a refrigerator (4°C) for 49 h to let *L. monocytogenes* establish itself in the meat before thermal processing. Each experiment was replicated twice.

Initial counts of *L. monocytogenes* were determined by surface plating on LPM (lithium chloride phenylethanol moxalactam) agar, LPM plus tellurite (LPMT), MVJ (modified Vogel Johnson) agar, and MPEF (mannitol-phenol red-esculin-ferric ammonium citrate) agar (Miller et al., 1990; Yen et al., 1991, 1992). Since no major differences were detected among counts recovered by different media, the results were combined and their averages are presented for each treatment.

The samples in the jars were heated by immersing in a water bath, the temperature of which was set 2-20°C higher than the target internal temperatures. Thermocouples were set in the center of jars of all treatments to record the internal meat temperatures while heating. Samples (two jars per treatment) were removed from the water bath to analyze for viable *L. monocytogenes* cells when the target internal sample temperatures were reached. All samples were cooled in an ice bath and plating for detection of viable cells was initiated immediately after cooling.

All cooked samples, including controls, were subjected to the enumeration procedure described above for uncooked samples. Twenty-five grams of sample were taken from the middle of the jar and put into a sterile stomacher bag. Phosphate buffer solution (225 ml) was added to each bag and the sample was stomached for two min. Suitable dilutions were made and surface plated on each selective medium. All plates were incubated at 35°C for 24-48 h and checked for colonies of *L. monocytogenes*. Samples of heated meat that yielded no *L. monocytogenes* colonies by direct plating on the selective media were enriched according to USDA procedures, using UVM as the primary enrichment broth, Fraser broth for secondary enrichment and MOX as the selective agar (McLain and Lee, 1988; Yen et al., 1991, 1992). Representative colonies from all media were picked and grown in TSB before being subjected to confirmation tests including Gram stain, catalase, beta-hemolysis, CAMP test and motility at 21°C (Lachica, 1990).

RESULTS AND DISCUSSION

The results of the study are presented in Tables 1-4. Increasing the average fat content of uncured ground pork from 5-7% to 31.1% resulted in a need for the heating time to be increased by 3.4, 3.6 and 4.2 min to reach the target temperatures of 50, 55 and 60°C. After each target temperature was reached, however, extent of thermal destruction of *L. monocytogenes* cells was similar ($P > 0.05$) between low (5.7%) and high (31.1%) fat products (Table 1). The extent of destruction was 0.52-0.57, 1.26-1.46 and 4.65-4.72 logCFU/g at 50, 55 and 60°C, respectively. The average pH of all products was 6.15 ± 0.06. These results indicated that if a product is heated to a given final temperature, extent of thermal destruction of *L. monocytogenes* should be similar, irrespective of fat content in the formulation. Thus, since low fat formulations cook faster, they should be as effective in *L. monocytogenes* elimination as higher fat products.

Table 1. Effect of fat content in uncured ground pork cooked to different temperatures on extent of thermal destruction of *L. monocytogenes*.[a]

Fat (%)	Water bath (°C)	Final product (°C)	Time to reach temperature (min)	Reduction of *L. monocytogenes* (logCFU/g)
5.7	70	50	24.8	0.57 ± 0.18b
	70	55	29.4	1.46 ± 0.44c
	70	60	36.4	4.65 ± 0.70d
31.1	70	50	29.0	0.52 ± 0.20b
	70	55	32.8	1.26 ± 0.17c
	70	60	40.0	4.72 ± 0.39d

[a]Initial inoculum: 6.49 ± 0.04 logCFU/g; pH: 6.15 ± 0.06.
bcd - Significantly different ($P < 0.05$).

With addition of 25% water to low fat (6.2%) uncured ground pork, the average time to reach a given temperature (60, 63, 66°C) decreased from 51.2, 54.4 and 54.5 min to 46.4, 49.6 and 50.6 min, respectively (Table 2). Extent of destruction of the pathogen,

however, was similar and exceeded 4 logCFU/g at 60°C and 7 log CFU/g at 63 and 66°C, respectively. Thus, addition of water reduces the cooking time but extent of destruction of the pathogen in uncured ground pork is still extensive at temperatures above 60°C (Table 2).

Addition of curing ingredients to meat has significantly reduced the extent of destruction of *L. monocytogenes* by heat (Farber, 1989; Mackey et al., 1990; Yen et al., 1991, 1992). The results of this study confirmed that curing ingredients significantly ($P < 0.05$) reduced extent of thermal destruction in lean (5%), water-added (50%) or fat-added (50%) cured ground pork compared to lean, uncured product (Table 3). Cooking to 60°C destroyed 3.91 log CFU/g in lean, uncured pork, while in cured formulations, the extent of destruction was 1.17-2.52 logCFU/g.

Table 2. Effect of water content in uncured ground pork (6.2% fat) cooked to different temperatures on extent of thermal destruction of *Listeria monocytogenes*.[a]

Water added (%)	Water bath (°C)	Final product (°C)	Time to reach temperature (min)	Reduction of *Listeria monocytogenes* (log CFU/g)
0	62	60	51.2	4.75 ± 0.04
	65	63	54.4	> 7.00b
	68	66	54.5	> 7.00
25	62	60	46.4	4.77 ± 0.00
	65	63	49.6	> 7.00
	68	66	50.6	> 7.00

[a]Initial inoculum: 8.00 ± 0.25 log CFU/g; pH: 6.14 ± 0.04.
b - Undetected by direct plating or after enrichment.

When ground pork of intermediate fat (14.3%) was heated to 62°C over a period of 53.0 min, extent of destruction of *L. monocytogenes* was 6.46 and 4.36 logCFU/g for uncured and cured product, respectively (Table 4). When the same products were cooked to 67°C, however, thermal destruction was extensive (> 6 logCFU/g) in uncured as well as cured products. Thus, the protective effect of the curing agents against thermal destruction of *L. monocytogenes* appears to be significant only at lower temperatures (≤ 62°C), while at higher cooking temperatures (e.g., 67°C), thermal destruction is extensive and differences between cured and uncured products may be negligible.

Table 3. Effect of curing ingredients on thermal destruction of *Listeria monocytogenes* in lean, water-added and fat-added ground pork.[a]

Treatment	Water bath (°C)	Final product (°C)	Time to reach temperature (min)	Reduction of *Listeria monocytogenes* (log CFU/g)
Lean (5% fat)	62	60	11.4	3.91 ± 0.11b
Lean + cure	62	60	16.2	2.47 ± 0.33c
Lean + 50% water + cure	62	60	12.1	1.97 ± 0.18c
Lean + 50% fat + cure	62	60	14.5	2.52 ± 0.34c

[a]Initial inoculum: 7.28 ± 0.15 logCFU/g; pH: 6.21 ± 0.03. Cure: 2.5% sodium chloride, 1% dextrose, 0.055% sodium erythorbate and 0.015% sodium nitrate.
bc - Significantly different ($P < 0.05$).

Table 4. Effect of curing ingredients on thermal destruction of *Listeria monocytogenes* in ground (14.3% fat) pork cooked to different temperatures.[a]

Product type	Water bath (°C)	Final product (°C)	Time to reach temperature (min)	Reduction of *Listeria monocytogenes* (log CFU/g)
Uncured	65	62	53.0	6.46 ± 0.04b
Cured	65	62	53.0	4.36 ± 0.11c
Uncured	70	67	50.5	> 6.00b
Cured	70	67	50.5	> 6.00b

[a]Initial inoculum: 7.36 ± 0.13 logCFU/g; pH: 6.41 ± 0.07. Cure: 2.5% sodium chloride, 1.0% dextrose, 0.055% sodium erythorbate and 0.015% sodium nitrite.
bc - Significantly different ($P < 0.05$).

CONCLUSION

The results of the studies reported here have indicated that: 1) ground pork with reduced fat is heated faster than pork with more fat, but extent of destruction of *L. monocytogenes* is similar when a target cooking temperature is reached; b) addition of water

to low fat ground pork reduces cooking time to reach a target temperature, but it does not affect extent of thermal destruction of *L. monocytogenes*; c) addition of curing ingredients reduces extent of thermal destruction of *L. monocytogenes* at 62°C in lean, water-added and fat-added ground pork; and d) cooking to higher temperatures (e.g., 67°C) results in extensive (> 6 logCFU/g) destruction of *L. monocytogenes* in both uncured and cured ground pork. In general, thermal destruction of *L. monocytogenes* can be adequate even in low-fat, high-moisture, cured and uncured products, if appropriate rates of heating and final cooking temperatures are established for specific formulations.

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