CHANGES IN PATHOGENIC BACTERIA COUNTS DURING LIMITED ABUSE OF FRESH PORK

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Background

Although public awareness of food safety issues has increased, consumers still continue to abuse their food (McIlvenen et al., 1999; Worsfold and Griffith, 1997). Fresh meat may be exposed to improper holding temperatures during transportation, at loading and unloading points, and in coolers that are not properly operating or insulated. Critical factors affecting the safety and shelf life of fresh pork include initial microbial loads and storage temperatures. Minimizing microbial contamination during product preparation and maintaining proper refrigeration from packing plant to home refrigerator/freezer will help prevent proliferation of pathogenic or spoilage microorganisms. Cabedo et al. (1997) demonstrated that storage at proper temperature can retard growth or inhibit survival of *E. coli* O157:H7 on beef. Proper refrigeration temperatures extend the lag phase and minimize growth of most spoilage and pathogenic bacteria; however, some microorganisms can grow, albeit slowly, at refrigeration temperatures and mesophilic pathogens can survive under refrigeration and grow during temperature abuse of food (Marth, 1998). Psychrotrophic (growth below 5°C) and mesophilic growth at 10-45°C) pathogens that could grow during extended refrigeration and temperature abuse, respectively (Marth, 1998) are food-safety concerns. Major efforts are undertaken in recent years to enhance the safety of foods such as pork products. A major component of this effort to enhance food safety is development of quantitative risk assessment models (Cassin et al., 1998; Lammerding and Paoli, 1997). Such models are useful in determining risks and applicable critical control points that can be managed through the hazard analysis critical control point (HACCP) system (Buchanan and Whiting, 1998; NACMCF, 1998).

Objectives

This study investigated the fate of *Campylobacter jejuni, Escherichia coli* O157:H7, *Salmonella* spp., *Yersinia enterocolitica* and *Listeria monocytogenes* inoculated on fresh ground pork and pork loin chops that were stored for 24 or 48 h at 0°C (not abusive) or at three progressively more abusive temperatures of 3, 7 or 10°C.

Methods

Pork center-cut loins were sliced to a 2.5 cm thickness with a meat slicer (Hobart Mfg. Co., Troy, OH), cut into 4 cm wide and 8 cm long pieces and placed in individual, plastic bags. Pork trim was ground (0.3 cm plate) in a grinder (Hobart Mfg. Co., Troy, OH) and 100 g portions were placed in individual plastic bags. Pathogens studied were *L. monocytogenes* (four pork variety meat isolates, LCDC 81861 and Scott A), *Salmonella* (group B, *S. enteritidis*, four pork carcass isolates and two pork variety meat isolates), *Y. enterocolitica* (one pork variety meat isolate and ATCC 51871), *C. jejuni* (five pork variety meat isolates) and *E. coli* O157:H7 (ATCC 43895, 43888, 43889, 43890, 51657, 51658). Each pathogen strain was incubated individually in tryptic soy broth with 0.6% yeast extract at 37°C for 24 h, except for of *C. jejuni* which was incubated at 42°C in a microaerophilic environment. Pathogen strains were combined in a sterile container and serially diluted in 9 ml Butterfield's Phosphate Buffer to obtain the desired inoculum level for inoculation of products. Each sample was inoculated with 10^3- 10^5 CFU/cm^2 (chops) or CFU/g (ground) pathogen (each sample was inoculated with one pathogen). After inoculation, bagged samples were divided appropriately among four incubators (0, 3, 7 or 10°C) and stored for 24 or 48 h.

Samples (two per treatment in each of three replicates) were analyzed at 0 h (immediately after inoculation to determine the inoculum level), and 24 or 48 h following storage at 0, 3, 7 or 10°C. For analysis, 100 ml of Butterfield's Phosphate Buffer was added to each sample and the samples were shaken in a 30 cm arc, 30 times (chops) or stomached (IUL Instruments, Barcelona, Spain) for 2 min (ground). The ground sample was diluted by adding 10 g of the previously generated slurry to 40 ml of sterile Butterfield's Phosphate Buffer and stomached for 2 min to yield the initial 1:10 dilution. Each sample was serially diluted in 9 ml sterile Butterfield's Phosphate Buffer . Each sample was plated on a general growth medium (TSA), lactic acid bacteria medium (MRS), 3M™ Total Coliform Count Petrifilm™, and selective agar media appropriate for each organism by depositing 0.1 ml of three consecutive dilutions on duplicate plates and spreading the sample with a sterile, bent glass rod. Plates were inverted and incubated at 25 (aerobically), 35 (aerobically), or 42°C (microaerophilic environment) depending on the temperature appropriate for the organism. Colonies were counted following incubation after 24 or 48 h and results expressed as log CFU/cm^2 (chops) or log CFU/g (ground) for calculation of the means and standard deviations.

Results and Discussion

Bacterial populations on inoculated pork chops (Table 1) remained relatively constant during storage at 3, 7 or 10°C for 24 or 48 h, with the exception of populations on chops inoculated with *Salmonella* and stored for 48 h at 10°C (increase of 0.6 log) or *Y. enterocolitica* and stored for 48 h at 7°C (increase of 1.1 log) or 10°C (increase of 0.5 log).. Storage of inoculated ground pork for 24 h at all temperatures allowed increases (from 1.3 to 2.6 log) in counts in samples inoculated with *C. jejuni* but lesser increases (0.4 to 1.0 log) occurred at 48 h (Table 2). Counts in ground pork inoculated with *Y. enterocolitica* increased (by 0.9 log at 6.7°C for 24 h and 1.6 log at 6.7°C for 48 h and by 1.1 log at 10°C for 24 h and by 2.1 log at 10°C for 48 h) in response to mild temperatures abuse, while counts in ground pork inoculated with *L. monocytogenes* and *Salmonella* remained relatively constant in spite of the mild temperature abuse. Counts in ground pork inoculated with *E. coli* O157:H7 increased (especially at 48 h) when stored at 3-10°C, but this may reflect growth of non-*E. coli* sorbitol negative bacteria. Pathogen survival and growth at 3-10°C was greater in ground pork (bacteria dispersed throughout the product) than on pork chops (bacteria present only on the product surface); counts increased by 1.0

log or more in 11 of 30 comparisons for ground pork but only 1 of 30 comparisons for pork chops, in response to mild temperature abuse.

Table 1: Mean [log CFU/cm^2 (SD)] bacterial counts on pork chops inoculated with *Campylobacter jejuni, Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* spp. or *Yersinia enterocolitica* and plated on selective agar media during aerobic storage at 0, 3, 7 or 10°C for 24 or 48 h.

			Bacterial Counts [log CFU/cm^2 (SD)]				
Temperature (°C)	Time (h)	N	*Campylobacter jejuni* (MCCDA)	*Escherichia coli* O157:H7 (SMAC)	*Listeria monocytogenes* (PALCAM)	*Salmonella* (XLT4)	*Yersinia enterocolitica* (CIN)
--	0	6	4.6 (0.9)	5.3 (0.4)	4.9 (0.1)	5.1 (0.4)	4.9 (0.1)
0	24	6	4.5 (1.4)	5.2 (0.4)	4.6 (0.4)	4.6 (0.3)	4.4 (0.6)
0	48	6	4.3 (1.7)	5.3 (0.0)	4.8 (0.1)	5.2 (0.6)	4.5 (0.2)
3	24	6	3.9 (1.8)	5.1 (0.3)	4.8 (0.2)	4.6 (0.3)	4.7 (0.4)
3	48	6	4.5 (1.4)	4.9 (0.8)	4.8 (0.1)	4.7 (0.6)	4.9 (0.5)
7	24	6	5.2 (0.3)	5.2 (0.5)	5.0 (0.2)	4.6 (0.6)	4.5 (0.4)
7	48	6	4.9 (0.6)	5.3 (0.4)	4.7 (0.4)	4.9 (0.5)	6.0 (0.6)
10	24	6	5.1 (0.2)	5.6 (0.3)	4.9 (0.1)	4.9 (0.4)	5.0 (0.7)
10	48	6	4.4 (1.2)	5.6 (0.5)	5.0 (0.3)	5.7 (0.5)	5.4 (0.8)

MCCDA = Modified Campylobacter Charcoal Differential Agar, SMAC = Sorbitol MacConkey Agar, XLT4 = Xylose Lysine Tergitol 4 Agar, CIN = Yersinia Selective Agar.

Conclusions

Mild temperature abuse (up to 7-10°C for as long as 24-48 h) resulted in increased counts (of 1.0 log or more) of *Campylobacter jejuni* (in 3 of 12 comparisons), *E. coli* O157:H7 (in 2 of 12 comparisons), *Listeria monocytogenes* (in 0 of 12 comparisons), *Salmonella* (in 0 of 12 comparisons) and *Yersinia enterocolitica* (in 5 of 12 comparisons), confirming the need to minimize exposure of pork products to microbial contamination during product preparation and maintenance of proper refrigeration temperatures during transportation and storage of fresh pork. The results of this study should be useful in the development of quantitative risk assessment models which are needed for more targeted control of hazards to improve food safety.

Table 2: Mean [log CFU/cm^2 (SD)] bacterial counts in ground pork inoculated with *Campylobacter jejuni, Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* spp. or *Yersinia enterocolitica* and plated on various selective agar media during aerobic storage at 0, 3, 7 or 10°C for 24 or 48 h.

			Bacterial Counts [log CFU/cm^2 (SD)]				
Temperature (°C)	Time (h)	N	*Campylobacter jejuni* (MCCDA)	*Escherichia coli* O157:H7 (SMAC)	*Listeria monocytogenes* (PALCAM)	*Salmonella* (XLT4)	*Yersinia enterocolitica* (CIN)
--	0	6	2.1 (1.8)	5.9 (1.4)	4.4 (0.6)	4.0 (0.7)	4.1 (0.6)
0	24	6	4.4 (0.3)	6.2 (1.3)	4.4 (0.8)	3.9 (0.7)	4.3 (1.1)
0	48	6	2.5 (1.2)	6.3 (1.2)	4.5 (0.8)	4.0 (0.7)	4.6 (0.5)
3	24	6	4.4 (0.4)	6.3 (1.3)	4.6 (0.7)	4.0 (0.7)	4.8 (1.1)
3	48	6	2.5 (1.4)	6.7 (0.9)	4.3 (0.9)	4.0 (0.9)	5.3 (0.4)
7	24	6	3.4 (2.0)	6.6 (1.1)	4.5 (0.6)	4.1 (0.5)	5.0 (0.7)
7	48	6	3.1 (1.4)	7.2 (1.2)	4.2 (1.1)	4.2 (0.8)	5.7 (0.7)
10	24	6	4.7 (0.7)	6.5 (1.5)	4.7 (0.4)	4.6 (0.4)	5.2 (1.0)
10	48	6	2.6 (1.9)	7.0 (1.0)	4.3 (1.1)	4.3 (0.4)	6.2 (0.6)

MCCDA = Modified Campylobacter Charcoal Differential Agar, SMAC = Sorbitol MacConkey Agar, XLT4 = Xylose Lysine Tergitol 4 Agar, CIN = Yersinia Selective Agar.

Pertinent Literature

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