

Changes in Inoculated Bacterial Pathogens on Fresh Pork Stored at Temperatures to Simulate Mild Distribution Abuse

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SUMMARY

Fresh meat products may be exposed to improper holding temperatures during transportation, at loading and unloading points, and in coolers that are not properly operating or insulated. A study was designed to investigate 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 subjected to 24 or 48 h of storage at 32°F (not abusive) or at three progressively more abusive temperatures of 38, 44 or 50°F. Bacterial populations in inoculated samples remained relatively constant or increased slightly, with samples inoculated with *Y. enterocolitica* showing higher bacterial counts than other samples. This emphasizes the importance of minimizing microbial contamination at the production stage as well as maintenance of proper refrigeration temperature during transportation and storage of fresh pork.

Key Words: pork, contamination, transportation, storage

INTRODUCTION

Critical factors that affect the safety and shelf life of perishable food products include storage temperature as well as the initial number of pathogenic and spoilage microbes. Therefore, efforts should be made to minimize microbial contamination during production as well as maintaining proper refrigeration temperature during processing, transportation/distribution and display of product. Improper storage and distribution conditions of potentially contaminated fresh pork may promote proliferation of pathogenic or spoilage microorganisms causing serious

deterioration of products, thereby increasing the potential of food-borne outbreaks. Although several steps in the food processing chain can influence the likelihood of causing food-borne illness (Cabedo et al., 1997), one important step that needs to be controlled is storage temperature. Refrigeration is normally considered to provide a useful hurdle in retarding growth or inhibiting survival of bacteria; and failure to maintain proper refrigeration temperature during transportation and storage constitutes product abuse. Proper refrigeration temperatures extend the lag phase of microorganisms, minimizing growth and preserving the products for limited times. Some microorganisms can grow, albeit slowly, at refrigeration temperatures and mesophilic pathogens can survive under refrigeration and grow during any temperature abuse of the food (Marth, 1998). The chief microbiological concerns associated with food safety center upon psychrotrophic (organisms that grow at refrigerated temperatures below 5°C) and mesophilic (organisms that grow at higher temperatures 10-45°C) pathogens that could grow during extended refrigeration and temperature abuse, respectively (Marth, 1998). Assessment of the response of specific pathogenic microorganisms to temperature abuse, as could occur during mishandling of potentially contaminated products, should be performed. This study was conducted to determine changes in bacterial counts in fresh pork products inoculated with *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., *Yersinia enterocolitica* or *Listeria monocytogenes* during non-abusive and abusive temperature conditions chosen to simulate circumstances likely to occur during transportation, distribution and holding storage.

Materials and Methods

Pork center-cut loins were sliced to a 2.5 cm thickness with a meat slicer (Hobart Mfg. Co., Troy, OH) then cut into 4 cm wide, 8 cm long pieces and placed into individual, plastic bags. Pork trim was processed into ground

pork using a 1/8 inch plate grinder (Hobart Mfg. Co., Troy, OH) and divided into 100 g portions in individual plastic bags. The inoculated pathogens included *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 individual pathogen strain was incubated individually in tryptic soy broth with 0.6% yeast extract at 37°C for 24 h, with the exception of *Campylobacter* which was incubated at 42°C in a microaerophilic environment. The pathogen strains were then combined in a sterile container and serially diluted in 9 ml Butterfield's Phosphate Buffer to obtain the desired inoculum level for inoculation of the product. Each sample was inoculated with approximately 10³- 10⁵ Colony Forming Units (CFU)/cm² (chops) or CFU/g (ground) of each of the pathogens (each sample was inoculated with one pathogen). After inoculation, bagged samples were divided appropriately among four incubators calibrated at four different temperatures (32, 38, 44 and 50°F) and stored for 24 and 48 h.

Samples (two per treatment in each of three replicates) were analyzed at 0 h (immediately after inoculation to determine the inoculum level in the samples), and 24 and 48 h following storage at 32, 38, 44, and 50°F temperatures. 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 further 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 then serially diluted in 9 ml sterile Butterfield's Phosphate

Buffer . Each sample was then 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 then 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 24-48 h and results expressed as log CFU/cm² (chops) or log CFU/g (ground) for calculation of the means and standard deviations.

RESULTS

Bacterial populations of inoculated pork chop samples remained relatively constant during storage at various temperatures (32, 38, 44 and 50°F) for 24 or 48 h, with the exception of populations on *Salmonella* inoculated samples stored for 48 h at 50°F and *Y. enterocolitica* inoculated samples stored for 48 h at 44 and 50°F (Table 1).

Storage of inoculated ground pork for 24 h at all temperatures appeared to allow increases in bacterial counts in samples inoculated with *C. jejuni* but populations were reduced after 48 h (Table 2). Populations of bacteria in samples inoculated with *Y. enterocolitica* increased at 44 and 50°F, while those of *L. monocytogenes* and *Salmonella* remained relatively constant. Bacterial counts on samples inoculated with *E. coli* O157:H7 appeared to have increased at 38-50°F, but may reflect growth of non-*E. coli* sorbitol negative bacteria. Pork chops were inoculated on their surface whereas the organisms were dispensed on and within the ground pork samples. Therefore, ground pork could have provided some form of buffer to the organism embedded within the product increasing the chances of pathogen survival and growth.

IMPLICATION

Results of this study reveal that, with one exception (*Y. enterocolitica*) short-term (24-48 h), mild temperature abuse of pork chops (44-50°F) is not likely to cause proliferation of meatborne pathogens, but it should be noted that they retained their viability. This study emphasizes the importance of minimizing microbial contamination at the production stage as well as maintenance of proper refrigeration temperature during handling, and should be useful in risk assessment studies for enhancement of food safety. The results also demonstrate the importance of consumer education with respect to safe food handling practices.

REFERENCES

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Table 1: Mean [log CFU/cm² (SD)] bacterial counts in pork chops 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 32, 38, 44 or 50°F for 24 or 48 h.

		Bacterial Counts [log CFU/cm ² (SD)]					
		<i>Campylobacter jejuni</i>		<i>Escherichia coli</i>	<i>Listeria monocytogenes</i>	<i>Salmonella</i>	<i>Yersinia enterocolitica</i>
Temperature (°F)	Time (h)	N	(MCCDA)	O157:H7 (SMAC)	(PALCAM)	(XLT4)	(CIN)
--	0	6	4.6 (0.9)	5.3 (0.4)	4.9 (0.1)	5.1 (0.4)	4.9 (0.1)
32	24	6	4.5 (1.4)	5.2 (0.4)	4.6 (0.4)	4.6 (0.3)	4.4 (0.6)
32	48	6	4.3 (1.7)	5.3 (0.0)	4.8 (0.1)	5.2 (0.6)	4.5 (0.2)
38	24	6	3.9 (1.8)	5.1 (0.3)	4.8 (0.2)	4.6 (0.3)	4.7 (0.4)
38	48	6	4.5 (1.4)	4.9 (0.8)	4.8 (0.1)	4.7 (0.6)	4.9 (0.5)
44	24	6	5.2 (0.3)	5.2 (0.5)	5.0 (0.2)	4.6 (0.6)	4.5 (0.4)
44	48	6	4.9 (0.6)	5.3 (0.4)	4.7 (0.4)	4.9 (0.5)	6.0 (0.6)
50	24	6	5.1 (0.2)	5.6 (0.3)	4.9 (0.1)	4.9 (0.4)	5.0 (0.7)
50	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.

Table 2: Mean [log CFU/cm² (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 32, 38, 44 or 50°F for 24 or 48 h.

		Bacterial Counts [log CFU/cm ² (SD)]					
		<i>Campylobacter jejuni</i>		<i>Escherichia coli</i>	<i>Listeria monocytogenes</i>	<i>Salmonella</i>	<i>Yersinia enterocolitica</i>
Temperature (°F)	Time (h)	N	(MCCDA)	O157:H7 (SMAC)	(PALCAM)	(XLT4)	(CIN)
--	0	6	2.1 (1.8)	5.9 (1.4)	4.4 (0.6)	4.0 (0.7)	4.1 (0.6)
32	24	6	4.4 (0.3)	6.2 (1.3)	4.4 (0.8)	3.9 (0.7)	4.3 (1.1)
32	48	6	2.5 (1.2)	6.3 (1.2)	4.5 (0.8)	4.0 (0.7)	4.6 (0.5)
38	24	6	4.4 (0.4)	6.3 (1.3)	4.6 (0.7)	4.0 (0.7)	4.8 (1.1)
38	48	6	2.5 (1.4)	6.7 (0.9)	4.3 (0.9)	4.0 (0.9)	5.3 (0.4)
44	24	6	3.4 (2.0)	6.6 (1.1)	4.5 (0.6)	4.1 (0.5)	5.0 (0.7)
44	48	6	3.1 (1.4)	7.2 (1.2)	4.2 (1.1)	4.2 (0.8)	5.7 (0.7)
50	24	6	4.7 (0.7)	6.5 (1.5)	4.7 (0.4)	4.6 (0.4)	5.2 (1.0)
50	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.