RESEARCH REPORT

PORK SAFETY

Title: Effects of chilling methods for bacterial recovery and reducing bacteria on pork carcasses - **NPB # 00-015**

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I. Abstract

To date, very little information exists on recovery methods for freeze-injured cells and the effect of blast- or conventional-chilling for reducing specific pathogens associated with pork surfaces. To address these issues, two separate studies were conducted to determine 1) an effective recovery method for freeze-injured *Listeria monocytogenes*, *Salmonella* Typhimurium and *Campylobacter coli* associated with pork surfaces and to use this recovery method to 2) determine the effect of blast- versus conventional-chilling methods for reducing pathogens at two bacterial levels (high and low) associated with skin-on and skin-off pork surfaces. In the first study, cell suspensions of *L. monocytogenes, S.* Typhimurium or *C. coli* or cells associated with pork surfaces were subjected to a freeze-thaw cycle (-15ºC, 24 h; 4ºC, 4 h). Following treatments, freeze-injured cells were plated onto media incorporating the following recovery methods: overlay (OV); thin agar layer (TAL); or Lutri plate (LP) method. The recovery rates for the pathogens using the TAL, OV and LP methods following freeze treatments in cell suspensions or on pork surfaces were not statistically different ($P>0.05$) from recovery rates associated with nonselective media. The results also demonstrated that OV and TAL were reliable and consistent recovery methods for isolation of freeze-injured cells. The TAL method was not only easier to perform, but also allowed for improved isolation of single colonies for further biochemical and serological characterization.

In the second study, fecal slurries at inoculation levels of 3 and 5 $\log_{10}$ CFU/cm^2, with and without pathogens (*L. monocytogenes*, *S.* Typhimurium and *C. coli*), were inoculated onto skin-on and skin-off pork surfaces, and subjected to blast- and conventional-chilling using the TAL method developed in the first study. Results indicated there were no statistically significant ($P > 0.05$) differences between blast- or conventional-chilling in reducing APC, total coliforms, *E. coli*, *L. monocytogenes*, *S.* Typhimurium and *C. coli* at a low inoculation level. However, both blast- and conventional-chilling were more effective against bacterial populations when pork samples were inoculated with a high inoculation level (5 $\log_{10}$ CFU/cm^2). Specifically, *C. coli* associated with pork surfaces and subjected to blast-chilling was reduced to undetectable levels. Given the low levels of pathogens associated with

fresh pork products, chilling regimens may provide pork processors with an added measure to improve the microbiological safety of their product.

II.Introduction

The pork industry is committed to producing a high quality, wholesome product that is safe for human consumption. During the slaughtering process, microbial contamination of animal carcasses is unavoidable (Bell, et al., 1986; Chung et al., 1989). This phenomenon represents one of the most critical quality and safety issues faced by the pork industry. While most of the microflora transferred to carcasses during the slaughtering process is non-pathogenic, pathogens such as *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* may be present (Epling et al., 1993).

While it has been demonstrated that chilling can improve the quality and shelf life of pork, very little information exists as to the effect of chilling parameters on the microbiological safety of pork. Since microbial activities are influenced by chemical and enzymatic reactions, the effect of low temperatures on microorganisms is essentially a growth retardation process. Most microorganisms will not grow at freezing temperatures due to reduced metabolic activities; however, these microorganisms will begin to grow again when placed in warmer temperatures (Ingram and Mackey, 1976; Cunningham and Cox, 1987, Mountney and Gould, 1988).

Several reports have demonstrated that chilling the pork extends the lag phase of bacterial growth resulting in lower bacterial load (Troller, 1976; Vanderzant et al., 1985). Using chilling temperatures slightly above ice formation or by freezing to 0 ºC, death or injury to bacteria can occur. Therefore, chilling is an important preservation method for foods (Smith, 1995). It is thought that freezing damages cells by penetration of ice crystals into the cell membrane, resulting in the release of cellular constituents, ultimately causing cellular death (Mazur, 1966). Death of organisms due to chilling also could be a function of a temperature change, especially when no ice crystals are formed (Smith, 1995). Smith (1995) also indicated that while there may be no physical disruption of the bacterial surface membranes, chemical changes may occur in the lipid bilayer of the cells that may result in permanent physical damage.

The pork industry currently utilizes either blast-chilling or conventional-chilling to reduce the temperature of carcasses immediately after slaughter and before fabrication. The most common procedure used by the pork industry is conventional-chilling which consists of placing the pre-rigor pork carcasses in a chiller at 1 to 4ºC with an air velocity of 1.5 to 3 feet/second for 18 to 24 h prior to processing or shipping (Greer and Dilts, 1987; Huff-Lonergan and Page, 2000). While this chilling method is widely adopted, it has several disadvantages, including substantial evaporative weight loss, considerable cooler space requirements, and the potential to increase the incidence of pale soft exudative (PSE) pork (Greer and Dilts, 1987; Crenwelge, et al., 1984). Conversely, conventional-chilling of pork surfaces was found to reduce contamination of *Campylobacter jejuni,* presumably due to the drying process, reduced relative humidity, or oxygen exposure that occurs under forced ventilation (Oosterom, et al., 1983: Bracewell et al., 1985).

Blast-chilling requires an air temperature of – 20 to – 40 ºC with an air velocity of 10 to 16 f/s for 1 to 3 h (Huff-Lonergan and Page, 2000). This method has been shown to reduce shrinkage, drip loss, incidence of PSE and produce a beneficial effect on muscle

appearance with a firmer structure, as compared to conventional-chilling (Greer and Dilts, 1987; Jones, et al., 1987). However, the effect of blast-chilling on the microbiological quality of the pork is debatable. Some researchers have found that blast-chilling reduced levels of carcass contamination and improved keeping quality (Dann, 1972; Price et al., 1976; Wernberg, 1972), whereas Greer (1987) and Dilts (1988) found that blast-chilling did not improve the quality of the pork product.

Previous published reports also demonstrate some disparity in the reported efficacy of blast-chilling compared to conventional-chilling. Cooper (1968) and James et al. (1983) found no differences using conventional-chilling or blast-chilling to reduce bacterial populations. Greer and Dilts (1987) examined mesophilic and psychrotrophic bacterial populations on carcasses, loins, and loin chops under retail display following conventional- and blast-chilling. These researchers demonstrated that chilling treatment had no effect on odor, appearance, or shelf life of fresh pork products and that blast-chilling could be applied without compromising the bacterial quality of pork products (Greer and Dilts, 1987). Carr et al (1998) established that populations of *Staphylococcus* spp. and lactic acid bacteria were not affected by either chilling treatment except when no fat trimming was used; APC and coliforms counts were not significantly different when chilling methods were compared. It has been hypothesized that the minimum growth temperature of 10 ºC may contribute to the reduced growth of *Staphylococcus* spp. and other bacteria on carcasses (Troller, 1976). Since inherent differences exist between the protocols, this research project will attempt to determine whether variables such as surface type, bacterial level, and chilling method affect bacterial reduction and pathogen recovery under laboratory controlled conditions.

Objectives

1. To determine recovery method (s) for pathogens associated with cell suspensions and pork surfaces following conventional-chilling.
2. Use the most efficient recovery method to determine whether conventional- or blast-chilling efficiently reduces bacterial levels associated with fecal contamination and pathogen contamination on skin-on and skin-off pork surfaces.

III. Procedures

Culture Storage and Preparation

Salmonella Typhimurium ATCC 14028 (American Type Culture Collection, Manassas, VA), *Campylobacter coli* ATCC 33559, and *Listeria monocytogenes* Scott A were obtained from the Food Microbiology Culture Collection at Pennsylvania State University. *S.* Typhimurium and *L. monocytogenes* were routinely grown aerobically and statically in tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) at 37ºC for 24 h and stored in TSB containing 10% glycerol at -76°C. *C. coli* was grown in Brucella broth (BB, Difco) at 42ºC for 48 h under microaerophilic conditions using anaerobic jars (BBL, Becton Dickinson, MD) and CampyPak Plus generating envelopes (BBL) and stored in BB containing 10% glycerol at -76ºC. Cells were washed and diluted in buffered peptone water (BPW, Difco) prior to experiments.

Media

Brucella agar, (Difco) was chosen as a non-selective medium, while *Campylobacter* Blood-free selective medium (modified CCDA-Preston) Agar (mCCDA; Oxoid, Ltd., Basingstoke, England) was used as selective media for isolation and recovery of *C. coli*. Trypticase soy agar (TSA, Difco) was used as the non-selective medium, whereas Xylose Lysine Decarboxylase agar (XLD, Difco) and Modified Oxford agar (MOX, Difco) were chosen as the selective media for *S.* Typhimurium and *L. monocytogenes,* respectively. Preliminary experiments indicated that the selective media were specific to the pathogens tested since none were able to grow on any of the other selective media.

Thin Agar Layer (TAL) Recovery Method

The TAL method consisted of pre-pouring one layer of selective media (20 ml) into a petri dish, allowing for solidification, followed by the addition of two layers, 7 ml each, of non-selective media (Kang and Fung, 2000). By pouring two separate layers of non-selective media onto the selective medium, the diffusion of the selective agents was retarded by a physical barrier generated by the two separate layers. Samples containing freeze-injured cells were inoculated onto the non-selective media layer and incubated for 24 to 48 h at 37ºC for isolation of *S.* Typhimurium and *L. monocytogenes*, and for 48 h at 42ºC under microaerophilic conditions for isolation of *C. coli.*

Overlay (OV) Recovery Method

The OV method required the inoculation of injured bacteria on top of 20 mls of solidified, non-selective media, incubation for 3 h, followed by the addition of selective media (10 mls), which was poured directly over the inoculated surface (Kang and Fung, 1999). By pouring selective media over recovered cells, the direct contact of selective agents allowed for isolation of specific microorganisms. For *S.* Typhimurium and *L. monocytogenes,* plates were incubated aerobically at 37°C for 21 h. *C. coli* were grown under microaerophilic conditions at 42°C for 45 h.

Lutri plate (LP) Recovery Method

The LP method consisted of using a specially designed lutri plate (Starkville, Miss), consisting of dual chambers. After solidification of 20 ml of non-selective agar, samples containing injured bacteria were inoculated directly onto the solidified media in compartment A, incubated at the appropriate temperature for 3 h, at which time, the divider was removed and 40 ml of selective agar poured into compartment B. During incubation, the selective agar diffuses across the grid system into the non-selective agar (Kang and Siragusa, 1999). For *S.* Typhimurium and *L. monocytogenes,* Lutri plates were held at 37°C for 21 h while *C. coli* was grown under microaerophilic conditions at 42°C for 45 h.

Recovery of Injured Cells from Suspensions

For each of the pathogens tested, serially diluted overnight cultures were added to test tubes containing 9 ml of BPW. Two test tubes were subjected to the freeze treatment and one tube was used as the control (no treatment). Two test tubes were placed in a conventional freezer (Kenmore, Ontario, Canada) at –15 °C for 24 h and thawed at 4°C for

4 h. Cultures were serially diluted in BPW and spread plated onto non-selective media, selective media, TAL, OV, or LP. The inoculated plates were incubated at the appropriate time and temperature as stated above, based on the recovery method and pathogen. After incubation, all plates were counted manually.

Recovery of Injured Cells from Pork Surfaces

Based on the findings from experiments using cell suspensions, the TAL method was chosen as the recovery method of choice for subsequent experiments with pork surfaces. Fresh, non-injected pork loins were purchased from a local grocery and transported to the laboratory. Pork surfaces were subjected to UV sterilization for 15 minutes (Cutter and Siragusa, 1994). Two 25-cm^2 areas were marked with edible ink using a sterile cotton tipped swab (Hardwood Products, Co., Guilford, MN) and a sterile stainless-steel template. Each of the marked areas was inoculated with 1 ml of a suspension of a serially diluted pathogen, spread using a sterile bent glass rod, and allowed to attach for 15 min at 25ºC under a biological safety hood. Using a sterile scalpel and tissue forceps, one 25-cm^2 area was aseptically excised from the loin. The loin was placed in a conventional freezer at -15°C for 24 h and then thawed at 4°C for 4 h. The remaining 25 cm^2 area was aseptically excised, the sample stomached (Stomacher 400, Tekmar, Cincinnati, OH) for 2 min in 25 ml of BPW with 0.1% Tween 20 (Fisher, St. Louis, MO), and serial dilutions were prepared in BPW. Dilutions were spread plated in duplicate onto the respective media for recovery of pathogens on TAL, selective, and non-selective media. The inoculated plates were incubated at the appropriate time, environment, and temperature as described previously and remaining organisms counted manually

Chilling treatments

Pork samples were obtained from hogs that were slaughtered and processed using conventional procedures at The Pennsylvania State University Meats Lab. After evisceration and splitting, skin-off and skin-on samples were obtained from the belly section. The pork samples were stored aseptically and transported within an hour to the laboratory for experiments.

Blast- and conventional-chilling treatments were conducted based on industry standards as determined by visits, phone calls, and shared information with companies. To obtain the industry-specific parameters for blast-chilling, the following set-up was devised. An air temperature of approximately –20ºC was maintained by adding 50 pounds of dry ice to a plastic bag and placing the bag on the bottom of a conventional –20°C freezer (Kenmore, Hoffman Estates, IL). Carbon dioxide gas was exhausted through a port in the bottom of the freezer. Inoculated pork surfaces were situated approximately 20 cm from a three-speed fan (Honeywell, Southborough, MA) to obtain an air velocity of 1,200 f/m. The blast-chilling treatment consisted of 3 h under blast-chilling conditions described above and 21 h in a conventional-chiller. For conventional-chilling, inoculated surfaces were subjected to industry-simulated conditions in an upright refrigerator (Kenmore, Hoffman Estates, IL) with an air velocity of 100 ft/m as determined using a Thermo-anemometer (Model DAFM2, Beaverton, OR) and maintained by a 2-speed oscillating fan (Windmere, Englewood, CO) to maintain an air temperature of 4°C (±2°C). All inoculated samples subjected to conventional-chilling were placed approximately 40 cm from the fan and chilled for 21 or 24 h.

Sampling and microbiological analyses

For each experiment, skin-on and skin-off pre-rigor pork surface samples were marked with edible ink using a sterile, cotton-tipped swab (Hardwood Products, Co., Guilford, MN) and a sterile 25-cm^2 stainless steel template. Freshly defecated pork feces was obtained from Swine Production Facilities at Penn State University and transported to the laboratory. Approximately 100 g of feces were diluted with BPW and a cocktail consisting of diluted, overnight cultures of *S.* Typhimurium, *L. monocytogenes*, and *C. coli* was added to obtain pathogen levels of approximately 4 and 6 $\log_{10}$ CFU/g. Inoculation of pork surfaces were performed by applying the feces with a sterile paintbrush to the pre-marked area and allowing the surfaces to remain undisturbed for 15 at 25ºC for bacterial attachment before being subjected to the blast- or conventional-chilling treatments. Approximately 3 (low level) and 5 (high level) $\log_{10}$ CFU/cm^2 were obtained using this method.

To determine levels of background microflora, surface samples were obtained prior to paintbrush inoculation by aseptically excising a 25-cm^2 by 0.5 cm thick piece with a sterile scalpel (Sakura, Torrence, CA) and forceps. Following inoculation and chilling treatment(s), surface samples were excised and analyzed for *E. coli*, coliforms, mesophilic bacteria, *S.* Typhimurium, *L. monocytogenes,* and *C. coli.* Samples were diluted in BPW prior to plating in duplicate onto respective media. Trypticase soy agar (TSA, Difco) was selected for growth of mesophilic bacteria, plated using an AutoPlate 4000 Spiral Plater (Advanced Instruments, Norwood, MA) and enumerated using the Q-count Image Analyzer (Advanced Instruments) after incubation for 24 h at 37ºC. The lowest level of detection for mesophilic bacteria using this methodology was 1.30 $\log_{10}$ CFU/cm^2. *E. coli* and coliforms were plated in duplicate onto Petrifilm™ (3M, Minneapolis, MN), incubated and enumerated according to the manufacturer's instructions. *S.* Typhimurium, *L. monocytogenes* and *C. coli* plates were incubated at the appropriate time, environment, and temperature using the TAL method as described previously and remaining organisms counted manually.

To ensure detection of low levels of pathogens following chilling procedures, samples were enriched. For *S.* Typhimurium, 1 ml of stomachate was added to 9 ml of lactose broth (Difco) and incubated 24 h at 37ºC. One ml of the pre-enrichment was added to 9 ml of selenite cysteine (SC; Difco) medium and another 1 ml added to 9 ml of tetrathionate broth (TT; Difco). The broths were incubated for 24 h at 37ºC. After incubation, samples from TT and SC were streaked for isolation onto XLD agar. Typical *S.* Typhimurium colonies were verified serologically using *Salmonella* agglutination latex test (Oxoid, Ogdensburg, NY). For *L. monocytogenes*, 1 ml of stomachate was added to 9 ml of Fraser broth (Difco), incubated for 24 h at 37ºC. The enriched culture was struck onto MOX agar and incubated for 24-48 h at 37ºC. Typical *L. monocytogenes* colonies were verified using Visual Immunoassay for *Listeria* (TECRA Diagnostics, Roseville, Australia) according the manufacturer's instructions. For *C. coli*, 1 ml of stomachate was added to 9 ml of *Campylobacter* enrichment broth (Bolton's formula) with lysed horse blood and antibiotic supplement (Oxoid) and incubated for 4 h at 37ºC and then for 20 h at 42ºC under microaerophilic conditions. The enriched culture was struck onto mCCDA agar and incubated for 24 h at 42ºC, under microaerophilic conditions. Typical *C. coli* colonies were verified serologically using *Campylobacter* agglutination test kit (Oxoid).

Surface pH Determination

The surface pH of all pork surfaces was measured using a flat surface electrode, (Accumte, Model 25 pH/ion meter, Fisher Scientific, Pittsburgh, PA) before and after receiving the chilling treatments (Table 8).

Statistical Analyses

After enumeration, bacterial populations were converted to $\log_{10}$ CFU/cm^2. Least squared means (LSM) of bacterial populations were calculated from four experimental replications. Statistical data analysis (analysis of variance, ANOVA) was performed using Minitab (Minitab, release ver. 13.20, State College, PA). Statistical significance was defined as $P \leq 0.05$, unless otherwise noted. Differences among variables were examined for levels of significance using Tukey's HSD Multiple Comparison Test.

Results

Objective 1: To determine recovery method (s) for pathogens associated with cell suspensions and pork surfaces following conventional-chilling.

In pure culture experiments, the S method produced a lower rate of recovery for *L. monocytogenes, S.* Typhimurium, and *C. coli* as compared with the TAL or OV method (Tables 1, 2 and 3) following freeze treatments of cell suspensions. Overall, the LP method was difficult to conduct and interpreting results was problematic. Conversely, OV and TAL methods recovered a similar level of injured cells for *L. monocytogenes, S.* Typhimurium and *C. coli.* The TAL was chosen for further experiments with inoculated pork surfaces due to the ability of cells to grow on top of the non-selective agar layer, allowing colonies to be picked easily for further biochemical confirmation.

Recovery experiments for *L. monocytogenes* and *S.* Typhimurium were performed with fresh pork loin roasts. Under the treatments described, the TAL method was found to be an acceptable recovery method and produced similar results as compared to non-selective media for *L. monocytogenes* (Table 4), *S.* Typhimurium (Table 5) and *C. coli* (Table 6).

Objective 2: Use the most efficient recovery method from Objective 1 to determine whether conventional- or blast-chilling efficiently reduces bacterial levels associated with fecal contamination and pathogen contamination on skin-on and skin-off pork surfaces.

For the next series of experiments, the experimental design was devised to mimic industry chilling conditions and the analyses separated to determine the effects of treatment and skin type based on inoculation level. Surface pH values of conventional- versus blast-chilling associated with skin-on or skin-off surfaces were taken and are presented in Table 7. As indicated by the information, pH values were higher prior to chilling, with reductions after the chilling process was completed.

For mesophilic bacteria (APC), total coliforms (TC), *E. coli* (EC), *L. monocytogenes*, *S.* Typhimurium and *C. coli,* the interactive effects of 3 treatments (control, blast- and conventional-chilling) and 2 types of surface (skin-on and skin-off) were analyzed. Table 8 demonstrates that both chilling regimens were equally detrimental to APC, TC, or EC when inoculated initially at either a high or low level. When inoculated at a low level and

subjected to either chilling regimen, remaining populations of *L. monocytogenes*, *S.* Typhimurium, or *C. coli* were not statistically different (Table 8). However, blast-chilling was more effective in reducing populations of *L. monocytogenes*, *S.* Typhimurium, or *C. coli* when initial inoculation levels were high.

Table 9 demonstrates that the type of surface (skin-on or skin-off) was not a significant ($P > 0.05$) factor in the reduction of low inoculation levels of APC, TC, CF, EC, or *L. monocytogenes.* However, for the reduction of low levels of *S.* Typhimurium or *C. coli,* blast-chilling a skin-on surface was more injurious to the organism than conventional-chilling a skin-off surface. More importantly, *C. coli* was not detected in enriched samples subjected to blast-chilling on either skin type.

With regard to the effect of surface type on high inoculation levels, there were no statistically significant differences between chilling treatments against populations of APC, TC, or EC (Table 10). The efficacy of the chilling treatments against high levels of *L. monocytogenes*, *S.* Typhimurium, and *C. coli* were not affected by skin surface; however, remaining populations following blast-chilling were significantly different from populations subjected to conventional-chilling (Table 10).

Discussion

The pork industry currently utilizes either conventional- or blast-chilling to reduce the temperature of the carcasses. Extensive research has been done regarding the effect of blast- and conventional-chilling on microorganisms, however the results are debatable (Copper, 1968; Dann, 1972; Price et al., 1976; Wernberg, 1972; James et al., 1983; Greer and Dilts, 1987; Carr et al, 1998). To date, no extensive studies have compared conventional-chilling to blast-chilling for reducing specific pathogen populations on skin-on or skin-off pork surfaces or whether chilling is more effective against different levels of specific pathogens on these surfaces.

Researchers have demonstrated that the cell wall of Gram-positive microorganisms is composed of 80% of peptidoglycan, which serves as a protection against the ice crystals formed during freezing (Rosset, 1982); thus the outer wall composition of *L. monocytogenes* is thought to be more resistant to freezing than Gram-negative microorganisms such as *S.* Typhimurium and *C. coli* (Rosset, 1982; Mountney and Gould, 1988). The results from this study concur with previous studies in that freezing does not reduce *L. monocytogenes* substantially (Palumbo, 1991; Saide-Albornoz, 1995), whereas *S.* Typhimurium and *C. coli* were reduced significantly (Bracewell et al., 1986; Van Laack, et al., 1993; Saide-Albornoz, 1995; Smith, 1995; Escartín, 1999). Previous researchers have demonstrated that *C. coli* is very susceptible to drying and following exposure to oxygenated conditions (Oosterom et al., 1983; Bracewell et al., 1985). In the present study, *C. coli* at low inoculation levels (3 $\log_{10}$ CFU/cm^2) was not recovered after blast-chilling by either direct plating or enrichment. When low inoculation levels of *C. coli* were subjected to conventional-chilling, cells were detected after recovery methods were performed. The difference in the level of recovery of freeze-injured *C. coli* between the two chilling treatments could be due to the high air velocity associated with blast chilling (13.64 m/h) as compared with conventional-chilling (1.14 m/h). These results are in agreement with Oosterom et al (1983) and Bracewell et al (1985) who hypothesized that a high air-flow creates a drier environment that could affect the survival rate of *C. coli.*

It has been hypothesized that the type of pork surface (skin-on or skin-off) may play a role in the level of attachment of the bacteria to the surface. Specifically, skin-on surfaces may allow the bacteria to more firmly attach to hair follicles (Butler, et al., 1979; Lillard, 1989). In this study, we determined that surface type significantly affected low levels of *S.* Typhimurium and *C. coli* populations following blast- and conventional-chilling treatments.

We also demonstrated that blast-chilling reduced a significant number of *L. monocytogenes*, *S.* Typhimurium and *C. coli*, as compared to conventional-chilling. We also observed that differences between blast- and conventional-chilling were significant only at high inoculation levels. It is possible that the efficacy of the chilling regimen is influenced by the type of growth medium, cultures conditions, extent and rapidity of temperature drop, and also by the concentration of cells present on a food surface (Marth, 1973).

Conclusions

We have demonstrated that the thin agar layer (TAL) method is suitable for recovery of freeze-injured pathogens from pork surfaces. Using the TAL method to isolate freeze-injured pathogens, we also were able to conduct experiments to determine the efficacy of two chilling regimens. Our results did not demonstrate a significant difference between blast- and conventional-chilling in the reduction of low levels of mesophilic bacteria, total coliforms, *E. coli*, *L. monocytogenes*, or *S.* Typhimurium. Our results also demonstrated that pre-rigor pork surfaces inoculated with a low level (3 $\log_{10}$ CFU/cm^2) of *C. coli* and subjected to blast-chilling with an air velocity of 13.64 m/h at – 20 ºC for 3 h followed by 21 h at 2 ºC with an air velocity of 1.14 m/h reduced the levels of *C. coli* to undetectable levels. We also were able to demonstrate that high initial inoculation levels of *L. monocytogenes*, *S.* Typhimurium, and *C. coli* were reduced at a significantly ($P < 0.05$) higher rate using blast-chilling, as compared to conventional-chilling. The results obtained from this research may provide pork processors with additional information to improve chilling processes as a step towards producing a microbiologically safer product. To further validate these laboratory findings, microbiological surveys of carcasses from commercial establishments utilizing either blast- or conventional-chilling should be conducted.

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Key for Tables 1-3:

NS= non-selective method (trypticase soy agar; TSA)
S = selective method (modified oxford agar; MOX)
OV = overlay method (TSA and MOX)
TAL = thin agar layer (TSA and MOX)
LP = lutri plate (TSA and MOX)
Treatment = -20°C for 24 hours followed by thawing at 4°C for 4 hours

Table 1. ***Listeria Monocytogenes*****- pure culture**

Method	Before treatment (log_{10}CFU/ml)	After treatment (log_{10}CFU/ml)	Log_{10} reduction
NS	5.24	4.68	0.56
S	5.21	4.33	0.88
OV	5.17	4.64	0.53
TAL	5.22	4.69	0.53
LP	5.13	4.56	0.57

Table 2. ***Salmonella*** **Typhimurium – pure culture**

Method	Before treatment (log_{10}CFU/ml)	After treatment (log_{10}CFU/ml)	Log_{10} reduction
NS	5.23	3.69	1.54
S	5.19	3.07	2.12
OV	5.19	3.65	1.54
TAL	5.22	3.66	1.56
LP	5.12	3.56	1.56

Table 3. ***Campylobacter coli*** **– pure culture**

Method	Before treatment (log_{10}CFU/ml)	After treatment (log_{10}CFU/ml)	Log_{10} reduction
NS	8.37	5.37	3.00
S	8.32	4.43	3.89
TAL	8.3	5.23	3.07
LP	8.34	4.8	3.54

Key for Tables 4-6:

NS = non-selective method (TSA)
TAL = thin agar layer (TSA and MOX)
Treatment = freeze at -20°C for 24 hours and thaw at 4°C for 4 hours

Table 4. Pork loin roast inoculated with *Listeria monocytogenes*

Method	Before treatment ($log_{10}CFU/cm^2$)	After treatment ($log_{10}CFU/cm^2$)	Log_{10} reduction
NS	2.89	2.55	0.34
TAL	2.87	2.51	0.36

Table 5. Pork loin roast inoculated with *Salmonella* Typhimurium

Method	Before treatment ($log_{10}CFU/cm^2$)	After treatment ($log_{10}CFU/cm^2$)	Log_{10} reduction
NS	3.20	2.62	0.58
TAL	3.19	2.60	0.59

Table 6. Pork loin roast inoculated with *Campylobacter coli*

Method	Before treatment ($log_{10}CFU/cm^2$)	After treatment ($log_{10}CFU/cm^2$)	Log_{10} reduction
NS	8.37	5.32	3.05
TAL	8.3	5.22	3.08

Table 7. The effect of blast- and conventional-chilling on the pH of skin-on and skin-off pork carcasses before and after chilling treatment.

Treatment	Before	After
Blast-chilling (skin-on)	6.51*	5.98
Conventional-chilling (skin-on)	6.47	5.84
Blast-chilling (skin-off)	6.49	5.81
Conventional-chilling (skin-off)	6.39	5.74

* Numbers represent mean values of four replications.

Table 8. The effect of chilling treatments (blast- and conventional-chilling) on high and low inoculation levels of populations (log_{10} CFU/cm^2) of aerobic, mesophilic plate counts (APC), total coliforms (TC), *Escherichia coli* (EC), *Listeria monocytogenes* Scott A (LM), *Salmonella* Typhimurium ATCC 14028 (ST), and *Campylobacter coli* ATCC 33559 (CC) on pork surfaces.

Treatment	Organism	High inoculation level	Low inoculation level
Untreated	APC	5.41 ± 0.15 [a] A	3.43 ± 0.34 A
Blast-chilling	APC	3.31 ± 0.35 B	2.43 ± 0.35 B
Conventional-chilling	APC	3.76 + 0.31 B	2.26 + 0.31 B
Untreated	TC	4.6 ± 0.09 A	2.39 ± 0.17 A
Blast-chilling	TC	1.2 ± 0.11 B	0.00 B
Conventional-chilling	TC	1.29 ± 0.31 B	0.00 B
Untreated	EC	4.39 ± 0.16 A	2.06 ± 0.16 A
Blast-chilling	EC	0.88 ± 0.11 B	0.00 B
Conventional-chilling	EC	1.1 ± 0.31 B	0.00 B
Untreated	LM	5.7 ± 0.14 A	3.3 ± 0.03 A
Blast-chilling	LM	5.10 ± 0.13 C	2.95 ± 0.06 B
Conventional-chilling	LM	5.3 ± 0.12 B	3.16 ± 0.04 B
Untreated	ST	5.75 ± 0.07 A	3.29 ± 0.10 A
Blast-chilling	ST	4.53 ± 0.12 C	2.46 ± 0.11 B
Conventional-chilling	ST	4.71 ± 0.02 B	2.62 ± 0.04 B
Untreated	CC	5.08 ± 0.08 A	3.23 ± 0.12 A
Blast-chilling	CC	1.81 ± 0.15 C	* 1.3 ± 0.0 B
Conventional-chilling	CC	2.13 ± 0.07 B	+ 1.3 ± 0.0 B

Means within a column for a given organism on a given inoculation level sharing the same letter are not significantly different ($p>0.05$). * Direct plating method negative, enrichment method negative. + Direct plating method negative, enrichment method positive. [a] denotes the standard error of the mean population of bacteria.

Table 9. The effect of chilling treatments (blast- and conventional-chilling) and type of surface (skin-on and skin-off) on low inoculation level of populations (log_{10} CFU/cm^2) on aerobic, mesophilic plate counts (APC), total coliforms (TC), *Escherichia coli* (EC), *Listeria monocytogenes* Scott A (LM), *Salmonella* Typhimurium ATCC 14028 (ST), and *Campylobacter coli* ATCC 33559 (CC) on pork surfaces.

Treatment	Organism	Low inoculation level / skin-on	Low inoculation level / skin-off
Untreated	APC	3.65 ± 0.29 A	3.43 ± 0.34 A
Blast-chilling	APC	2.23 ± 0.78 B	2.64 ± 0.30 B
Conventional-chilling	APC	1.34 ± 0.13 B	2.26 ± 0.31 B
Untreated	TC	2.39 ± 0.17 A	1.87 ± 0.14 A
Blast-chilling	TC	0.00 B	0.00 B
Conventional-chilling	TC	0.00 B	0.075 ± 0.13 B
Untreated	EC	2.06 ± 0.23 A	1.46 ± 0.32 A
Blast-chilling	EC	0.00 B	0.00 B
Conventional-chilling	EC	0.00 B	0.00 B
Untreated	LM	3.3 ± 0.03 A	3.31 ± 0.09 A
Blast-chilling	LM	2.95 ± 0.06 B	3.13 ± 0.07 B
Conventional-chilling	LM	3.16 ± 0.04 B	3.23 ± 0.06 B
Untreated	ST	3.29 ± 0.10 A	3.73 ± 0.13 A
Blast-chilling	ST	2.46 ± 0.11 B	2.71 ± 0.11CD
Conventional-chilling	ST	2.62 ± 0.04 BC	2.8 ± 0.12 D
Untreated	CC	3.23 ± 0.12 A	3.09 ± 0.05 A
Blast-chilling	CC	* 1.3 ± 0.00 B	* 1.3 ± 0.00 B
Conventional-chilling	CC	+ 1.3 ± 0.00 B	+ 1.3 ± 0.00 B

Means within a column for a given organism on a given inoculation level/surface type sharing the same letter are not significantly different ($p > 0.05$). Means within rows with different letter denote a significant difference ($p > 0.05$) due to surface type. * Direct plating method negative, enrichment method negative. + Direct plating method negative, enrichment method positive.

[a] denotes the standard error of the mean population of bacteria.

Table 10. The effect of chilling treatments (blast- and conventional-chilling) and type of surface (skin-on and skin-off) on high inoculation level of populations (log_{10} CFU/cm^2) on aerobic, mesophilic plate counts (APC), total coliforms (TC), *Escherichia coli* (EC), *Listeria monocytogenes* Scott A (LM), *Salmonella* Typhimurium ATCC 14028 (ST), and *Campylobacter coli* ATCC 33559 (CC) on pork surfaces.

Treatment	Organism	High inoculation level / skin-on	High inoculation level / skin-off
Untreated	APC	5.41 ± 0.16 A	5.5 ± 0.34 A
Blast-chilling	APC	3.31 ± 0.35 B	3.27 ± 0.19 B
Conventional-chilling	APC	3.76 ± 0.31 B	3.78 ± 0.38 B
Untreated	TC	4.9 ± 0.16 A	4.6 ± 0.09 A
Blast-chilling	TC	0.93 ± 0.71 B	1.2 ± 0.11 B
Conventional-chilling	TC	2.18 ± 0.11 B	1.29 ± 0.31 B
Untreated	EC	4.4 ± 0.24 A	4.39 ± 0.16 A
Blast-chilling	EC	0.49 ± 0.53 B	0.88 ± 0.11 B
Conventional-chilling	EC	1.22 ± 0.13 B	1.1 ± 0.32 B
Untreated	LM	5.7 ± 0.14 A	5.79 ± 0.07 A
Blast-chilling	LM	5.03 ± 0.13 C	5.18 ± 0.06 C
Conventional-chilling	LM	5.19 ± 0.12 B	5.4 ± 0.10 B
Untreated	ST	5.75 ± 0.07 A	5.77 ± 0.12 A
Blast-chilling	ST	4.61 ± 0.12 C	4.46 ± 0.16 C
Conventional-chilling	ST	4.71 ± 0.02 B	4.64 ± 0.17 B
Untreated	CC	5.08 ± 0.08 A	5.18 ± 0.08 A
Blast-chilling	CC	1.81 ± 0.15 C	1.63 ± 0.27 C
Conventional-chilling	CC	2.13 ± 0.07 B	2.06 ± 0.11 B

Means within a column for a given organism on a given inoculation level/surface type sharing the same letter are not significantly different ($p > 0.05$). [a] denotes the standard error of the mean population of bacteria.