“Effects of Chilling Methods on Bacterial Recovery and Reducing Bacteria on Pork Carcasses”

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Tyson Foods, Inc.
Funding

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Outline

Foodborne Pathogens Associated with Pork
Pork Processing
Low temperatures
Conventional- vs. Blast-Chilling
Hypotheses
Objectives
Experimental Designs
Results
Conclusions
Future Research
I. Foodborne Pathogens Associated with Pork

Salmonella Typhimurium: 54.4% tested positive from pork carcasses surfaces samples

Listeria monocytogenes: 52% tested positive from pork carcasses surfaces samples

Campylobacter spp.: 79.5% tested positive from pork carcasses surface samples

(Nationwide Pork Microbiological Study, 1996)
Salmonella Typhimurium

- Rod shaped
- Gram negative
- Facultative anaerobe
- Non spore forming
- Symptoms: diarrhea, fever and vomiting
- Mildly sensitive to heating and freezing
- Associated with pork, poultry and dairy products
Listeria monocytogenes

- Rod shaped
- Gram positive
- Facultative anaerobe
- Non spore forming
- Symptoms: vomiting, diarrhea and fever
- Resistant to effects of drying, freezing, heating, low pH and high salinity
- Associated with pork, milk, cheese, ready to eat meats, water and vegetables
Campylobacter coli

- Gram negative
- Microaerophilic organism
- Non spore forming
- Sensitive to heating, freezing and drying
- Symptoms: abdominal pain, fever and watery/bloody diarrhea
- Associated with pork
II. Pork Processing

- Stunning
  - Mechanical
  - Electrical
  - Loss of consciousness
Pork Processing

- Bleeding
  - Six inch knife
  - Seven pounds of liquid blood
  - Nine minutes
Pork Processing

- **Scalding**
  - Immerse in water at 60°C
  - 4 minutes
  - Loosen hair follicle
Pork Processing

- Dehairing
Pork Processing

- Remove of head
  - Tongue
  - Tonsils
  - Esophagus
  - Trachea
  - Jowl
Pork Processing

- Evisceration
  - Prevent cut or tear
    - Small and large intestine
    - Stomach
    - Liver
    - Spleen
Pork Processing

- Splitting
  - Removal of kidneys and leaf fat
  - Trim blood clots and loose lymph glands
Pork Processing

- Washing
  - water and/or 1-3% lactic/acetic acid

- Prepare carcass for chilling
  - Blast chill
  - Conventional chill
III. Low Temperatures

- Chilling temperature (10 to 15°C)
- Refrigeration temperature (0 to 10°C)
- Conventional-chilling (air temperature 1 to 4°C)
- Blast-chilling (air temperature –7 to -40°C)

(Mountney & Gould, 1988)
Low Temperatures

- Cold is used to preserve food for long periods of time

- Is not an effective means of destroying microorganisms in foods

- Maintains the food in a good physical state

(Brown, 1982)
Effects of Freezing

- Fast versus slow freezing
  - Size of the ice crystal

- Injury of microorganisms
  - Inability to replicate in selective environments
Effects of Freezing

- Type of organism
  - Gram positive
    - Listeria monocytogenes
  - Gram negative
    - Salmonella Typhimurium and Campylobacter coli
Gram-Positive Cell Wall

The Gram-positive Envelope

- Polysaccharide
- Teichoic acid
- Peptidoglycan
- Cytoplasmic membrane
- Phospholipid
- Protein
Gram-Negative Cell Wall

The Gram-negative Envelope

- Outer membrane
- Periplasmic space
- Cytoplasmic membrane
- Phospholipid
- O-antigens
- Porin trimer
- Lipopolysaccharide
- Brown's lipoprotein
- Peptido-glycan
- Protein
Cold Shock

- Rapid decline of the temperature
- No adaptation to low temperatures
- Loss of selective permeability of the cellular membrane

(Rosset, 1982)
IV. Conventional- vs. Blast-Chilling

Conventional-chilling

- Temperature 1 to 4°C
- Air flow velocity 90 – 180 f/m
- 24 – 48 hrs
Conventional-Chilling

- Advantages
  - Commonly used in the pork industry
  - Less expensive

- Disadvantages
  - Requires additional cooler/storage space
  - Results in substantial evaporative weight loss
  - Time consuming
  - Increases incidence of pale soft exudative (PSE) pork
Conventional- vs. Blast-Chilling

Blast-chilling

- Temperature – 7 to – 40 °C
- Air velocity 600 – 960 f/m
- 30 – 90 minutes
- Conventional-chilling
Blast-Chilling Tunnel

Washer

Air Fans

Cooling Room
Conventional- vs. Blast-Chilling

Blast-Chilling

- **Advantages**
  - Reduces chilling time by 30 – 50%
  - Reduces incidence of pale soft exudative (PSE) pork

- **Disadvantages**
  - Expensive
  - Air fans in order to obtain high air flow velocity rates
  - Space requirements for the tunnels
Conventional- vs. Blast- Chilling

- Previous studies found:
  - Blast-chilling decreases levels of carcass contamination and improves keeping quality (Price et al., 1976)
  - Conventional-chilling is more detrimental to the psychrotophic cells than blast-chilling on pork carcasses (Brown, 1982)
  - Blast-chilling reduced Campylobacter spp. on pork to below detectable levels (Oosterom et al., 1982)
Conventional- vs. Blast- chilling

- Previous studies found:
  - No difference between blast- and conventional-chilling for reduction of mesophilic bacterial populations on pork carcasses. (James et al., 1983)
  - Blast-chilling on pork surfaces with fat tissue produced lower counts of coliform and Staphylococcus spp. (Carr et al., 1998)
V. Hypotheses

- Differences exist between blast- and conventional-chilling of pork with regard to:
  - Skin surface (skin-on vs. skin-off)
  - Inoculation level (10^5 vs. 10^3 CFU/cm^2)
  - Type of microorganisms (Salmonella Typhimurium, Campylobacter coli, Listeria monocytogenes, Escherichia coli, Coliform and mesophilic bacteria)
Skin-On vs. Skin-Off

- Skin on

- Skin off
VI. Objectives

- To determine the best recovery method for pathogens associated with cell suspensions and pork surfaces following freeze-thaw cycle.
Objectives

- Use the most efficient recovery method to determine whether conventional- or blast-chilling effectively reduces bacterial levels associated with fecal contamination and pathogen contamination on skin-on and skin-off pork surfaces.
VII. Experimental Design

- Recovery Methods Experiments
- Blast- and Conventional-Chilling Experiments
Materials and Methods

- **Listeria monocytogenes Scott A**
  - Non-selective (trypticase soy agar, TSA)
  - Selective (modified oxford, MOX)
- **Salmonella Typhimurium ATCC 14028**
  - Non-selective (trypticase soy agar, TSA)
  - Selective (xylose lysine decarboxylase, XLD)
- **Campylobacter coli ATCC 33559**
  - Non-selective (brucella agar, Bru)
  - Selective (Campylobacter blood-free selective medium, CCDA)
Materials and Methods

Recovery Methods

- Non selective (NS)
- Selective (S)
- Overlay method (OV)
- Thin agar layer method (TAL)
- Lutri plate (LP)
Non-selective Method

- Non-selective (TSA) layer (24 h)
Selective Method

- Selective XLD agar layer (24 h)
Overlay Method

- Selective XLD agar layer (21 h)

- Non selective TSA layer (3 h)

(Kang and Fung, 1999)
Thin Agar Layer Method

- 2 layer of non-selective TSA layers (24 h)

- Selective XLD agar layer

(Kang and Fung, 2000)
Lutri Plate Method

- Non selective TSA layer (2 h)

- Selective XLD agar layer (22 h)

(Kang and Siragusa, 1999)
Recovery Method Experiments

- 4 replications
- Pathogens (Listeria monocytogenes, Salmonella Typhimurium and Campylobacter coli)
- Cell suspension
- Pork loin roast experiments (covered with adipose tissue)
Cell Suspension Experiments Protocol

Overnight culture

Place tube in a freezer at -15 °C for 24 h

Thaw at 4°C for 4h

Serially dilute in BPW

Plate on 5 different recovery methods

Incubate

Count plates
Statistical Analyses for Recovery Methods on Cell Suspensions

- Analysis of variance (ANOVA)
- General linear model procedure
- Tukey pairwise comparison test
Listeria monocytogenes
Cell Suspension Experiments

Recovery method

<table>
<thead>
<tr>
<th>Recovery method</th>
<th>Control (log10 CFU/ml)</th>
<th>Treatment (log10 CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-selective</td>
<td>5.23a</td>
<td>4.68b</td>
</tr>
<tr>
<td>Selective</td>
<td>5.20a</td>
<td>4.33c</td>
</tr>
<tr>
<td>Thin agar layer</td>
<td>5.17a</td>
<td>4.64b</td>
</tr>
<tr>
<td>Lutri plate</td>
<td>5.22a</td>
<td>4.70b</td>
</tr>
<tr>
<td>Overlay</td>
<td>5.21a</td>
<td>4.56b</td>
</tr>
</tbody>
</table>
Salmonella Typhimurium Cell Suspension Experiments

Control | Treatment

Recovery method:
- Non-selective
- Selective
- Thin agar layer
- Lutri plate
- Overlay

Log 10 CFU/ml:
- Non-selective: 5.22 ± 0.0
- Selective: 5.19 ± 0.0
- Thin agar layer: 5.19 ± 0.0
- Lutri plate: 5.22 ± 0.0
- Overlay: 5.19 ± 0.0

Legend:
- Control
- Treatment
Campylobacter coli

Cell Suspension Experiments

Recovery method

- Non selective
- Selective
- Thin agar layer
- Lutri plate

Control
Treatment

Log10 CFU / ml

8.37
8.32
8.30
8.34

5.37
4.43
5.23
4.80

0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
Conclusions

- The TAL, OV and LP methods were not statistically different ($P > 0.05$) as compared to the NS method.

- The TAL method was easier to perform and allowed for improved isolation of single colonies.
Pork Loin Roast Experiments Protocol

Overnight culture

- UV sterilized pork surfaces, mark 2, 10 x 10 cm areas
- Inoculate the areas with pathogen, for 15 min at 25°C
- Excise sample, stomach and plate (control sample)
- Place inoculated meat in a freezer at -15°C for 24h
- Thaw at 4°C, 4 h
- Excise sample, stomach
- Serially dilute, plate and incubate
- Count
Statistical Analyses for Recovery Methods

- Analysis of variance (ANOVA)
- General linear model procedure
- Tukey pairwise comparison test
Pork Loin Roast Inoculated with Listeria monocytogenes

Non selective

Thin agar layer

Recovery method

<table>
<thead>
<tr>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.89</td>
<td>2.55</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>2.87</td>
<td>2.45</td>
</tr>
</tbody>
</table>

log10 CFU / cm²

0.0  0.5  1.0  1.5  2.0  2.5  3.0  3.5

Legend:
- Red: Control
- Blue: Treatment
Pork Loin Roast Inoculated with Salmonella Typhimurium

![Bar chart showing log10 CFU/cm² for non-selective and thin agar layer recovery methods.]

- **Non-selective**
  - Control: 3.20 (a)
  - Treatment: 2.61 (b)

- **Thin agar layer**
  - Control: 3.19 (a)
  - Treatment: 2.57 (b)
Pork Loin Roast Inoculated with Campylobacter coli

<table>
<thead>
<tr>
<th>Recovery method</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non selective</td>
<td>8.37 a</td>
<td>5.37 b</td>
</tr>
<tr>
<td>Thin agar layer</td>
<td>8.30 a</td>
<td>5.22 b</td>
</tr>
</tbody>
</table>
Conclusions

- Thin agar layer (TAL) method was not significantly different, as compared to the Non-selective (NS) method

- TAL method presents selective isolation of foodborne pathogens
Materials and Methods for Blast- and Conventional-Chilling Experiments

- 4 replications of each experiment
- Variables
  - Treatments (untreated, blast- and conventional-chilling)
  - Pathogens (Listeria monocytogenes, Salmonella Typhimurium, Campylobacter coli)
  - Indicator organisms (Escherichia coli, coliforms and mesophilic bacteria)
  - Type of skin (skin-off and skin-on)
  - Inoculation level for pathogens (10^3 and 10^5 log_{10} CFU/ml)
General Protocol for Blast- and Conventional-Chilling Experiments

Pork carcass sample with skin-on/off

With USDA blue edible ink, mark 4, 12 x 12 cm area

Test one area for natural flora

Inoculate 3 areas with fresh sterile dilute feces inoculated with pathogen “cocktail” or non sterile dilute feces
General Protocol for Blast- and Conventional-Chilling Experiments

Aseptically excise one area and plate for initial bacterial level (control)

| Place inoculated surfaces in the blast or conventional chiller following industry parameters |
| Aseptically excise and sample remaining 25cm² |
General Protocol for Blast- and Conventional-Chilling Experiments

Enrichment (qualitative)

| Plate (quantitative)
| Incubate
| Count colonies
Statistical Analyses for Blast- and Conventional-Chilling Experiments

- Analysis of variance (ANOVA)
- General linear model procedure
- Tukey pairwise comparison test
Results for Indicator Microorganisms

- No statistically significant difference between blast- and conventional-chilling in reducing indicator microorganisms at $10^3$ and $10^5 \log_{10} \text{CFU/cm}^2$.
Results for Indicator Microorganisms

- No statistically significant difference between skin-on and skin-off in reducing indicator microorganisms at $10^3$ and $10^5$ CFU/cm²
### Results for Listeria monocytogenes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>High Inoculation</th>
<th>Low Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>skin-on</td>
<td>skin-off</td>
</tr>
<tr>
<td>Untreated</td>
<td>5.70 ± 0.14 A</td>
<td>5.79 ± 0.07 A</td>
</tr>
<tr>
<td>Blast-chilling</td>
<td>5.03 ± 0.13 C</td>
<td>5.18 ± 0.06 C</td>
</tr>
<tr>
<td>Conventional-chilling</td>
<td>5.19 ± 0.12 B</td>
<td>5.40 ± 0.10 B</td>
</tr>
</tbody>
</table>

High inoculation ~ 5 log_{10} CFU/cm²
Low inoculation ~ 3 log_{10} CFU/cm²
## Results for Salmonella Typhimurium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>High Inoculation</th>
<th>Low Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>skin-on</td>
<td>skin-off</td>
</tr>
<tr>
<td>Untreated</td>
<td>5.75 ± 0.07 A</td>
<td>5.77 ± 0.12 A</td>
</tr>
<tr>
<td>Blast-chilling</td>
<td>4.61 ± 0.12 C</td>
<td>4.46 ± 0.16 C</td>
</tr>
<tr>
<td>Conventional-</td>
<td>4.71 ± 0.02 B</td>
<td>4.64 ± 0.17 B</td>
</tr>
<tr>
<td>chilling</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

High inoculation ~ 5 \( \log_{10} \) CFU/cm\(^2\)
Low inoculation ~ 3 \( \log_{10} \) CFU/cm\(^2\)
### Results for Campylobacter coli

<table>
<thead>
<tr>
<th>Treatment</th>
<th>High Inoculation</th>
<th>Low Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>skin-on</td>
<td>skin-off</td>
</tr>
<tr>
<td>Untreated</td>
<td>5.08 ± .08 A</td>
<td>3.23 ± .12 A</td>
</tr>
<tr>
<td>Blast-chilling</td>
<td>1.81 ± .15 C</td>
<td>1.3 B</td>
</tr>
<tr>
<td>Conventional-chilling</td>
<td>2.13 ± .07 B</td>
<td>+ 1.3 B</td>
</tr>
</tbody>
</table>

High inoculation ~ 5 log\(_{10}\) CFU/cm\(^2\)
Low inoculation ~ 3 log\(_{10}\) CFU/cm\(^2\)

* Direct plating method negative, enrichment method negative
+ Direct plating method negative, enrichment method positive
Conclusions

- Using TAL method, no significant difference between blast- and conventional-chilling in reducing indicator microorganisms
Conclusions

- Blast-chilling was significantly different in reducing pathogen microorganisms at $10^5 \log_{10} \text{CFU/cm}^2$
Conclusions

- Pork samples inoculated with $3 \log_{10} \text{CFU/cm}^2$ of Campylobacter coli and subjected to blast-chilling were reduced to undetectable levels.
X. Future Research

- To validate our results, commercial processors utilizing blast- or conventional-chilling should be surveyed and samples collected for bacteriological analyses.

- If viable but nonculturable cells (VBNC) are present after chilling regimen.
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Vivian Chang, born in Guatemala City in 1975, obtained a B.S in Food Science from Universidad del Valle de Guatemala in 1994. In December 2002, she earned a M.S degree in Food Science from Penn State University and her major advisor was Dr. Catherine Cutter. Ms. Chang’s thesis project was concentrated on pathogens associated with pork and was funded by the National Pork Board. Since March 2002, she has been working at Tyson Foods Inc.