Title: Project #645: Blast Surface Freezing Combined with Antimicrobials to Eliminate Listeria Monocytogenes on Ready-to-Eat Meat

Investigator: Paul Dawson

Clemson University, Clemson, SC 29634-0316

Department: Food, Nutrition and Packaging Sciences, 224 Poole Hall

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Submit final reports to:
Henry Marks, Ph.D.
U.S. Poultry & Egg Association
1530 Cooledge Road
Tucker, GA 30084-7303
Industry Summary: Problem Studied: Effect of surface freezing on survival of bacteria on chicken meat.

The objectives of the original proposal were modified to:

1. Determine the survival of artificially-inoculated *Escherichia coli* and *Salmonella Typhimurium* on the surface of raw poultry products subjected to crust freezing
2. Determine the quality shelf life of chicken breasts subjected to crust freezing with and without skin.

The project attempted to determine the effect of surface freezing on the survival of *Escherichia coli* and *Salmonella Typhimurium* on fresh chicken breast as well as the effect of surface freezing on native spoilage bacteria. In the 1st study, these pathogens were inoculated onto the meat, allowed to attach then the live cells were recovered after exposure to surface freezing and compared to inoculated bacteria on meat that was only refrigerated or completely frozen. In addition, since bacteria in the processing environment are likely to have been previously exposed to low temperatures, a half of the samples were inoculated with bacteria that had been cold-shocked or exposed to cold temperatures prior to inoculation. No significant differences were observed in the reduction of cold-shocked or non-cold-shocked bacteria on products that were crust- or completely frozen, with or without skin. The average reduction for *E. coli* was 0.15 log_{10} CFU/mL of rinse and for *S. Typhimurium* 0.10 log_{10} CFU/mL of rinse; therefore, none of the final reductions were greater than the desired target (1 log). Data showed minimal initial reduction of these pathogens due to crust freezing. In the 2nd objective the effect of crust freezing (20 min, -85 °C) on the quality of raw chicken breasts, with or without skin, during aerobic, refrigerated storage for up to 18 days was assessed by means of the International Commission on Illumination (CIE) color parameters L*, a* and b*; tenderness; and total aerobic (APC) and yeasts and molds counts (YMC). Microbial load increased over time and exceeded 8.0 log_{10} CFU/ml, which occurred before 12 days of storage. Under the experimental conditions used, crust freezing did not affect color or tenderness of raw chicken breasts, with or without skin but no significant increase in microbiological shelf life was observed either.

**Scientific Report**
Materials and Methods Study 1

Culture preparation

Experiments were performed using an ampicillin-resistant (AR) *E. coli* JM 109 strain and a nalidixic acid-resistant (NAR) *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain (*S. Typhimurium*). Antibiotic-resistant strains were used as a means to minimize potential interference with background microflora when monitoring survival of both bacteria (Dominguez and Schaffner, 2009). For *E. coli*, AR stock cultures were maintained at -85 ºC in Difco Tryptic Soy Broth (TSB; Becton Dickinson, Sparks, MD) plus 20% glycerol. Intermediate cultures were prepared by transferring 0.1 mL of the stock culture to 9.9 mL of sterile TSB containing 500 ppm of ampicillin (Sigma-Aldrich, St. Louis, MO). Incubation was at 37 ºC for 24 h with agitation at 200 rpm. Then, 0.1 mL of the growing (24-h) culture was transferred to a tube with 9.9 mL of fresh, sterile TSB containing 500 ppm of ampicillin (Sigma-Aldrich, St. Louis, MO) and maintained at 37 ºC for 16 to 18 h with agitation at 200 rpm. Similarly, NAR *S. Typhimurium* stock cultures were maintained at -85 ºC in TSB plus 20% glycerol. Intermediate cultures were prepared by transferring 0.1 mL of the stock culture to 9.9 mL of sterile TSB containing 200 ppm of nalidixic acid (Sigma-Aldrich, St. Louis, MO). Incubation was at 37 ºC for 24 h with agitation at 200 rpm. Then, 0.1 mL of the growing (24-h) culture was transferred to a tube with 9.9 mL of fresh, sterile TSB containing 200 ppm of nalidixic acid and maintained at 37 ºC for 16 to 18 h with agitation at 200 rpm. After the second incubation, the intermediate cultures of each bacterium were centrifuged for 20 min at 3000 rpm, the supernatant was discarded, and the pellet resuspended in 9.9 mL of sterile Difco Bacto Peptone water (0.1% wt/vol; Becton Dickinson, Sparks, MD). Late-exponential-phase cultures were used in order to
mimic the state of natural contaminants in poultry (Dominguez and Schaffner, 2009). The resuspended pellet of cells was defined as the working culture.

**Sublethal injury**

After incubation at 37 °C for 16 to 18 h as previously described, sets of intermediate cultures of both AR *E. coli* and NAR *S. Typhimurium* were placed in refrigeration at 4±2 °C and stored for 10 days in order to provoke sublethal injury by cold stress as described by Jasson et al. (2007). After this period, final working cultures were prepared as described above.

**Food product preparation and inoculation**

Skinless chicken breasts and skin-on chicken thighs of the same commercial (non-frozen) brand were purchased at a local supermarket and maintained in refrigeration at 4±2 °C for no more than one day until the experiments were performed. Each product package was wiped with sterile paper towels to remove excess water before opening under a Bioflow Chamber (Germfree, Ormond Beach, FL). Chicken products were aseptically removed with a sterile forceps and placed onto sterile paper towel. Products were inoculated using 0.50 mL of AR *E. coli* and 0.50 mL of NAR *S. Typhimurium* (of approximately 10^6 CFU/mL each) placed on separate sections of the same product, both working cultures being either cold-stressed or unstressed. Inoculation spots were located at the center and edges of each sample. Inoculated, non-frozen units were used as controls. Cultures were allowed 20 min for attachment then individual samples were placed in clear, plastic commercial freezer bags for further processing.

**Freezing, storage and thawing**

Samples were individually placed in an ultra-freezer at -85 °C for either 20 (crust-frozen) or 60 min (complete frozen). In the first case, the 20 min time was selected because it was the
minimum amount of time required for the formation of a visible crust around the product (approximately 20 mm deep). In the latter, the samples were completely frozen in 60 min. Afterwards, bags containing the samples were refrigerated at 4±2 °C during 18 to 20 h prior to microbial enumeration.

**Microbial enumeration**

Fifty milliliters of sterile Bacto Peptone water (0.1% wt/vol; Becton Dickinson, Sparks, MD) were added to each freezer bag containing either a chicken breast or a chicken thigh. The pieces were then massaged gently by hand for 30 s to wash bacteria into the peptone water within the bag. The solution was serially diluted in sterile Bacto Peptone water (0.1% wt/vol; Becton Dickinson, Sparks, MD) and enumeration was carried out as follows:

- For the recovery of AR *E. coli*, serial dilutions were surface-plated on Difco Tryptic Soy Agar (TSA; Becton Dickinson, Sparks, MD) and Difco Violet Red Bile Glucose Agar (VRBGA, Becton Dickinson, Sparks, MD), with both media containing 500 ppm of ampicillin. All dilutions were plated in duplicate. Colonies were counted on duplicate plates with 25 to 250 colonies after incubation at 37 ºC for 48 h on a Quebec colony counter. Bacterial populations were converted to log_{10} CFU/mL of rinse.

- Enumeration of NAR *S. Typhimurium* was performed by surface-plating the corresponding serial dilutions on TSA and Brilliant Green Sulfa agar (BG Sulfa), both media containing 200 ppm of nalidixic acid. All dilutions were plated in duplicate. Colonies were counted on dilution plates with 25 to 250 colonies after incubation at 37 ºC for 48 h on a Quebec colony counter. Bacterial populations were converted to log_{10} CFU/mL of rinse.
Metabolically and structurally injured cells are able to produce colonies on nonselective agar (Roszak and Colwell, 1987); therefore, a selective medium was used for each bacterium to assess the number of injured cells. All antibiotic solutions used in the experiments were filter-sterilized using a 0.45 μm–pore-size syringe filter.

**Log reduction and extent of injury**

All counts were converted to log CFU/mL of rinse before analyzing the data. The decimal reduction attained by crust or complete freezing was defined as the difference between the values of the inoculated, non-frozen samples (controls) and the populations of either *E. coli* or *S. Typhimurium* in TSA containing the corresponding antibiotic, after either freezing treatment. The extent of injury for *E. coli* was defined as the difference between the counts on TSA and VRBGA. For *S. Typhimurium*, the extent of injury was considered to be the difference between the counts on TSA and BG Sulfa. The decimal reductions and the extent of injury were estimated for experiments carried out with cold-stressed and non-stressed cultures, in skin-on or skinless units. All reductions were estimated in CFU/mL of sample rinse and then converted to logarithmic values for statistical analysis.

**Statistical analysis**

The experiment was designed as a completely-randomized study with four factors: bacteria (*E. coli* or *S. Typhimurium*), stress (non-stressed or cold-stressed), presence of skin (skin-on or skinless), and freezing type (control, crust frozen, or complete frozen). The experiments were replicated three times using three breasts per treatment (n=9) from separate lots of meat and bacterial cultures. The experimental unit was defined as a single chicken product. An analysis of variance was performed separately on the the log reduction data and the
extent of injury results using PROC MIXED on the Statistical Analysis System (SAS) version 9.2 (SAS Institute, Cary, NC). Results were deemed significant at a p<0.05.

Materials and Methods Study 2

Fresh, clean chicken carcasses were kindly provided by a local commercial processing plant in Greenville, SC and transported directly in ice to the laboratory about one hour after slaughter. All of the chicken carcasses used for a given experimental replicate were procured from the same process lot in order to reduce variability among samples. Samples were not frozen prior to performing the experiments so that the normal microflora and microbial load would remain as intact as possible. Upon arrival to the laboratory, the chicken carcasses were kept under refrigeration at 4±2 °C for no more than four hours until preparation. Using sterile technique, whole chicken breasts in each batch were manually separated from the carcasses and randomly assigned to a “skin-on” or a “skinless” group and to one of four possible storage times (3, 6, 12, or 18 days). Five breasts with either skin-on or skin-off were then split longitudinally into two halves and each half was randomly assigned to one of two possible freezing treatments (control or crust-freezing).

Crust freezing, thawing, and sampling periods

All chicken breasts assigned to the crust freezing group, with or without skin, were placed in clear, plastic freezing bags and individually frozen at -85 °C for 20 min, time needed for a visible crust to be formed homogenously around the product (approximately 20 mm deep, as determined by preliminary tests). After the crust freezing treatment, bags containing the samples were placed under refrigeration at 4±2 °C for 18 h, along with their non-frozen counterparts, prior to sampling and quality analyses. Control or non-frozen samples were handled the in same
way except they were not crust frozen and placed directly under refrigeration. Microbial and quality analyses were performed on treatment and control samples at 3, 6, 12, and 18 days of refrigerated storage.

**Cooking**

Refrigerated chicken breasts were cooked prior to measuring instrumental texture in order to determine treatment effects on meat tenderness. Samples were wrapped in aluminum foil, placed on a tray and steamed in an autoclave for 15 min so that the internal temperature in the thickest part of the breast reached at least 74 °C. After cooling down to room temperature (22-25 °C) in open air for at least 60 min, instrumental texture was measured on each breast as described below.

**Microbiological and quality analyses**

Instrumental color and texture, aerobic plate counts (APC), and total yeasts and molds counts were measured at every sampling period. All of the measurements were taken on a breast in the following order: color, APC along with yeasts and molds, and finally texture, which was the only destructive assay.

**Color.** Instrumental color analysis was based on at least five measurements of light reflected from each chicken breast surface, measured at the center and edges of each sample. International Commission of Illumination (CIE) lightness (L*), redness (a*), and yellowness (b*) values were obtained using a Chroma Meter with an 8 mm viewing port and illuminant D_65_ (CR-300, Minolta Corp, Ramsey, NJ). The instrument was calibrated against a standard white ceramic tile immediately before the measurements were taken.
**Microbial analyses.** Fifty ml of sterile Bacto Peptone water (0.1% wt/vol; Becton Dickinson, Sparks, MD) were added to each freezing bag containing a chicken breast. The pieces were then massaged gently by hand for 30 s to disperse bacteria into the peptone water within the bag. The recovery solution was then serially diluted in sterile Bacto Peptone water (0.1% wt/vol; Becton Dickinson, Sparks, MD) and enumerated as follows:

- For Aerobic Plate Counts, serial dilutions were surface-plated on Difco Plate Count Agar (PCA; Becton Dickinson, Sparks, MD). Colonies were counted on dilution plates with 25 to 250 colonies after incubation at 37 ºC for 48 h on a Quebec colony counter. Bacterial populations were converted to $\log_{10}$ CFU/ml of sample rinse.

- For yeasts and molds, serial dilutions were surface-plated on Difco Dichloran-Rose Bengal-Chloramphenicol (DRBC; Becton Dickinson, Sparks, MD). Plates were incubated at room temperature (22-23 ºC) protected from light. Colonies were counted on dilution plates with 10 to 150 colonies after 5 days of incubation on a Quebec colony counter. Microbial populations were converted to $\log_{10}$ CFU/ml of sample rinse.

**Instrumental Texture Analysis.** Chicken meat tenderness was evaluated by shear energy using a TX.XT Texture Analyzer (Stable Micro Systems Inc, Surrey, UK) connected to a PC for data logging via Texture Exponent (TEE) 32 version 4.0.8.0, following the procedure described by Cavitt *et al.* (2004) with modifications. Briefly, a razor blade probe with a height of 24 mm and a width of 8.9 mm set to a penetration depth of 20 mm was used to compress the muscle tissue perpendicularly to the muscle fibers after equilibration to room temperature. Instrumental blades were replaced after every 50 samples and recalibrated to eliminate error due to dulling of blades. Shear energy and shear force on intact cooked breasts were recorded in at least five different locations on each breast to obtain mean and standard error data. The
instrument settings were: maximum cell load: 2 kg; probe pre-test speed: 2 mm/s; test speed: 10 mm/s; post-test speed: 10 mm/s; trigger force: 10-g contact force. Shear force (g) was defined as the maximum force recorded and shear energy (g*mm) was considered to be the area under the force-deformation curve from the beginning to the end of the test.

**Statistical analysis**

The experiment was designed as a split-plot study with two whole plots (presence of skin and storage time) and one sub-plot (freezing type) and replicated two times from lots collected on different days of processing using a total of eight chicken carcasses for each replicate. The lot from which the carcasses were obtained was considered a blocking factor. Analysis of variance of the APC, yeasts and molds counts, CIE color parameters L*, a* and b*, and shear energy were performed using the PROC MIXED command on the Statistical Analysis System (SAS) version 9.2 (SAS Institute, Cary, NC). Bonferroni’s inequality was used to estimate multiple comparison error rates at a 5% level of significance.

**Results and Discussion Study 1**

In the present study, cold-stressed sets of *E. coli* and *S. Typhimurium* were prepared by maintenance of late-exponential phase cultures in refrigeration at 4 °C for 10 days in order to simulate conditions in the processing and retail environment (Jasson et al., 2007). Furthermore, cultures were used in their late exponential phase, under aerobic conditions in relatively high concentration (10^5-10^6 CFU/mL). Additionally, the freezing rate was considered high, as the temperature of the products was lowered to about -20 °C within 30 minutes (Jay, 2005). This method was selected in order to evaluate a freezing treatment that would produce minimum quality changes.
The results of this study show that the presence of skin was not a significant factor in determining survival of either bacterium (p=0.11). Consequently, no differences were observed between skinless chicken breasts and skin-on chicken thighs. The freezing treatment was a significant factor in the reduction of both bacteria on the surface of raw poultry products (Table 1). However, none of the reductions were greater than 1 log<sub>10</sub>CFU/mL; therefore, this factor was deemed insignificant from a practical perspective. According to the United States Department of Agriculture Food Safety and Inspection Service (FSIS, 2005), reductions of at least one logarithmic unit are considered of relevance to food processors, meaning that the treatment employed could potentially be used as a decontamination technique. Unstressed *E. coli* tended to yield greater population reductions than unstressed *S. Typhimurium*; however, the same behavior was not observed with cold-stressed cultures, indicating a significant interaction (p<0.05) between the factors “stress” and “bacteria” in reducing the numbers of bacteria on the surfaces of raw poultry products (Figure 1). From a practical point of view, bacteria are more likely to be stressed due to refrigeration or freezing conditions at the production or retail level. Consequently, it is expected that the two species will behave similarly under real conditions.

Using antibiotic resistant and non-resistant strains of *S. Kentucky* and *S. Typhimurium*, Dominguez and Schaffner (2009) found that *Salmonella* can readily survive during frozen storage at -20 °C for up to 16 weeks when inoculated into frozen chicken nuggets and frozen chicken strips, with structural injury as a consequence. The results of the present study agree with those reported by Dominguez and Schaffner (2009) since *Salmonella* numbers were not reduced significantly by the freezing process. Escatín et al. (2000) studied survival of native *Salmonella* for up to 42 weeks of frozen storage of pork at -15 °C and observed significant reductions in the populations throughout time. These researchers found that *S. Heidelberg* and *S.
Agona were the most commonly isolated serotypes (naturally contaminated samples, throughout time).

However, the same findings were not reported by Dykes and Moorhead (2001) who evaluated survival of three *Salmonella* serotypes (S. Brandenberg, S. Dublin and S. Typhimurium, artificial inoculation) on beef trimmings during simulated commercial freezing and frozen storage at -18 and -35 °C for up to 9 months. The authors attributed the unexpected high survival rates to the highly protective level of the subcutaneous fat layer of the product, allowing insulation of bacteria and up to a 100% survival rate. Raj and Liston (1961) proposed that the protein-rich matrix of meat and poultry might bind free water and act as a hydrocolloid, protecting the cells against harmful effects.

Under the experimental conditions used in the present study, neither crust nor complete freezing reduced the population of artificially inoculated *E. coli* or *S. Typhimurium* (< 1 log reduction). In this respect, it would be expected that neither treatment would be useful in reducing the populations of naturally contaminated samples, no matter how low. According to Georgala and Hurst (1963), food poisoning bacteria do not differ greatly from non-pathogens in their survival at low temperatures, as it was observed by using a pathogenic *Salmonella* serovar versus a non-pathogenic *E. coli* strain.

In the present investigation, between 50 and 70% of the initial bacterial populations were injured after refrigeration, crust freezing (20 min, -85 °C) or complete freezing (60 min, -85 °C), with no significant differences detected among any of the treatments. Refrigeration alone was expected to cause cell injury due to the mesophilic nature of *E. coli* and *Salmonella*. Quick freezing could result in injury due to the rapid decrease in temperature. No differences were observed between stressed and unstressed cultures of either bacterium possibly due to the high
freezing rate used in the present experiments. Wesche et al. (2009) indicated that bacterial injury could be defined as the effect of one or more sub-lethal treatments on a microorganism. Significant differences in survival to prolonged frozen and refrigerated storage of cold-adapted *Salmonella* cultures have been reported by Jeffreys et al. (1998).

Structural injury due to crust freezing was assessed by difference in growth on non-selective versus selective media (Brashears et al., 2001; Straka et al., 1959; Wesche et al., 2009; Wuytack et al., 2003). Populations of *S. Typhimurium* that were not cold-stressed tended to have the smallest extent of injury of all followed by stressed populations of *E. coli*. This reflects a significant interaction (p<0.0001) between the application of stress and the bacteria species (Figure 2). The presence of skin was not significant in determining microbial injury. According to Brashears et al. (2001), structural damage results in the loss of the permeability barrier in the cell wall and the cell membrane due to loss of mono- and divalent cations causing conformational and structural change of lipopolysaccharides in the outer membrane of gram-negative cells and teichoic acids in the cell wall of gram-positive microorganisms. Data from the present study demonstrate that quick freezing causes structural injury in bacterial populations however, under the crust freezing conditions used in this study, no significant reduction in inoculated *E. coli* or *S. Typhimurium* were observed. Crust freezing injures bacteria and when combined with other inhibitory hurdles may offer added protection from common pathogens.

**Results and Discussion Study 2**

Crust freezing had little effect on the color of chicken breasts as determined by CIE instrumental chromatic attributes. Unfrozen, skinless samples had significantly higher L* values (p=0.0033) relative to their crust-frozen, skinless counterparts (Table 1). However, average lightness differences were smaller than two units and were not deemed significant from a
practical perspective. Unfrozen and crust-frozen samples that retained skin did not show significant differences in L* values (average 74.64 and 75.14, respectively). Differences in L* values were observed between skinless and skin-on chicken breasts (p<0.0001). On average, skin-on samples were 20 units lighter than skin-off units (75 vs. 55), regardless of the application of a freezing treatment. These results are in agreement with those of Sirri et al. (2010) who found an average L* value of 75.40 for 2,300 yellow-skinned broiler chicken breasts in Italy. Likewise, the mean L* values of approximately 55 for skinless and 75 for skin-on samples reported here are comparable to those obtained by Mielnik et al. (1999) of between 66 and 77 lightness units, depending on whether the measurement was made on the front or back side of the breast and on the type and rate of chilling used. This suggested a possible interaction between skin and freezing method in determining L* values. This interaction was found to be significant in the present study (p=0.0046), mainly due to major differences among samples with skin off compared to those with skin on, as described above. In this study, all of the values were taken from the front or upper side of the chicken breasts in order to be consistent with the location of the measurements and be able to provide objective evidence of treatment effects.

On the other hand, only small variations were observed in L* values throughout the storage period. Like in the present study, Rotabakk et al. (2006) found that average L* values of skinless chicken breasts fillets stored aerobically in refrigeration for up to 24 days was 56 and did not change significantly over storage time. Similarly, in a study on the effect of freeze chilling and modified atmosphere packaging on quality parameters of raw chicken fillets, Patsias et al. (2008) did not find differences in L* values during aerobic, refrigerated storage with values ranging from 50 to 60, similar to those found in the present investigation.
Redness values ($a^*$) remained fairly stable throughout storage with a notable difference at 18 days of storage, when samples with skin on had significantly higher $a^*$ values than their counterparts sampled at day 12 for both unfrozen and crust-frozen units (Table 2). This indicates a significant interaction ($p<0.0001$) between presence of skin and freezing in $a^*$ values of chicken breasts, particularly at the latter stages of aerobic, refrigerated storage. As time increased, significantly lower $a^*$ values were seen for crust-frozen samples, particularly in the absence of skin. However, there was no discernible pattern to indicate why the interaction was significant. Yellowness values ($b^*$) on chicken breasts were determined by the three-way interaction between the factors, which turned out to be was significant ($p<0.01$). No differences in yellowness values ($b^*$) were seen between unfrozen and crust-frozen units, regardless of the presence of skin (Table 3). The only notable difference occurred at day 18, when skin-on, crust-frozen units had higher $b^*$ values than their corresponding skin-on, unfrozen pairs. Average $b^*$ values for unfrozen, skin-on samples were consistently lower than their crust-frozen counterparts and $b^*$ values of chicken breasts tended to increase throughout time becoming significantly higher by day 12 of storage; however, values tended to decrease by day 18.

The $a^*$ values obtained for samples in this study are similar to those reported by Sirri \textit{et al.} (2010) who obtained a mean of 1.16 for chicken breasts, whereas thighs and shanks had lower values. However, $b^*$ values here were much lower than the average 22.77 reported by the same authors. The overall range for redness was -2.10 to 0.78. This range was considered small compared to reports in the literature of up to 12 units (Petracci \textit{et al.} 2004). One possible reason is the natural difference in the concentration of xanthophylls and other pigments present in the feed and later deposited in the epidermis. Furthermore, low myoglobin content of the chicken breasts might have influenced their low $a^*$ values. Still, visually, samples were considered to be
slightly red or pink. The range for yellowness was 1.38 to 3.79, consistent with an average $b^*$ of 3.62 obtained by Wattanachant et al. (2004) and 2.08 reported by Petracci et al. (2004) for broilers chicken breasts in Thailand and Italy, respectively. The latter authors also report a range for $b^*$ values from -3 to 12, indicating that high natural variability among samples play an important role in color determination. Studies have shown higher $a^*$ and $b^*$ values for chicken breasts subjected to blast chilling, of approximately 3 and 12, respectively (Patsias et al. 2008), whereas freezing and frozen storage for up to eight months yielded $a^*$ and $b^*$ mean values of 3.5 and 2.5, respectively (Lee et al. 2008). Reduced freezer burn compared to prolonged frozen storage could potentially be the reason why crust-frozen units in this study presented lower $b^*$ values than in other reports.

Color variation is a major problem in the retail environment because consumers are more sensitive to color variation than to absolute color. Instrumental color data was supported by the fact that no visual discoloration was observed throughout the storage period. Although the processing method, the packaging conditions, the degree of exposure to light and other interactive effects can influence changes in visual color of the products (Petracci et al. 2004; Modi et al. 2006). In the present investigation, the reflectance was stable during storage. Thus, differences observed in other studies could be attributed to natural variability among the chicken meat or skin, to random location of color readings on each unit, or to process variability during scalding, and not necessarily to the treatments (Ellis et al. 2006). The results indicate that chicken breast lightness, redness and yellowness undergo only small changes during aerobic, refrigerated storage and therefore, they are not conclusive parameters in determining shelf life of this kind of food product.
Tenderness is another critical quality and palatability attribute for chicken breast meat (Barbanti and Pasquini 2005; Zhuang et al. 2007). Chicken meat texture has been measured instrumentally using several different probes, the most common being Warner-Bratzler shear-type blade, Allo-Kramer shear, Razor Blade shear and needle puncture. The use of a razor blade shear, like the one used in this study, has the advantage of being less time consuming than the other tests as it requires no weighing or further sample preparation other than cooking (Young and Lyon 1997; Cavitt et al. 2004; Cavitt et al. 2005a; Thielke et al. 2005; Zhuang et al. 2007; Del Olmo et al. 2010). Additionally, the razor blade probe has proven to perform similar to Warner-Bratzler shear-type blade, the typical reference method. Studies by Cavitt et al. (2005b) and Xiong et al. (2006) conclude that all of the instrumental shear stress probes previously mentioned perform similarly for predicting the tenderness of cooked broiler breast meat and correlate well with descriptive and sensory analysis, therefore making the razor blade probe suitable option for measuring tenderness.

As shown in Table 4, differences in tenderness of unfrozen and crust-frozen samples were observed on days 3 and 6 of aerobic, refrigerated storage, particularly for skin-on units \((p<0.05)\). When these differences were present, crust-frozen samples tended to have significantly lower shear energy than unfrozen units, in other words, the latter samples were more tender (McKee 2007). Shear energy values for skin-on samples remained stable throughout time likely because the test accounts for the firmness levels of the skin, not the meat directly. Even when the meat underneath could potentially lack firmness, the skin might remain firm, as reflected by the data presented. The values measured at day 18 for skin-on samples were higher than their crust-frozen counterparts at day 12. On the other hand, skinless samples showed erratic behavior and tended to have lower shear energy values than their skin-on pairs, which is consistent with the fact that
skin would oppose higher resistance to biting and chewing than the meat tissue itself. Skinless, crust-frozen samples had unusually lower shear energy by day 6 and regained firmness by day 18 of storage. Differences noted in tenderness measured as shear energy may be attributed to greater or lower activation rates of calpains acting on proteolysis of the meat muscle. This change in calpains activity may be due to pH variation in the muscle as the tissue ages and to change in the concentration of Ca$^{2+}$ ion during storage (Lee et al. 2008).

According to Lee et al. (2008), refrigerated storage does not significantly cause muscle shrinkage and therefore, softening of the tissue is expected, as opposed to toughening, which occurs during prolonged frozen storage. Loss of firmness as determined by decreasing shear energy values may be a consequence of microbial enzymatic activities, particularly proteolysis caused by Pseudomonas and yeasts and molds. Charles et al. (2006) indicated that when total microbial counts reach $10^8$ logCFU/g, decomposition of the muscle tissue is evident by surface slime formation. In the present investigation, the loss of firmness of the tissue as storage time increases may be due to increasing levels of bacteria, yeasts, and molds throughout time. This relationship, however, was not evaluated statistically.

Table 5 shows the results for aerobic plate counts (APC) of chicken breasts subjected to crust freezing and stored in refrigeration for up to 18 days. Freezing along with prolonged frozen storage have proven to be effective in reducing the number of bacteria in processed chicken products. This was reported by Modi et al. (2006), who froze chicken curry for up to 6 months. In the present study, rapid surface freezing was performed and refrigerated storage did not achieve differences in microbial growth between crust-frozen units and their unfrozen pairs. Time of storage, along with freezing, significantly influenced APC values of chicken breasts. At the two first stages of refrigerated storage, higher bacterial counts (p<0.05) were determined on
crust-frozen chicken breasts compared to their unfrozen pairs, specifically for skin-on samples. However, the same did not occur at and after 12 days of storage, when the counts became stable across time and did not increase significantly by day 18. This means that freezing also interacted significantly with skin for total aerobic bacterial counts of the chicken breasts (p=0.0062). Skin-on, crust-frozen samples had consistently higher bacterial counts than their skin-on, unfrozen counterparts, possibly due to higher number of bacteria being attached primarily to connective tissue, rather than to myofibrils, allowing greater recovery rates (Benedict et al. 1991). It has been hypothesized that the quick decrease in temperature to the freezing state opens up the product structure, resulting in a greater recovery of bacteria trapped in crevices and this may be the reason why crust-frozen samples showed higher bacterial counts that their unfrozen counterparts (Thomas and McMeekin 1981; Lillard 1988; Fagan et al. 2003; Patsias et al. 2008).

Initial low levels of APC (mean of $3.5 \log_{10} \text{CFU/ml}$ 0.1% peptone water) in chicken breasts either with skin off or skin on are indicative of good hygiene during processing. Since samples were brought directly to the laboratory shortly after slaughter and kept on ice during transport, handling was minimal, thus reducing the potential for contamination and bacterial growth rates (Modi et al. 2006). An average increase of $3 \log_{10} \text{CFU/ml}$ between 6 and 12 days was observed for all groups. No significant differences were noted in APC values between 12 and 18 days of storage, as described before. The average increase between these two sampling times was $1.4 \log_{10} \text{CFU/ml}$. Maximum APC values were in the order of $10^{10}$ with an average increase of $2.0 \log_{10} \text{CFU/ml}$ between consecutive sampling times. Similarly, Rotabakk et al. (2006) found that time of storage was a significant factor in determining APC values, no matter what other factors were tested. Their results, however, tended to be lower for equivalent storage times compared to the results in the present investigation. These differences might be due to
methodology employed for bacterial recovery or initial levels of contamination and handling practices prior to enumeration. In this study, bacterial recovery was done by rinsing. Therefore, the results are expressed as log_{10}CFU/ml 0.1% rinse water, whereas in the Rotabakk et al. study, the recovery was done by excision of muscle tissue.

For a given sampling time, no significant differences were seen among skin-off or skin-on samples no matter the application of a freezing treatment. Initially, this was not considered to be the expected outcome, since the skin of poultry is known to retain a great proportion of the total number of bacteria, and certainly the bacterial levels are expected to be higher for skin than for muscle (Daud et al. 1979; Thomas and McMeekin 1981). However, the results of this study are supported by the observations of Berrang et al. (2000) who noted that bacterial populations on skin plus meat, skin alone or meat alone, recovered from split breasts, thighs and drumsticks of broilers purchased at a retail outlet and aseptically skinned in the laboratory were not significantly different from one another. The authors conclude that processing, particularly immersion in the chill tank, allows the counts on the bone-in meat, which is mainly covered by skin, to equalize to that on the skin itself (Thomas and McMeekin, 1981). Additionally, when cutting-up the carcass the muscle surfaces are compromised by exposing them to skin and allowing transfer of water and dissolved substances from skin to meat tissue (Berrang et al. 2000). This is certainly a potential hypothesis for the results of this investigation. Other temperature reduction methods, such as evaporative air chilling and freeze-chilling have also proven ineffective in reducing spoilage microorganisms in chicken carcasses (Mielnik, et al. 1999; Patsias et al. 2008).

Finally, total Yeasts and Molds Counts (YMC) results paralleled those of Aerobic Plate Counts (Table 6). In general, small differences were observed between unfrozen and crust-frozen
chicken breasts, regardless of the presence of skin. In the cases where the differences were significant, crust-frozen units tended to have higher counts possibly due to open pores allowing greater recovery, as previously described for bacterial counts. Presence of skin alone was not of great influence in determining total yeasts and molds counts but interacted significantly with freezing and time (p=0.0023), which were individually significant. The average increase in counts from 6 to 12 days was $1.6 \log_{10} \text{CFU/ml}$. From 12 to 18 days of storage, the APC counts increased on average $1 \log_{10} \text{CFU/ml}$ in average. Maximum YMC were in the order of $10^5 \text{CFU/ml}$ with an average increase of $1.0 \log_{10} \text{CFU/ml}$ between consecutive sampling times.

Initial mean population of yeasts and molds in raw, unprocessed chicken breasts was $3.0 \log_{10} \text{CFU/ml}$, which are similar to those reported by Ismail et al. (2000). The Ismail et al. study found that after two weeks of storage, the final mean yeasts and molds counts of raw chicken breasts and carcasses were $3.7$ and $5.0 \log_{10} \text{CFU/g}$, respectively, equivalent to the results of the present study. The same authors also identified *Yarrowia lipolytica*, *Candida zelanoides* and basidiomycetous yeasts as major isolates, the first one being partly responsible for proteolytic and lipolytic spoilage of chicken meat surfaces. Total counts of yeasts and molds remain a small part of the spoilage microflora throughout time, as noted above. According to Thomas and McMeekin (1981), these microorganisms are able to grown on the skin and muscle of chicken products stored under refrigeration but fail to compete with the pseudomonads and remain an insignificant proportion of the spoilage microflora. The equilibration in competitive flora may be the reason why yeasts and molds counts decreased from day 3 to 6 and then increased again by day 12.

In general, microbial counts increased during storage time as expected, with total bacteria loads being higher than those of yeasts and molds. Before 12 days of aerobic, refrigerated
storage, APC values reached unacceptable levels of $8 \log_{10}\text{CFU/ml}$ 0.1% peptone water, which is generally considered a cut-off point for shelf life of raw chicken meat (Charles et al. 2006; Patsias et al. 2008; Rotabakk et al. 2006), with or without skin, unfrozen or subjected to crust freezing.

**Conclusion**

Crust freezing is a commercially available application aimed to improve quality and extend shelf life of highly perishable foods, such as raw poultry products. In the present study, color attributes L*, a*, and b* did not change greatly over 18 days of refrigerated, aerobic storage and were mostly affected by the presence of skin. Bacterial counts reached unacceptable levels before 12 days of storage. Yeasts and molds counts remained low throughout time. Finally, tenderness, measured by razor blade shear energy, tended to decrease progressively due to deterioration of the meat tissue and was the quality measure most affected by storage time. Under the conditions used, crust freezing did not extend shelf life nor affect quality of raw chicken breasts, with or without skin. In the current study, a static crust freezing method was employed that was closely monitored to mimic the process used commercially and to adhere to the “fresh, not frozen” criteria used commercially. Other factors used in commercial crust freezing of chicken may impact shelf life and may warrant further investigation.

**Tables and Figures Study 1**
TABLE 1. Average population reductions (log10 CFU/mL of rinse) of cold stressed and unstressed *E. coli* and *S. Typhimurium*;
<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Stress</th>
<th>Reduction (log$_{10}$CFU/mL$^1$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>No</td>
<td>0.3±0.1 a</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.2±0.1 b</td>
</tr>
<tr>
<td><em>S. Typhimurium</em></td>
<td>No</td>
<td>NR$^2$</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.2±0.1 b</td>
</tr>
</tbody>
</table>

$^1$ Reductions estimated as bacterial counts recovered from the unfrozen samples minus bacterial counts recovered from crust-frozen or complete-frozen. Numbers for skin and freezing factors were pooled as these factors were not statistically significant (p>0.05). Differences were estimated as CFU/mL and then transformed to logarithmic values.

$^a,b$ means followed by different letters were significantly different (p<0.05). N=9.

$^2$ NR: No reduction.
Figures and Tables for Study 2

Table 1. CIE Lightness (L*) values of refrigerated skinless and skin-on chicken breasts subjected to crust freezing.

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Skinless Unfrozen</th>
<th>Skinless Crust-frozen</th>
<th>Skin-on Unfrozen</th>
<th>Skin-on Crust-frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>56.84&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>54.90&lt;sup&gt;A2&lt;/sup&gt;</td>
<td>73.30&lt;sup&gt;B2&lt;/sup&gt;</td>
<td>75.26&lt;sup&gt;B12&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>57.58&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>57.10&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>76.24&lt;sup&gt;B1&lt;/sup&gt;</td>
<td>76.48&lt;sup&gt;B1&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>55.86&lt;sup&gt;A12&lt;/sup&gt;</td>
<td>53.66&lt;sup&gt;A2&lt;/sup&gt;</td>
<td>75.70&lt;sup&gt;B1&lt;/sup&gt;</td>
<td>74.63&lt;sup&gt;B12&lt;/sup&gt;</td>
</tr>
<tr>
<td>18</td>
<td>54.54&lt;sup&gt;A2&lt;/sup&gt;</td>
<td>53.64&lt;sup&gt;A2&lt;/sup&gt;</td>
<td>73.33&lt;sup&gt;B2&lt;/sup&gt;</td>
<td>74.20&lt;sup&gt;B2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with the same letter in a row or numerical value in a column are not significantly different for a significance level of 0.05. Standard error for individual means is 0.82.

Table 2. CIE redness (a*) values of refrigerated skinless or skin-on chicken breasts subjected to crust freezing.

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Skinless Unfrozen</th>
<th>Skinless Crust-frozen</th>
<th>Skin-on Unfrozen</th>
<th>Skin-on Crust-frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>-0.49&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>-1.78&lt;sup&gt;B1&lt;/sup&gt;</td>
<td>-0.48&lt;sup&gt;AB1&lt;/sup&gt;</td>
<td>-1.14&lt;sup&gt;AB1&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>-1.00&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>-1.37&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>-0.80&lt;sup&gt;A2&lt;/sup&gt;</td>
<td>-0.50&lt;sup&gt;A2&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>-0.52&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>-1.58&lt;sup&gt;B1&lt;/sup&gt;</td>
<td>-0.93&lt;sup&gt;AB2&lt;/sup&gt;</td>
<td>-0.76&lt;sup&gt;AB2&lt;/sup&gt;</td>
</tr>
<tr>
<td>18</td>
<td>-0.34&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>-2.10&lt;sup&gt;B1&lt;/sup&gt;</td>
<td>0.78&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>0.76&lt;sup&gt;A2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with the same letter in a row or numerical value in a column are not significantly different for a significance level of 0.05. Standard error for individual means is 0.32.
Table 3. Yellowness (b*) values of refrigerated skinless or skin-on chicken breasts subjected to crust freezing.

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Skinless</th>
<th>Skin-on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfrozen</td>
<td>Crust-frozen</td>
</tr>
<tr>
<td>3</td>
<td>1.67&lt;sup&gt;A2&lt;/sup&gt;</td>
<td>2.20&lt;sup&gt;A2&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>1.88&lt;sup&gt;A2&lt;/sup&gt;</td>
<td>2.99&lt;sup&gt;A12&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>3.70&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>3.60&lt;sup&gt;A1&lt;/sup&gt;</td>
</tr>
<tr>
<td>18</td>
<td>3.79&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>3.29&lt;sup&gt;A12&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>A,B,1,2</sup> Values with the same letter in a row or numerical value in a column are not significantly different for a significance level of 0.05. Standard error for individual means is 0.34.

Table 4. Shear energy values (g-mm) of refrigerated skinless and skin-on chicken breasts subjected to crust freezing.

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Skinless</th>
<th>Skin-on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfrozen</td>
<td>Crust-frozen</td>
</tr>
<tr>
<td>3</td>
<td>12375&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>10303&lt;sup&gt;A12&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>11251&lt;sup&gt;AB1&lt;/sup&gt;</td>
<td>4624&lt;sup&gt;C2&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>8734&lt;sup&gt;AB1&lt;/sup&gt;</td>
<td>8294&lt;sup&gt;AB2&lt;/sup&gt;</td>
</tr>
<tr>
<td>18</td>
<td>13984&lt;sup&gt;A1&lt;/sup&gt;</td>
<td>13053&lt;sup&gt;A1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>A,B,1,2</sup> Values with the same letter in a row or numerical value in a column are not significantly different for a significance level of 0.05. Standard error for individual means is 1144.
Table 5. Aerobic plate counts (log$_{10}$CFU/ml) of refrigerated skinless and skin-on chicken breasts subjected to crust freezing.

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Skinless</th>
<th>Skin-on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfrozen</td>
<td>Crust-frozen</td>
</tr>
<tr>
<td>3</td>
<td>3.5 $^{AB3}$</td>
<td>4.0 $^{AB3}$</td>
</tr>
<tr>
<td>6</td>
<td>5.4 $^{AB23}$</td>
<td>5.4 $^{AB23}$</td>
</tr>
<tr>
<td>12</td>
<td>8.7 $^{A12}$</td>
<td>8.6 $^{A12}$</td>
</tr>
<tr>
<td>18</td>
<td>10.1 $^{A1}$</td>
<td>10.3 $^{A1}$</td>
</tr>
</tbody>
</table>

$^{A,B,1,2}$Values with the same letter in a row or numerical value in a column are not significantly different for a significance level of 0.05. Standard error for individual means is 0.6.

Table 6. Yeast and mold population (log$_{10}$CFU/ml) on refrigerated skinless and skin-on chicken breasts subjected to crust freezing.

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Skinless</th>
<th>Skin-on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfrozen</td>
<td>Crust-frozen</td>
</tr>
<tr>
<td>3</td>
<td>2.9 $^{B2}$</td>
<td>3.8 $^{A12}$</td>
</tr>
<tr>
<td>6</td>
<td>2.8 $^{A2}$</td>
<td>2.8 $^{A2}$</td>
</tr>
<tr>
<td>12</td>
<td>4.6 $^{A12}$</td>
<td>4.4 $^{A12}$</td>
</tr>
<tr>
<td>18</td>
<td>5.3 $^{A1}$</td>
<td>5.7 $^{A1}$</td>
</tr>
</tbody>
</table>

$^{A,B,1,2}$Values with the same letter in a row or numerical value in a column are not significantly different for a significance level of 0.05. Standard error for individual means is 0.4.

References Study 1


References Study 2


Presentations


Gary, C., Chaves, B. Han, I. and Dawson, P. 2009. The effect of quick freeze-thaw cycles on the growth of E. coli and on RTE turkey bologna. Summer Program for Research Interns, SC Governor’s School Science and Mathematics, Clemson University July 18th.

Publications
