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EFFECT OF CRUST FREEZING ON THE QUALITY AND SURVIVAL OF ESCHERICHIA COLI AND SALMONELLA TYPHIMURIUM IN RAW POULTRY PRODUCTS

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EFFECT OF CRUST FREEZING ON THE QUALITY AND SURVIVAL OF
ESCHERICHIA COLI AND S*ALMONELLA* TYPHIMURIUM IN RAW POULTRY
PRODUCTS

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ABSTRACT

Industry has widely used freezing as a strategy to halt pathogen growth and more recently, crust freezing has been suggested as a means to improve mechanical operations, quality, and safety of poultry products. Two separate studies were conducted to evaluate the effect of crust freezing (20 min, -85 °C) on the survival of *Escherichia coli* and *Salmonella* Typhimurium and on quality and shelf life of raw chicken breasts, with or without skin. For the first study, ampicillin-resistant *E. coli* JM 109 and nalidixic acid-resistant *S*. Typhimurium were used in the experiments. A set of cultures was subjected to cold-shock stress by storage at 4 °C for 10 days. Commercial chicken breasts without skin and chicken thighs with skin were inoculated with each bacterium in separate experiments after being either cold-shocked or non-cold-shocked prior to inoculation. Samples were crust frozen at -85 °C for 20 min or completely frozen at -85 °C for 60 min. *E. coli* and *S*. Typhimurium were recovered on appropriate selective and non selective media containing the corresponding antibiotic. Log reductions and injury extent were calculated and treatments were compared using ANOVA. For the second study, quality of fresh chicken breasts during aerobic, refrigerated storage for up to 18 days was assessed by means of International Commission on Illumination (CIE) color parameters L*, a* and b*; tenderness; and total aerobic (APC) and yeasts and molds counts (YMC). Skin-on breasts had significantly higher L* values compared to skinless units (average 75 vs. 55), whereas a* and b* remained relatively constant no matter the presence of skin, freezing or time. Values oscillated between -2.10 to 0.78 and 1.38 to 3.77, respectively. Shear energy varied erratically for skinless samples but tended to remain constant

throughout time for skin-on units. Microbial load increased over time and was considered unacceptable over 8.0 $\log_{10}$CFU/ml, which occurred before 12 days of storage. Under the experimental conditions used, crust freezing did not show practical significance for initial reduction of *E. coli* or *S.* Typhimurium in raw poultry products and was not useful in extending shelf life of raw chicken breasts, with or without skin.

DEDICATION

I dedicate this work to my parents, my sister and my aunt, without whose support and encouragement, this would have not been possible.

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CHAPTER I

REVIEW OF LITERATURE

Introduction

Poultry meat is a very popular food commodity around the world and its

consumption in many countries has increased over the last few decades. At present,

approximately 30% of the world's total meat consumption is poultry (del Rio et al.,

2007). Some of the reasons why poultry is such a popular food item are the low cost of

product (passed onto consumers with low purchase price), the low fat content, and the

high nutritional value. Because poultry products are highly perishable foods, one of the

main concerns for the industry is the shelf life extension. Ways to achieve shelf life

extension include the application of the hurdle technology concept and the use of natural

food preservatives to ensure protection from both spoilage and pathogenic

microorganisms (Chouliara et al., 2007). Although more efficient and technologically

advanced processing methods have resulted in a greater availability and a wider variety

of poultry products for consumers, these advances can have both desirable and

undesirable effects on sensory, functional, chemical, and microbiological properties of

the finished products. Food safety and desirable sensory perception have become a

primary issue for many consumers and play a significant role in the consumption of

poultry products (McKee, 2007a). The quality of poultry products is determined by their

sensory attributes, chemical composition, physical properties, and level of

microbiological and toxicological contaminants, shelf life, and packaging. Among of all these factors, the sensory attributes have the crucial influence on the market success of the product (Probola and Zander, 2007). Additionally, the microbiological safety of meat and poultry products has assumed paramount importance for industry, consumers, and public health officials because associated foodborne bacterial infections remain of great concern worldwide in terms of economic losses and health effects (Bohaychuk et al., 2006; Hernández et al., 2005). It is well known that the consumption of poultry products is a vehicle for the transmission of foodborne pathogens, especially *Campylobacter jejuni*, *Salmonella* spp., *Listeria monocytogenes*, and to a lesser extent, *Escherichia coli* and *Shigella* (Bryan and Doyle, 1995; Sackey et al., 2001). The relative risk of foodborne diseases transmission through poultry is high since contamination with different pathogenic organisms may occur at several production levels (Moore, 2004; Skandamis et al., 2007).

Freezing is a food preservation method that can potentially deliver a high degree of safety, nutritional value, sensory quality, and convenience. In the past, most foods were cooked and immediately consumed; however, freezing technology provides consumers and processors with alternatives for highly perishable products. More recently, the focus has shifted to convenience and a much wider range of foods are available. However, quality is an increasing concern for consumers and the challenge for the food industry is to maximize the sensory and nutritional value without compromising microbiological safety (Archer, 2004; Skandamis et al., 2007).

Freezing as a Food Preservation Method

Freezing is a method of food preservation that slows the physical, chemical and microbiological activity that causes deterioration in foods. The essential step in freezing is to lower the temperature of foods with the intention of extending useful storage life and increasing product safety. During freezing, microbial growth is halted, although residual microbial or endogenous enzyme activity may persist and eventually spoil a product. Although low temperature storage slows deleterious reactions, it does not completely stop them. Holding foods at low temperatures merely reduces the rate at which these changes take place (Berry et al., 2008). Foods begin to freeze somewhere in the range of -0.5 to -3 ℃. As water is converted into ice during freezing, the concentration of solutes in the unfrozen water increases, further decreasing its freezing point. Low temperature is not the only inhibitory factor operating in frozen food. They also have a low water activity (*Aw*) produced by removal of water in the form of ice (Adams and Moss, 2008; Ray and Bhunia, 2008). In addition, the freezing of foods is accompanied by changes in properties such as pH, titratable acidity, ionic strength, viscosity, osmotic pressure, vapor pressure, freezing point, surface and interfacial tension, and oxidation-reduction potential. Therefore, the freezing of foods results in complex physical and chemical changes and depends upon three factors: nature of the process, nature of the food, and type of microorganisms present in the food system (Jay, 2005).

The freezing process could be either rapid or slow. In rapidfreezing, the temperature of foods is lowered to about -20 °C within 30 minutes. Slow freezing refers to the process whereby the desired temperature is achieved within three to 72 hours. Most

domestic freezers employ a slow freezing method. Slow freezing favors large extracellular crystal formation, while quick freezing promotes the formation of small intracellular ice crystals. Ice crystals grow in size and cause cell damage by disrupting membranes, cell walls, and internal structures to the point where the thawed product is quite unlike the original product in texture and flavor, limiting the freezer life of certain foods. Upon thawing, foods frozen by the slow freezing method tend to lose more water in the form of drip than quick-frozen foods held for comparable periods of time (Jay, 2005). The composition, pH, Aw, and presence of microbial inhibitors or preservatives in a food can greatly influence chemical changes and viability of microorganisms during storage at freezing temperature. Lastly, the nature of the microorganism defines their susceptibility to freezing. In general, Gram-negative bacteria are more susceptible to the damaging effect of freezing than Gram-positive bacteria. Gram-positive cell walls are relatively simple in structure, comprising several layers of peptidoglycan connected to each other by cross-linkages to form a strong, rigid scaffolding. In addition, they contain acidic polysaccharides called teichoic acids. Gram-negative cells have a much thinner layer of peptidoglycan, making the wall less sturdy. In addition, cells from the early exponential phase of growth are more susceptible to freezing than those from the early stationary phase. Species and strains of microorganisms also differ greatly in sensitivity and resistance to freezing damage. Bacterial spores are virtually unaffected by freezing (Ray and Bhunia, 2008). Food materials often act as cryoprotectants for bacteria allowing them to survive for long periods in the frozen state (Adam and Moss, 2008).

Shelf Life of Frozen Poultry Products

Extending the shelf life of poultry products is a major concern for the industry. The shelf life of a frozen food is a complex concept that depends on the characteristics of the food product and the environmental conditions to which the food is exposed after being subjected to the freezing process. In addition to freezing rate, the three main factors that affect product quality of any given frozen food are: initial quality of the original foodstuff, particularly initial microbial loads; processing and packaging of the product, including pre-freezing treatments and formulation; and temperature and duration of storage. Shelf life testing consists basically of selecting the quality characteristics which deteriorate most rapidly in time (Berry et al., 2008).

Color, appearance, and texture of poultry products, both fresh and frozen, are important factors that consumers will consider before making a purchase decision According to Kennedy et al. (2005), tissue color is the predominate factor for consumer preference for poultry. Color measurements are mainly aimed to support visual observations and to provide objective evidence of treatment effects that can be statistically analyzed. In general, poultry skin color ranges from cream to yellow appearance (Sirri et al., 2010). Poultry meat color is affected by factors such as bird age, gender, strain, dietary carotenoids such as xanthophylls, intramuscular fat, meat moisture content, preslaughter conditions, and processing variable such as cooking, and chilling/freezing (Del Olmo et al., 2010; McKee, 2007b; Pérez-Álvarez and Fernández-López, 2009; Totosaus, 2007). Scalding temperature during processing of poultry also affects color. According to Petracci and Fletcher (2002), three types of scalding are

generally used depending upon the type of poultry and the difficulty of picking: semi-scald or slack scald (120 sec; 125-130 °F/52-54 °C); sub-scald (90 sec, 138-140 °F/58-60 °C); and hard or full scald (45 s, 140-150 °F/60-65 °C). In general, as the temperature of scalding increases, appearance of the flesh becomes more bleached. Raw muscle typically has a pink to reddish color due to myoglobin and, to a lesser extent, hemoglobin. When cooked, however, muscles that are used more extensively, including the leg and thigh, appear darker due to the higher quantity of myoglobin while less-used muscles such as the breast are referred to as white meat (McKee, 2007b). The essential difference is between fibers with a predominantly oxidative metabolism and those with a mainly glycolytic metabolism. Oxidative fibers have more mitochondria, containing red cytochromes, and a higher concentration of the red pigment myoglobin in their sarcoplasm. Oxidative fibers therefore appear red in color. Myoglobin transports oxygen within the cell, just as the hemoglobin of the red blood cells transport oxygen from the lungs to the rest of the body. Glycolytic fibers contain relatively small amounts of cytochromes or myoglobin and thus appear white (Warriss, 2000).

On the other hand, texture is comprised of a complex set of properties that include thickness, gumminess, chewiness and fracturability. One of the most important textural properties in meats is tenderness, which is defined as the ease with which a piece of meat can be cut and chewed. Juiciness, a property related to the fat and moisture content of meat, and water holding capacity are also important textural components in poultry meats (Barbut, 2009; McKee, 2007a).

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Microbial Spoilage of Chilled and Frozen Poultry Products

The growth and development of the microbiota of poultry, and consequently, the rate of spoilage, is governed by intrinsic parameters of the product, such as pH and buffering capacity, water activity, redox potential, presence of antimicrobial compounds, and nutrient composition. Extrinsic factors also affecting spoilage rates include temperature, relative humidity, the composition of the surrounding gaseous atmosphere, and type and extent of processing. Of all these factors, temperature is by far the most important for the control of microbial growth (James and James, 2002; Guerrero-Legarreta, 2009; Paramithotis et al., 2009). Microbiological food spoilage is manifested in several different ways. According to Thomas and McMeekin (1981), visible microbial growth may be apparent in the form of surface slime or colonies, loss of texture due to degradation of structural components of the food, or production of chemical substances of microbial metabolism such as gas, pigments, polysaccharides, off-odors and flavors. A general feature of microbial spoilage is its relatively sudden and unexpected onset. This is a reflection of the exponential nature of microbial growth (Adam and Moss, 2008).

As with most meat processing, poultry slaughter involves a shift from a mesophilic environment to a psychrotrophic environment at the end of the production cycle. Thus, spoilage of poultry is most often associated with psychrotrophic microorganisms. Poultry harbors a large number of different microorganisms capable of spoiling both raw and processed products. However, because meat and poultry are stored at temperatures between –1 and 5 ℃, psychrotrophs constitute major spoilage microflora. In general, the microbiota reflects the slaughtering and processing environments. If the

product is kept under cold, aerobic conditions, psychrotrophic aerobes will grow rapidly,
especially Gram-negative rods, such as *Pseudomonas, Shewanella putrefaciens*,
Alteromonas, Proteus, Psychrobacter and *Alcaligenes*. Other organisms are usually only
a minor component of the spoilage microflora, but include psychrotrophic
Enterobacteriaceae such as *Serratia liquefaciens* and *Enterobacter agglomerans* (Arnaut-
Rollier et al., 1999; Guerrero-Legarreta, 2009; Paramithotis et al., 2009). *Pseudomonas*
spp. are the most common organisms associated with spoilage of refrigerated poultry
meat stored under aerobic conditions. In addition to being psychrotrophic, the
pseudomonads are able to grow at pH levels of 5.5 to 7.0 and are highly oxidative, which
gives them the ability to use nitrogen compounds as an energy source (García-López et
al., 1998; McKee, 2007a). Yeast loads have been reported to increase in both chicken and
turkey during cold storage. Proteolytic and/or lipolytic species of yeasts such as *Candida
zeylanoides* and *Yarrowia lipolytica* are some of the most commonly isolated from fresh
and spoiled poultry (Dillon, 1998; Ismail et al., 2000; McKee, 2007). The psychrotrophic
microorganisms responsible for spoilage of poultry meat are typically present at low
levels immediately after slaughter but flourish rapidly during cold storage to produce
changes in the aroma, appearance, and texture of meat. Off-odors are typically one of the
first organoleptic characteristics to be detected in spoiling poultry and become noticeable
when microbial levels reach 10^6 to 10^8 CFU/cm^2. Off-odors are attributed primarily to
Shewanella putrefaciens A, B, and D; *Pseudomonas fluorescens* A, B, and D; and *Ps.
lundensis* and *Ps. fragi*. Bacterial metabolism produces a complex mixture of volatile
esters, alcohols, ketones and sulfur-containing compounds which collectively comprise

the off-odors detected (Daud et al., 1979; Dimick and Mac Neil, 1970; Rodríguez-Pérez et al., 2003). Sliminess on poultry typically occurs after off-odors are apparent and has been associated with microbial levels of 10^6 to 10^9 CFU/cm^2 (Charles et al., 2006; Rodríguez-Pérez et al., 2003).

Effects of Freezing on Bacteria Associated with Foods

Freezing is an effective means of controlling microbial growth. Limited death may occur during freezing, especially during slow freezing of Gram-negative bacteria. However, freezing is not a reliable means of inactivating microorganisms. From the strict standpoint of food preservation, freezing should not be regarded as a way to destroy foodborne microorganisms (Adams and Moss, 2008; Jay, 2005). The rate of freezing is known to influence the microbiological status of poultry. While it is generally true that total numbers of microorganisms decrease slightly during freezing, frozen storage and thawing, growth of some microorganisms can occur at temperatures below 0 °C, with frequent reports of growth down to −5 °C and less frequently down to −10 °C. Generally speaking, temperatures below −10 °C are considered to effectively inhibit growth of microorganisms associated with foods. Therefore, the rate of cooling is not only relevant to killing or reducing effects on microorganisms but is also important for preventing growth at sub-zero temperatures. Growth at temperatures below 0 °C is more likely to be that of yeasts and molds rather than bacteria. This is consistent with the growth of fungi under lower water activity conditions (Jay, 2005; Warriss, 2000). However, even though freezing inhibits the metabolism of microorganisms during frozen storage, enzyme activity still continues, particularly if temperature abuse occurs (Berry et al., 2008).

Maximum lethality is seen with slow freezing where exposure to high solute concentrations is prolonged. Survival is greater with rapid freezing where exposure to these conditions is minimized. However, food freezing processes are not designed to maximize microbial lethality but to minimize loss of product quality (Ray and Bhunia, 2008).

The ability of organisms to grow at low temperatures appears to be particularly associated with the composition and architecture of the plasma membrane. As the temperature is lowered, the plasma membrane undergoes a phase transition from a liquid crystalline state to a rigid gel in which solute transport is severely limited. The temperature of this transition is lower in psychrotrophs and psychrophiles largely as a result of higher levels of unsaturated and short chain fatty acids in their membrane lipids. If some organisms are allowed to adapt to growth at lower temperatures they increase the proportion of these components in their membranes (Adams and Moss, 2008). The following salient facts, presented by Georgala & Hurst (1963), Jay (2000) and Berry et al. (2008), summarize what happens to certain microorganisms upon freezing:

- There is a sudden mortality immediately upon freezing, varying with species.
- The proportion of cells surviving immediately after freezing die gradually when stored in the frozen state.
- Freezing results in a loss of cytoplasmic gases such as O_2 and CO_2.
- Freezing affects concentration of cellular electrolytes.

- There is a general alteration of the colloidal state of cellular protoplasm. The lowered water content, along with the concentration of electrolytes, affects the change in state of cellular proteins.

- Freezing induces temperature shock in some microorganisms. This is true more for thermophiles and mesophiles than for psychrophiles.

- Freezing causes metabolic injury to some microbial cells. Some bacteria have increased nutritional requirements upon thawing from the frozen state.

Microbial Cold Shock

Use of freezing to minimize growth of spoilage organisms and to control pathogens has resulted in increased attention to microbial adaptation to freezing conditions. Bacterial adaptation to low temperature, known as cold shock, is thought to involve modification of membrane lipid composition for the purpose of maintaining optimum membrane fluidity in a process called homeoviscous adaptation (Juneja et al., 2003). Although mesophiles cannot grow at chill temperatures, they are not necessarily killed. The extent of cold shock depends on a number of factors such as the organism (Gram-negatives appear more susceptible than Gram-positives), its phase of growth (exponential-phase cells are more susceptible than stationary phase cells), the temperature differential (the larger it is, the greater the damage), and the growth medium (cells grown in complex media are more resistant). The principal mechanism of cold shock appears to be damage to membranes caused by phase changes in the membrane lipids which create hydrophilic pores through which cytoplasmic contents can leak out.

An increase in single-strand breaks in DNA has also been noted as well as the synthesis of specific cold-shock proteins to protect the cell (Dodd et al., 2007).

One of the major concerns regarding freezing as a food safety technology is the fact that bacterial cells remain pathogenic after the treatment is applied. This phenomenon had been studied by Sorrells et al. (1970), who found that freezing a cell suspension of *S*. Gallinarum at -75 °C in a dry ice-acetone bath and even storing it at -20 °C for one day yielded uninjured and sublethally injured cells with no difference in pathogenicity compared to unfrozen cultures. These researchers determined pathogenicity by injecting 1 ml of the treated suspension in chicks 6-weeks old and concluded that the cells can survive freezing and are able to recover if the conditions are favorable, leading to a potential public health risk of frozen products (Sorrells et al., 1970). Metabolic injury of *S*. gallinarum in this study was manifested by an increase in nutritional requirements. This was based on a greater number of colonies appearing on the complete medium as compared to colony formation on the minimal medium. Metabolic injury was obtained by freezing and thawing the cells without storage. Storage of the cells at -20 C resulted in additional injury. The absence of a detectable difference between the effects of uninjured and injured cells on chick mortality suggests that metabolic injury due to freezing did not alter pathogenicity. Repair of the injury could have occurred while the cells were within the intraperitoneal cavity of the chick. This might be expected since replica plating indicated that injury was repairable in a nutritionally adequate environment (Sorrells et al., 1970). More information on bacterial stress and sublethal injury can be found in

reviews by Aldsworth, (1999); Everis (2001); Dodd et al. (2007); Wu (2008); and Wesche et al. (2009).

Foodborne Pathogens Associated with Poultry Products

Although various foods can serve as sources of foodborne illness, poultry and their products are important sources of human disease with a variety of foodborne pathogens. The origin of microbial contamination in poultry is varied: vertical or horizontal transmission on farms and during transport; slaughter and processing, particularly in scalding, defeathering and eviscerating, and separation into giblets, as well as cross contamination during deboning, slicing, chopping, grinding, and preparation for direct consumption at home (Kusumaningrum et al., 2004; Nørrung et al., 2009). In general, the presence of small numbers of pathogens in raw materials is not a problem because meat and poultry are normally cooked before consumption (James and James, 2002). It is generally agreed that the internal tissues of healthy slaughter animals are free of bacteria at the time of slaughter. However, under the current practices of meat and poultry processing, it is impossible to guarantee sterility of the final products (Jay, 2005). The main bacterial hazards of concern in meat and poultry include infectious Gram-negative bacteria such as salmonellae, *Campylobacter* spp., Verotoxigenic *Escherichia coli* and *Yersinia enterocolitica*; Gram-positive infectious bacteria such as *Listeria monocytogenes*, as well as Gram-positive toxicogenic bacteria such as *Staphylococcus aureus*, *Clostridium perfringens* and *Cl. botulinum* (Matagaras and Drosinos, 2009; Paramithotis et al., 2009; Tessi et al., 2002).

Coliforms, especially *E. coli*, are microorganisms of concern in almost every food product, since high counts of coliforms and presence of *E. coli* in foods usually reflect unhygienic handling during production, improper storage conditions and/or post-process contamination. Environmental cross contamination is one of the major sources of coliforms in food systems (Keeratipibul et al., 2009). On the other hand, *Salmonella* is a microorganism of paramount importance for the poultry industry, indicated by the large number of poultry products recalls due to possible or confirmed contamination with the pathogen. Animals other than humans are major reservoirs of salmonellae. Even when these bacteria are not a normal part of the animal's microflora, they are readily acquired from the environment and are harbored in the intestinal tract of mammals and birds. Intestinal colonization and contamination occur on farms and during transport for slaughter, and are favored by intensive rearing of animals kept together in the same facility. The sanitary conditions of the feed supply and of the environment to which livestock and poultry are exposed influence the proportion of animals infected with *Salmonella*. Furthermore, when moving animals from the farms to the slaughterhouses there is an additional transfer of salmonellae through feces, increasing the numbers of microorganisms prior to entering the processing plants (Bryan and Doyle, 1995). The mechanism of contamination of carcasses with *Salmonella* involves retention of bacteria in a liquid film on the skin from which they migrate into the skin and become entrapped in ridges and crevices. Moreover, this microorganism has the ability of rapidly adhering to the polysaccharide materials surrounding collagen fibers in the skin. Once they infiltrate under the skin and attach to the muscle tissue, they are inaccessible to

bacteriocidal substances or processes that may be applied, posing a major risk of contaminating subsequent food processing and preparation environments (Bryan and Doyle, 1995; Firstenberg-Eden, 1981; Thomas and McMeekin, 1981). The infective nature of salmonellae makes their presence in foods a matter of concern, even if only low numbers are present and no opportunity for multiplication arises. A heavily contaminated food would not necessarily have to be mishandled to become dangerous, although such abuse might increase an already existing danger (Georgala and Hurst, 1963).

The recovery of bacteria from poultry products

Among the techniques available for sampling poultry are those involving surface swabbing, whole carcass rinsing, tissue excision and maceration, repeated dipping of carcasses in diluent, collection of drip, high-pressure spraying or scraping of a defined area of skin, spraying the abdominal cavity, and lifting of skin contaminants with an agar contact plate or nitrocellulose membrane. Other techniques include removal of an area of skin and sample the underlying muscle and more recently, the whole carcass incubation Mead et al. (2010). The techniques most commonly used for sampling carcasses in the plant are those involving surface swabbing (especially for larger carcasses), whole carcass rinsing, and maceration of skin samples. Rinse sampling is used in the United States within the USDA Food Safety and Inspection Service pathogen reduction program, whereas sampling of neck skin is preferred in the European Union (EU). The EU method samples only a small proportion of the skin but has the advantage that carcasses can be sampled without their removal from the processing line (Mead et al. 2010). Thus, sample collection is faster and less laborious than whole carcass rinse sampling. Although

recovery of *Salmonella* tends to be lower with carcass swabbing, rinse sampling and neck skin maceration give comparable results, as proved by Kotula and Davis (1999) and Cox et al. (2010). These authors found that the 1% buffer peptone water carcass rinse method for detecting *Salmonella* on broiler carcasses yielded similar results to those obtained with the neck skin excision method. In general, no significant differences were observed between the two methods for *Salmonella* prevalence. However, both methods produced false-negative *Salmonella* results, which could potentially hinder efforts to globally standardize one particular method.

In contrast to the generally similar results obtained by the excision methods or by rinsing, carcass swabbing yields different results, as shown by Gill and Badoni (2005). As values obtained by swabbing could be either higher or lower than those recovered using other methodologies, there appears to be a variable relationship between swabbing and other techniques for carcass bacterial numbers. Therefore, swabbing would seem to be an inappropriate method for recovering bacteria from broiler carcasses. Conversely, as sampling of neck skin would usually be more convenient and less destructive of product than other methods, it would seem to be the preferable method of sampling for the enumeration of bacteria on broiler carcasses. Testing products at the retail stage rather than during processing is more relevant to the exposure of consumers to *Salmonella* via raw poultry meat. The results obtained can be of greater value in assessing the human health risk, which is required in risk assessments, and in verifying the effectiveness of *Salmonella* control measures for different types of product. All the main forms in which poultry products are marketed should be sampled, e.g., whole carcasses, portions, meat

preparations, and fresh and frozen products, and it will be important to distinguish between domestic and imported products (Mead et al. 2010).

Incidence of *Salmonella* in raw, chicken products

Salmonellae are found ubiquitously in the environment and are universally recognized as zoonotic agents. The prevalence of *Salmonella* in chicken products could be as low as one percent or as high as 60%. The environmental and processing conditions determine the final numbers once the product reaches the consumer (Bryan and Doyle, 1995). According to Uyttendaele et al. (1998), the serovars involved in salmonellosis vary geographically, but frequently include *S*. Typhimurium and *S*. Enteriditis as major causes of disease. The same authors found that the average prevalence of *Salmonella* in poultry carcasses in Belgium during a four-year period was 25.5%. Their study also showed that there is a 100%-increase in the number of *Salmonella*-positive samples when cutting the carcasses into individual parts, presumably due to cross-contamination, and that skinless parts are less likely to harbor pathogenic microorganisms compared with samples in which skin is retained. However, Capita et al. (2003) examined the incidence of *Salmonella* in chicken products in Spain and found a higher rate of contamination of the whole birds (55%) compared with that of the chicken parts (40%), with *S*. Enteriditis being the most common serotype. The recovery method might have influenced the results given that all the samples in the Belgian study had been frozen before enumeration. Soultos et al. (2003) report a 1.5% prevalence of *Salmonella* in Northern Ireland, including the presence of *S*. Infantis and *S*. Tennessee. They concluded that the measures undertaken by the poultry industry to control this pathogen have been effective in this

country. More recently, Pointon et al. (2008) found that the average prevalence of

Salmonella was 41.6% and varied according to the product type (whole bird vs.

individual parts) and retail mode (butcher, supermarket, or specialty store). They also

found that whole carcasses usually had lower contamination levels than individual parts,

as previously described by Uyttendaele et al. (1998). The dominant serotypes were Sofia,

Typhimurium, Subsp. I, and Infantis similar to those found by Eglezos et al. (2008), who

characterized the bacteriological profile of raw, frozen chicken nuggets from an

Australian processing plant. In the Canary Islands, Spain, Hernández et al. (2005)

analyzed imported frozen chicken carcasses, legs, and breasts and found a 16.5%

prevalence of *Salmonella*, with serotypes Enteriditis, Heidelberg, and Typhimurium

being the most commonly isolated. Likewise, Eglezos et al. (2008) enumerated *E. co*li to

count with a hygiene indicator of fecal contamination and *Salmonella* to count with a

prevalence reference. An *E. coli* prevalence of 47%, indicative of poultry carcass input

cross-contamination and proliferation, did not correlate with the semi-quantitative

determination of *Salmonella* (positive in 8.7% of the samples), which suggested a

possible non-fecal route of contamination of with *Salmonella*, possibly environmental

harboring. Serotypes Subsp. I, Typhimurium, and Sofia were among the positive isolates.

Additionally, poultry has been reported to be a vehicle for the transmission of

antimicrobial-resistant *Salmonella*. In Portugal, a study conducted by Antunes et al.

(2003) revealed a 60% incidence of *Salmonella*, in particular *S*. Enteriditis and *S*. Hadar.

Factors affecting survivability of Salmonella to freezing in meat and poultry products

Although freezing is a common process for meat and poultry products, its role in the control of pathogens has not been fully explored and the effect of cyclic freezing and thawing on most bacteria are not well documented. One of the reasons for this is that there are multiple factors to take into consideration to achieve a desired lethality by freezing. The cellular events that occur upon freezing are complex and varied and depend on the matrix, pH, holding time and temperature, presence of chemicals and cryoprotectants, the bacteria strains, and the process rate (Archer, 2004). Furthermore, the type of organisms that lose their viability in this state differ from strain to strain and depend on the type of freezing employed, the nature and composition of the food in question, the length of time of freezer storage, and other factors, such as temperature of freezing (Jay, 2005). The following describe some of the reported effects of freezing based on process, microorganism and matrix characteristics.

Freezing rates

The freezing rate and the lowest temperature of freezing during storage dictate the extent of microbial damage from ice crystals. Damage and death are more extensive at a slower rate of freezing and at –20 ℃ than at a rapid rate of freezing and at –78 °C or –196 °C. Death and sublethal injury are very high during initial storage (ca. 7 days) but subsequently slow down (Ray and Bhunia, 2008). With respect to crystal formation upon freezing, slow freezing favors large extracellular crystals, and quick freezing favors the formation of small intracellular ice crystals. Upon thawing, foods frozen by the slow

freezing method tend to lose more drip than quick-frozen foods held for comparable periods of time (Jay, 2005). The rate of freezing plays a major role in the kind of effects that certain processes may have on the survival of bacteria. A high survival rate of *Salmonella* has been reported in several freezing experiments. Dykes and Moorhead (2001) simulated commercial freezing of beef trimmings to -18 and -35 °C to compare survival of *Salmonella* under slow and rapid freezing rates, respectively. The products were then stored at -18 °C for up to nine months. No significant differences were observed throughout the storage time, independently from the freezing rate. The unexpected results were attributed to intrinsic properties of the matrix, particularly fat, or to the possibility of bacterial quick freezing relative to the rest of the meat, rendering a low injurious effect due to ice crystal formation. A study performed by Dominguez and Schaffner (2009) supports the previous findings. In this case, four *Salmonella* strains originally isolated from poultry were inoculated in processed chicken products. Fully cooked chicken nuggets and raw, breaded chicken strips were frozen and stored in a conventional freezer to -20 °C for up to 16 weeks. The authors evaluated the effect of slow freezing rates (7-10.5 °C/h) on the survival of *Salmonella* in chicken nuggets and chicken strips. This type of freezing is characterized by extracellular ice formation and osmotic dehydration of the cells and is expected to be more detrimental than fast freezing (50 °C/h or higher) in which intracellular ice formation determines the extent of damage (Ray and Bhunia, 2008). However, the researchers concluded that *Salmonella* are able to survival prolonged frozen storage when inoculated in frozen, processed chicken products. Similarly, Escatín et al. (2000) froze raw pork naturally contaminated with *Salmonella* to

-15 °C and stored it for up to 78 weeks. The rate and freezing method were not explicitly stated in this study but is presumably low due to the final temperature reached. The results show that *Salmonella* populations declined during frozen storage but survivors were detected throughout the whole study period (22, 42, and 78 weeks). In spite the inevitable variations resulting from using naturally contaminated samples, a clear trend to a decrease in counts was observed, though undetectable levels were never reached. These results are not in accordance with those of Dominguez and Schaffner (2009) and Dykes and Moorhead (2001) where *Salmonella* populations did not decrease significantly during prolonged frozen storage. In general, the literature reports high survivability rates of *Salmonella* to freezing and frozen storage no matter the process rate or the storage duration.

Variability within species

Dykes and Moorhead (2001) used three *Salmonella* serotypes to evaluate survival on frozen beef trimmings. No variability was seen between resistance of *S*. Brandenberg, *S*. Dublin, and *S*. Typhimurium to freezing and frozen storage in this study. However, that was not the expected result and the authors attributed survival to the products' fat insulating capacity. More recently, Dominguez and Schaffner (2009) used a culture of four mixed different strains in their experiments (serovars Kentucky and Typhimurium, either antibiotic-resistant or non-resistant). Growth of each of the four strains was not evaluated separately, however, no difference was observed on any of three non-selective media, suggesting that the prevalence of certain serovars in frozen products is not of concern from a public health standpoint. Contrary to these findings, in a study by Escatín

et al. (2000), *Salmonella* serovars naturally present in raw pork were serologically identified after freezing the samples for 22, 42, and 78 weeks at -15 °C. After the first period, *S*. Heidelberg and *S*. London were the most common out of 10 serovars identified. In the second trial, 14 *Salmonella* serovars were identified, of which *S*. Agona and *S*. Anatum were the most prevalent. Lastly, in a third trial conducted, 29 serovars were identified, with *S*. Agona being the most prevalent and abundant of all. Though the inherent variability in naturally contaminated pork samples may be the reason why so many different serovars were identified, it also likely that some of the them are more resistant to freezing than others and therefore, be able to persist for longer frozen storage periods on meat surfaces. Studies show inconclusive data on effect of freezing on individual *Salmonella* serovars. Further research is needed.

Protective effects of the matrix

According to Ray and Bhunia (2008), composition, pH, *Aw*, and presence of microbial inhibitors in a food can greatly influence growth, sublethal injury, and viability of microorganisms during storage at low temperature. It is obvious that the problem of bacterial cells surviving in a given frozen food is related to its composition. It would appear that there is a likelihood of substances being present in foods that would afford a high degree of protection from freezing damage to the bacterial cells of those species that are grouped as causes of foodborne illnesses. A study by Woodburn and Hussemann (1959) indicated that more than one type of substance conferred protection to cells of *S*. Typhimurium, *Staphylococcus aureus* and *Streptococcus faecalis* from the lethal effects of freezing. They used four different simplified substrates: waxy rice flour, low-dextrin

corn syrup, egg white and sodium alginate, all at 4% dry weight. For comparison, a

0.0003 mol/l buffer and a 0.85% NaCl solution were included in the study. In this

experiment, cells suspensions were frozen in test tubes at -11, -21 and -30 °C and the

tubes were subsequently stored at the same temperature of freezing for 24 h, one, four

and 10 weeks. The suspensions were then thawed in a water bath at 37 °C for 3 min and

held in ice water. All of the suspensions had an approximate concentration of 10^4 cells

per ml. The interaction between medium and temperature was highly significant meaning

that there was progressively less destruction as the temperature of frozen storage

decreased. In general, the greatest numbers of cells survived at each time period at the

lowest temperature (-30 °C) and least at the highest (-11 °C). As compared with the

buffer alone, the presence of added substances generally appeared to offered a marked

protection to the suspended cells during freezing, frozen storage and thawing. The

authors defined the highest degree of protection as the maintenance of the numbers of

cells at approximately the original levels. Under this definition rice flour and egg white

suspension appeared to be particularly effective since bacterial counts were significantly

higher ($p<0.05$) than the other four substrates over all the time periods at each of the three

temperatures. Corn syrup proved inferior to these two media in terms of recovery of

viable cell but was superior to buffer alone at each temperature (Woodburn and

Hussemann, 1959).

Raj and Liston (1961) proposed that the protein-rich matrix of meat and poultry

might bind free water and therefore act as a hydrocolloid, protecting the cells against

harmful effects. Massaging pork pieces might also affect the survival of *Salmonella*.

According to Escatín et al. (2000), release of protein by muscle tissue may confer protection to bacterial cells against freezing, contrary to what happens in intact meat. In the latter case, release of free amino acids that act as cryoprotectants was proposed as the major protective effect. Previously, Sheridan (1982) had studied the survival of *S.* Kentucky in minced pork, beef, and lamb was evaluated. For pork, the decrease in viability over time was not significant and it might be attributed to the protective effect of amino acids released from the muscle tissue upon mincing. The rapid tenderization of pork meat compared with beef and lamb may be the reason why the same protection level is only seen in this type of meat. Dykes and Moorhead (2001) attributed the unexpectedly high survival rates of three *Salmonella* serotypes to freezing and frozen storage of beef trimmings at -18 °C to the highly protective level of the subcutaneous fat layer of the product, allowing insulation of bacteria and up to a 100% survival rate, like in this case. When minced beef samples containing different fat amounts (5, 10, 20, 30, and 50 %) were artificially inoculated with *S.* Kentucky, blast-frozen to -35 °C, and stored for to 10 week, Sheridan (1997) observed that the survival of the pathogen improved as a result of increasing fat contents up to 20%. The author hypothesizes that fat itself is not a cryoprotectant since it does not penetrate into the cells, as in the case of glycerol. However, upon mincing, glycerol concentration may rise due to bacterial hydrolysis and to a natural release from fat cells creating a protective effect for bacteria. The fat level at which inhibition occurs might also be a free fatty acid concentration effect. The influence of free fatty acids on bacterial growth is well known, particularly for Gram positive

bacteria and to a lesser extent, to Gram negative ones. In general, fat was considered a primary protective component of *Salmonella* to freezing in poultry and meat products.

Contamination levels

Dominguez and Schaffner (2009) evaluated the survival of *Salmonella* in frozen chicken products during frozen storage at -20 °C for up to 16 weeks. They used an initial population of 10^4 to 10^5 CFU/g on chicken nuggets or chicken strips and found no significant reduction during the storage period. Relatively low (10^3 CFU/g) and high (10^5 CFU/g) inoculation levels were used by Dykes and Moorhead (2001) with no significant difference in the reduction after freezing and frozen storage. Survival was attributed to a matrix effect, rather than a concentration effect. According to Dominguez and Schaffner (2009) *Salmonella* counts on selective XLT4 media were significantly lower than counts on other three non-selective media, even after immediate inoculation for both chicken nuggets and strips. The results showed no significant difference in the numbers of *Salmonella* cells enumerated on non-selective media throughout the 16-week storage period, whereas decreasing numbers of cells were seen on XLT4 agar during the same time, suggesting that the permeability barriers in all the cells were damaged but their functional components remained intact (Ray, 1979). The results obtained by Dominguez and Schaffner (2009) conflict with those of Dykes and Moorhead (2001). While in the first study, the numbers of *Salmonella* on selective XLT4 agar tended to decrease during frozen storage, in the latter no significant bacterial injury was produced as determined by comparing counts on selective XLD agar and non-selective TSA, suggesting that *Salmonella* cells remained structural and metabolically uninjured. Escatín et al. (2000)

recovered *Salmonella* from frozen raw pork using the Most Probable Number (MPN) method. The authors state that due to the heterogeneous distribution of bacterial cells on the surface of raw pork, it is inappropriate to use the plate count method for *Salmonella*. Furthermore, in frozen products, pathogens may be physiologically stressed, creating difficulty in recovery. Disparities on the results of the effect of initial contamination levels were strongly related to the recovery methods used. Since several methods are currently under use, differences in results and limited conclusions can be drawn if standardized procedures are not enforced.

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CHAPTER 2

SURVIVAL OF ARTIFICIALLY-INOCULATED *Escherichia coli* AND *Salmonella* Typhimurium ON THE SURFACE OF RAW POULTRY PRODUCTS SUBJECTED TO CRUST FREEZING

Abstract

Escherichia coli and *Salmonella* spp. are ubiquitous to the poultry production environment and hence their transmission to poultry products is a concern. Industry has widely used freezing as a strategy to halt pathogen growth and more recently, crust freezing has been suggested as a means to improve mechanical operations, quality, and safety of poultry products. The purpose of this study was to evaluate the effect of crust freezing on the survival of *Escherichia coli* and *Salmonella* Typhimurium artificially inoculated on the surface of raw poultry products with or without skin. Ampicillin-resistant *E. coli* JM 109 and nalidixic acid-resistant *S*. Typhimurium were used in the experiments. A set of cultures was subjected to cold-shock stress by storage at 4 °C for 10 days. Commercial chicken breasts without skin and chicken thighs with skin were inoculated with each bacterium in separate experiments after being either cold-shocked or non-cold-shocked prior to inoculation. Samples were crust frozen at -85 °C for 20 min or completely frozen at -85 °C for 60 min. *E. coli* and *S*. Typhimurium were recovered on appropriate selective and non selective media containing the corresponding antibiotic. Log reductions and injury extent were calculated and treatments were compared using

ANOVA. 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 reductions tended to be greater for *E. coli* than for *S.* Typhimurium (average 0.15 vs. 0.10 $\log_{10}$CFU/ml of rinse) although none of the final reductions were greater than the desired target (1 log). Bacterial cell injury was not significantly different ($p>0.05$) among any of the treatments. In conclusion, data showed no practical significance for initial reduction of these pathogens due to crust freezing and thus, this technology should not be considered strategies for the reduction of these pathogens on poultry.

Key words: crust freezing, poultry products. *E. coli*, *Salmonella*, food safety

Introduction

Salmonella is a leading cause of foodborne illness in the United States and continues to be of public health significance because of its ability to withstand harsh environments (Chambliss et al., 2006). It is responsible for significant human suffering, loss of productivity, and mortality. Although the disease is underreported, an estimated 1.4 million people were affected in 2008 in the United States, with an overall health cost of $2.6 billion. Other considerations may include lost productivity, costs to food producers and caterers, and investigational costs (Mead et al., 2010). Coliforms, especially *Escherichia coli*, are microorganisms of concern in almost every food product since high counts of coliforms and the presence of *E. coli* in foods usually reflect unhygienic handling during production process, improper storage conditions and post-process contamination (Keeratipibul et al., 2009; Mackey et al., 1980). The

implementation of the hazard analysis and critical control point (HACCP) system and similar process control strategies in poultry processing has led to an increase in microbiological testing of chicken carcasses and parts (Cox et al., 2010). Poultry carcasses further processed into parts or used to make ground products may have a higher incidence of *Salmonella* because of possible cross contamination (Stopforth et al., 2007).

Raw chicken products are an important part of domestic and international food trade. The different criteria and subsequent actions in the case of noncompliance that address the presence of *Salmonella* depend upon the stage in the food chain, the sensitivity of the sampling plan and method, and the type of analytical method. Because of the health and economic implications of these foodborne pathogens, the food industry uses a variety of preservation treatments, including freezing, acidification, and heat treatments, to obtain a safer product (Chang et al. 2003). Freezing is a food preservation method that can potentially deliver a high degree of safety, nutritional value, sensory quality, and convenience. The main purpose of freezing is to lower the temperature of foods with the intention of preventing, or at least minimizing, microbial, biochemical, and chemical changes. However, such reduction in foods' temperature results in complex physical and chemical changes for the products themselves and for any living organism on or in them, depending upon the extent of freezing (Berry et al., 2008). Several freezing applications have been developed for such purpose, crust freezing being among them. The rapid drop in surface temperature of the product when chilling at very low temperatures not only limits evaporation from the surface but also leads to crust freezing. This frozen crust acts as a vapor barrier inhibiting further evaporation. Crust freezing

operations are used to produce the optimum texture in a chilled product so that it is suitable for mechanical processing (James and James, 2002). However, little is known about the degree of safety offered by this type of quick freezing. The goal of this study was to evaluate the effect of crust freezing on the survival of *Escherichia coli* and *Salmonella* Typhimurium artificially inoculated on the surface of raw poultry products with or without skin.

Materials and Methods

Culture preparation

Experiments were performed using an ampicillin-resistant (AR) *Escherichia coli* JM 109 strain and a nalidixic acid-resistant (NAR) *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain (*Salmonella* Typhimurium). Antibiotic-resistant strains were used as a means to minimize potential interferences with background microflora when monitoring survival of both bacteria (Dominguez and Schaffner, 2009). For *E. coli*, AR stock cultures were maintained at -85 ℃ in Difco Tryptic Soy Broth (TSB; Becton Dickinson, Sparks, MD) plus 20% glycerol. Intermediate cultures were prepared by transferring one-tenth of one 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 ℃ for 24 h with agitation at 200 rpm. Then, one-tenth of one 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 ℃ for 16 to 18 h with agitation at 200 rpm. Similarly, NAR *S.* Typhimurium stock cultures were maintained at -85 ℃ in TSB plus 20% glycerol. Intermediate cultures were prepared by transferring one-tenth of

one 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 ℃ for 24 h with agitation at 200

rpm. Then, one-tenth of one 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 ℃ 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

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* placed on separate sections of the same product, both working cultures being either non 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 to dry on the surface of the chicken products for at least 20 min and then individual samples were put in clear, plastic commercial freezing 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 units were frozen to completion in 60 min. Afterwards, bags containing the samples were put under refrigeration 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 freezing bag containing either a chicken breast or a chicken thigh. The pieces were then massaged gently by hand during 30 s to disperse 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), both media containing 500 ppm of ampicillin. All dilutions were plated in duplicates. Colonies were counted on duplicate plates with 25 to 250 colonies after incubation at 37 ℃ for 48 h on a Quebec colony counter. Bacterial populations were converted to $\log_{10}$ CFU/ml of sample 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 duplicates. Colonies were counted on dilution plates with 25 to 250 colonies after incubation at 37 ℃ for 48 h on a Quebec colony counter. Bacterial populations were converted to $\log_{10}$ CFU/ml of sample rinse.

Metabolically and structurally injured cells are able to produce colonies on nonselective agar; therefore, a selective medium was used for each bacterium to assess the number of structurally injured cells. All antibiotic solutions used in the experiments were filter-sterilized using a 0.45 μm –pore-size syringe filter.

Log reduction and injury extent

All counts were converted to log CFU/ml of sample 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 numbers of either *E. coli* or *S.* Typhimurium in TSA containing the corresponding

antibiotic, after either freezing treatment. The injury extent for *E. coli* was defined as the difference between the counts on TSA and VRBGA. For *S.* Typhimurium, the injury extent was considered to be the difference between the counts on TSA and BG Sulfa. The decimal reductions and the injury extent 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. The experimental unit was defined as a single chicken product. An analysis of variance was performed separately on the final survival values, the log reduction data and the injury extent results using PROC MIXED on the Statistical Analysis System (SAS) version 9.2 (SAS Institute, Cary, NC). Results were deemed significant at a $p < 0.05$.

<div align="center">

Results and Discussion

</div>

Microorganisms inhabiting foods that must be refrigerated for pre- and/or post-processing storage are subject to cold shock (Wesche et al., 2009). In the present study, cold-stressed *E. coli* and *S.* Typhimurium were provoked by maintenance of late-exponential phase cultures in refrigeration at 4 °C for 10 days in order to simulate

conditions that bacteria encounter in the processing and retail environment (Jasson et al., 2007). According to Packer et al. (1965) and Archer (2004), there are several factors that influence the survivability of bacteria to freezing in food systems. The phase of growth (logarithmic or exponential), the atmosphere (aerobic or anaerobic), the density of the culture (high or low inoculum), the freezing rate, and the protective effect of the matrix (pH, cryoprotectants) are some of such factors. In the present study, cultures were used in their late exponential phase, under aerobic conditions in high concentration (10^5-10^6 CFU/ml). Furthermore, the freezing rate was considered high, as the temperature of foods is lowered to about -20 °C within 30 minutes (Jay, 2005).

The results of this study show that the presence of skin was not a significant factor in determining survival of either bacteria (p=0.11). Consequently, no differences were observed between skinless chicken breasts and skin-on chicken thighs. *E. coli* tended to have larger reductions than *S*. Typhimurium, whose populations remained unchanged after the application of crust- or complete freezing. The freezing treatment was statistically significant in the reduction of both bacteria on the surface of raw poultry products. However, none of the reductions were greater than 1 $\log_{10}$CFU/ml of water rinse; therefore, this factor was deemed insignificant from a practical perspective and not taken into account for data report purposes.

According to the Food Safety and Inspection Service of the United Sates (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. Table 1.1 gives the average logarithmic reductions of each

bacterium depending on the application of cold stress. Unstressed *E. coli* tended to have greater reductions than *S*. Typhimurium; however, the same behavior was not observed with cold-stressed cultures of either bacteria, indicating a significant interaction ($p<0.05$) between these two factors in reducing the numbers of bacteria on the surfaces of raw poultry products (Figure 1.1). 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.

Only a limited number of studies have evaluated the survivability of *Salmonella* to freezing in meat and poultry matrixes. 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. In the present investigation, refrigerated storage was not considered an additional source of cell damage and death. On the other hand, prolonged frozen storage could potentially reduce the number of bacteria on meat and poultry products. 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. However, the same findings were not reported by Dykes and Moorhead (2001) who evaluated survival of three *Salmonella* serotypes on beef trimmings during simulated commercial freezing and frozen storage at -18 and -35 °C for up to 9 months. The authors

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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.

In the present study, it was found that under the experimental conditions used, neither crust nor complete freezing reduced the population of artificially inoculated *E. coli* or *S*. Typhimurium. 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), the 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 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 would 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 experiment. Wesche et al. (2009) indicated that bacterial injury could be defined as the effect of one or more sub-lethal treatments on a microorganism. Bacterial cells exposed to different physical and chemical treatments suffer injury that could be reversible in food materials during

storage. The injured cells can repair in a medium containing the necessary nutrients under conditions of optimum pH and temperature leading to outbreaks of foodborne disease and food spoilage. Significant differences in survival to prolonged frozen and refrigerated storage of cold-adapted *Salmonella* cultures have been reported by Jeffreys et al. (1998). In general, after the application of a physical preservation treatment, a population of bacteria contains a mix of physiologically different types of cells: the uninjured cells, that are capable of growth and multiplication in selective and non-selective culture media; the injured cells, that are capable of multiplication only in a nonselective medium; and the dead cells, which are incapable of multiplication under any conditions. The injured population can constitute a very high proportion of the total surviving bacteria, up to 99% and more, as it was observed in this study (Breasheats et al., 2001; Straka et al., 1959; Wesche et al., 2009; Wuytack et al., 2003).

Structural injury due to crust freezing was assessed by difference in growth on non-selective versus selective media. Populations of *S.* Typhimurium that were not cold-stressed tended to have the smallest injury extent 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 1.2). The presence of skin was not significant in determining microbial injury. According to Breasheats 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

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that quick freezing causes structural injury in bacterial populations. Metabolic injury is presumed not be of concern due to the quick nature of the freezing process.

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TABLE 1.1

Average reductions ($\log_{10}$CFU/ml of rinse water) of cold stressed and unstressed *E. coli*

and *S*. Typhimurium

Bacteria	Stress	Reduction ($\log_{10}$CFU/ml[1])
E. coli	No	0.3 a
	Yes	0.2 b
S. Typhimurium	No	ND[2]
	Yes	0.2 b

[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 pulled together as they were not significant statistically and

practically, respectively. Differences were calculated as CFU/ml and then transformed to
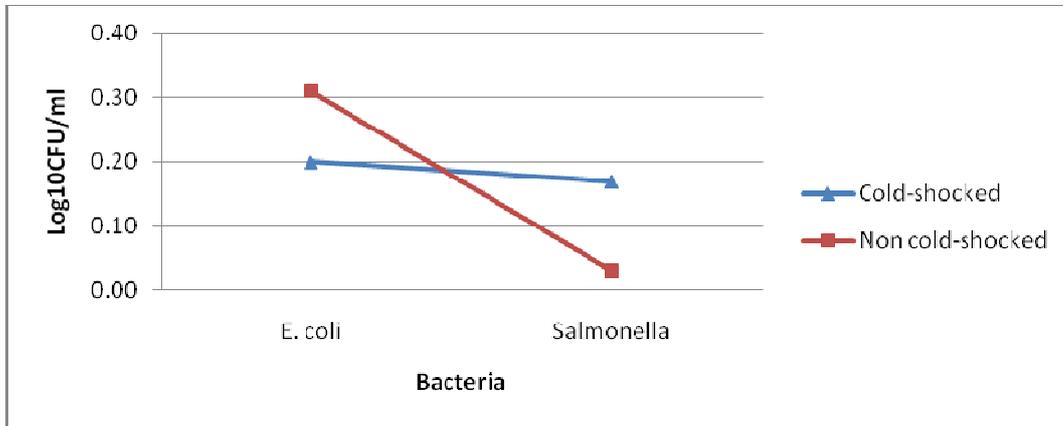
logarithmic values.

[2] ND: not detected.

Figure 2.1 Interaction plot for the reductions ($\log_{10}$CFU/ml) of unstressed or cold-stressed

E. coli and *S*. Typhimurium cultures
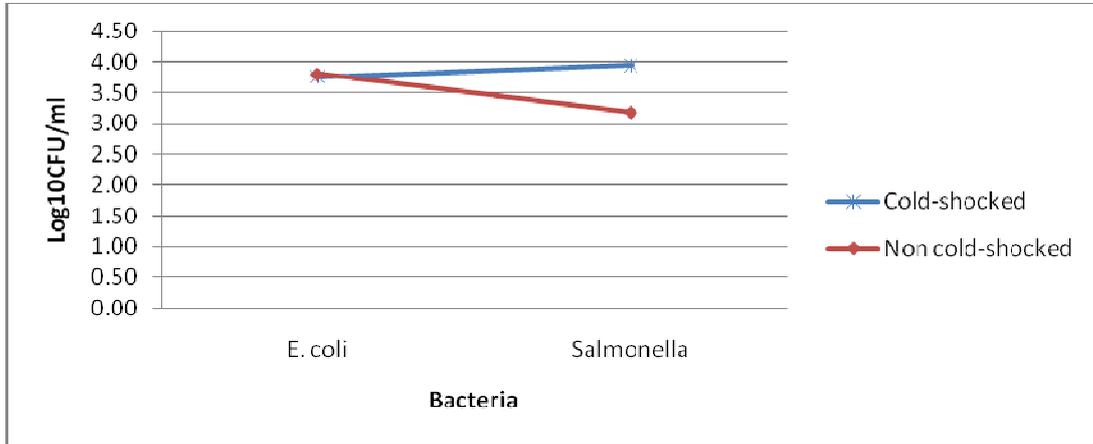
Figure 2.2 Interaction plot for the injury extent values ($\log_{10}$CFU/ml) of unstressed or cold-stressed *E. coli* and *S*. Typhimurium cultures

CHAPTER 3

QUALITY AND SHELF LIFE OF FRESH CHICKEN BREASTS SUBJECTED TO

CRUST FREEZING WITH AND WITHOUT SKIN

Abstract

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 International Commission on Illumination (CIE) color parameters L*, a* and b*; tenderness; and total aerobic (APC) and yeasts and molds counts (YMC). Skin-on breasts had significantly higher L* values compared to skinless units (average 75 vs. 55), whereas a* and b* remained relatively constant no matter the presence of skin, freezing or time. Values oscillated between -2.10 to 0.78 and 1.38 to 3.77, respectively. Shear energy varied erratically for skinless samples but tended to remain constant throughout time for skin-on units. Microbial load increased over time and was considered unacceptable over 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 was not useful in extending their shelf life.

Key words: crust freezing, shelf life, chicken breasts, skin

Practical Applications

Raw poultry products are susceptible to spoilage and deterioration. Several freezing applications at the commercial level claim to improve quality attributes or extend shelf life of these products. Crust freezing, where the product is semi-frozen and only at superficial layers of tissue, is one such application. The current study found that crust freezing did not affect quality attributes of raw chicken breasts, with or without skin, but neither did it extend their shelf life under aerobic, refrigerated storage, based on aerobic plate count values. Although further investigation is needed, this research serves as the foundation for other studies to benefit industry. Since crust freezing is widely accepted as a shelf life extending treatment, the results of this study imply that the freezing method, length of the crust freeze, and the product type and form may influence the effectiveness of crust freezing for shelf life extension.

Introduction

The increase in consumption and production of poultry meat has progressively moved from whole carcasses to cut-up parts and boneless meat. Boneless, skinless chicken breast fillets are of primary economical importance and more than 60% of consumers purchase boneless chicken breast meat when purchasing chicken products (Rotabakk *et al*. 2006; Zhuang *et al*. 2006; del Rio *et al*. 2007). However, poultry products are highly perishable foods. Depending on the degree of processing following slaughter, their spoilage varies between 4 and 10 days under refrigeration. Mesophilic aerobic counts, psychrotrophs, Enterobacteriaceae, coliforms, and yeasts and molds are

general indicators of processing hygiene, storage quality and potential shelf life in aerobic atmospheres (Charles *et al*. 2006; Patsias *et al*., 2006; Guevara-Franco *et al*. 2010). Quality, including taste, color, freshness and tenderness of chicken meat are major components of consumer satisfaction and the major marketing emphasis by chicken processors (Kumar *et al*. 2007). Freezing is a widely accepted preservation method used to store meat and poultry and is the safest and most efficient way to maintain product quality for long-term storage. However, poultry meat quality may decline during long periods of frozen storage (Lee *et al*. 2008). Crust freezing is defined as an operation in which only 10 to 20 mm deep in the tissue is frozen (James and James, 2002). The core of the unit remains unfrozen and it is used to produce optimum temperature in a chilled product so that it is suitable for cutting, portioning or cubing. In this case, the product is semi-frozen so that it is stiff enough to be portioned. Crust freezing can be used to temporarily stiffen products which are not to be totally frozen but are subsequently kept in refrigeration. The crust allows handling and packing with less damage and according to commercial claims, it could potentially improve quality attributes and extend the shelf life of raw poultry products. The goal of this study was to evaluate the effect of crust freezing on the color, tenderness and total microbial loads of raw chicken breasts with and without skin.

Materials and Methods

Materials and sample preparation

Fresh, clean chicken carcasses were kindly provided by a local commercial processing plant in Greenville, SC and transported directly 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 control or 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. 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 ℃. After cooling down to room temperature (22-25 ℃) 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 on each experimental unit at every sample period. All of the measurements were taken on each sample in the following order: color, APC along with yeasts and molds, and 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 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

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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 °C 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 Difo Dichloran-Rose Bengal-Chloramphenicol (DRBC; Becton Dickinson, Sparks, MD). Plates were incubated at room temperature (22-23 °C) 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 every 50 samples and recalibrated to eliminate error because of dulling of blades. Shear energy and shear force on intact cooked breasts were recorded in at least five different spots of 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 using

a total of eight chicken carcasses for each replicate. The batch 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

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

2.1). 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). Significant differences in L* values were observed between skinless and

skin-on chicken breasts (p<0.0001). In 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 2.2). 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 significant differences in yellowness values (b*) were seen between unfrozen and crust-frozen units, regardless of the presence of skin (Table 2.3). 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 *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 *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 of -3 to 12 units, 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 2.4, 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 2.5 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

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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 $\log_{10}$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 $\log_{10}$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 $\log_{10}$CFU/ml. Maximum APC values were in the order of 10^{10} with an average increase of 2.0 $\log_{10}$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 2.6). 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 $\log_{10}$ CFU/ml. From 12 to 18 days of storage, the APC counts increased on average 1 $\log_{10}$ CFU/ml in average. Maximum YMC were in the order of 10^5 CFU/ml with an average increase of 1.0 $\log_{10}$CFU/ml between consecutive sampling times. Initial mean populations of yeasts and molds in raw, unprocessed chicken breasts was 3.0 $\log_{10}$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 $\log_{10}$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 $\log_{10}$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.

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TABLE 3.1

CIE LIGHTNESS (L*) VALUES OF REFRIGERATED SKINLESS AND SKIN-ON

CHICKEN BREASTS SUBJECTED TO CRUST FREEZING[§]

Storage time	Skinless		Skin-on	
(days)	Unfrozen	Crust-frozen	Unfrozen	Crust-frozen
3	56.84 ^{A1}	54.90^{A2}	73.30 ^{B2}	75.26^{B12}
6	57.58 ^{A1}	57.10^{A1}	76.24^{B1}	76.48^{B1}
12	55.86^{A12}	53.66 ^{A2}	75.70^{B1}	74.63^{B12}
18	54.54^{A2}	53.64^{A2}	73.33^{B2}	74.20^{B2}

[§] 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 3.2

CIE REDNESS (a*) VALUES OF REFRIGERATED SKINLESS OR SKIN-ON

CHICKEN BREASTS SUBJECTED TO CRUST FREEZING[§]

Storage time	Skinless		Skin-on	
(days)	Unfrozen	Crust-frozen	Unfrozen	Crust-frozen
3	-0.49 ^{A1}	-1.78 ^{B1}	-0.48 ^{AB1}	-1.14 ^{AB1}
6	-1.00 ^{A1}	-1.37 ^{A1}	-0.80 ^{A2}	-0.50 ^{A2}
12	-0.52 ^{A1}	-1.58 ^{B1}	-0.93 ^{AB2}	-0.76 ^{AB2}
18	-0.34 ^{A1}	-2.10 ^{B1}	0.78 ^{A1}	0.76 ^{A2}

[§] 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 3.3

YELLOWNESS (b*) VALUES OF REFRIGERATED SKINLESS OR SKIN-ON

CHICKEN BREASTS SUBJECTED TO CRUST FREEZING[§]

Storage time	Skinless		Skin-on	
(days)	Unfrozen	Crust-frozen	Unfrozen	Crust-frozen
3	1.67^{A2}	2.20^{A2}	1.83^{A2}	2.27^{A2}
6	1.88^{A2}	2.99^{A12}	2.40^{A12}	2.69^{A12}
12	3.70^{A1}	3.60^{A1}	3.17^{A1}	3.77^{A2}
18	3.79^{A1}	3.29^{A12}	1.38^{B2}	3.30^{A12}

[§] 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 3.4

SHEAR ENERGY VALUES (g-mm) OF REFRIGERATED SKINLESS AND SKIN-

ON CHICKEN BREASTS SUBJECTED TO CRUST FREEZING[§]

Storage time	Skinless		Skin-on	
(days)	Unfrozen	Crust-frozen	Unfrozen	Crust-frozen
3	12375 [AI]	10303 [AI2]	14251 [AI]	12721 [AI]
6	11251 [AB1]	4624 [C2]	10963 [A1]	7541 [BC1]
12	8734 [AB1]	8294 [AB2]	12016 [A1]	7749 [B1]
18	13984 [A1]	13053 [A1]	10905 [A1]	10482 [A1]

[§] 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 3.5

AEROBIC PLATE COUNTS ($\log_{10}$CFU/ml) OF REFRIGERATED SKINLESS AND

SKIN-ON CHICKEN BREASTS SUBJECTED TO CRUST FREEZING[§]

Storage time	Skinless		Skin-on	
(days)	Unfrozen	Crust-frozen	Unfrozen	Crust-frozen
3	3.5 [AB3]	4.0 [AB3]	2.5 [B3]	3.9 [A3]
6	5.4 [AB23]	5.4 [AB23]	5.0 [B23]	5.7 [A23]
12	8.7 [A12]	8.6 [A12]	8.2 [A12]	8.5 [A12]
18	10.1 [A1]	10.3 [A1]	9.5 [A1]	9.6 [A1]

[§] 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 3.6

YEASTS AND MOLDS COUNTS ($\log_{10}$CFU/ml) OF REFRIGERATED SKINLESS

AND SKIN-ON CHICKEN BREASTS SUBJECTED TO CRUST FREEZING[§]

Storage time	Skinless		Skin-on	
(days)	Unfrozen	Crust-frozen	Unfrozen	Crust-frozen
3	2.9 ^{B2}	3.8 ^{A12}	3.2 ^{AB12}	2.9 ^{AB2}
6	2.8 ^{A2}	2.8 ^{A2}	2.6 ^{A2}	3.1 ^{A2}
12	4.6 ^{A12}	4.4 ^{A12}	4.6 ^{A12}	4.4 ^{A12}
18	5.3 ^{A1}	5.7 ^{A1}	5.1 ^{A1}	5.5 ^{A1}

[§] 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

CONCLUSION

The short shelf life of poultry products and their high rate of foodborne illness transmission continuously forces industry to develop methods for quality and safety extension. In the present investigation, the potential of crust freezing for reduction of bacterial pathogens on the surfaces of raw poultry products and for the shelf life extension of fresh chicken breast was evaluated. Under the conditions used in this study, crust freezing was not considered a way to increase safety of extend shelf life of raw poultry products, with or without skin.