Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of *Escherichia coli* O157:H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products

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Abstract

As meat consumption is increasing around the world, so do concerns and challenges to meat hygiene and safety. These concerns are mostly of a biological nature and include bacterial pathogens, such as *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter* in raw meat and poultry, and *Listeria monocytogenes* in ready-to-eat processed products, while viral pathogens are of major concern at foodservice. A major goal of scientists, industry, public health and regulatory authorities is to control pathogenic microorganisms and improve meat product hygiene and safety within a country and internationally. This paper is not a comprehensive or critical review of the scientific literature on the broad area of meat hygiene and safety, but it provides an overview of major current meat hygiene and safety issues, and then a summary of studies on biofilm formation by pathogens, control of *E. coli* O157:H7 in nonintact meat products, and control of *L. monocytogenes* in ready-to-eat meat products, conducted at the Center for Meat Safety & Quality and Food Safety Cluster of Colorado State University in recent years.

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1. Introduction

This paper is the basis for an invited presentation on meat hygiene and safety at the 56th International Congress of Meat Science and Technology, held on 15–20 August 2010 in Jeju, South Korea. As the subject of meat hygiene and safety is very broad and cannot be covered in a single lecture or paper, the presentation, and consequently the paper, were designed to provide a brief overview of major meat hygiene and safety issues, and then to concentrate on three timely meat hygiene and safety topics, namely biofilm formation by pathogens in meat processing and foodservice environments, control of *Escherichia coli* O157:H7 in nonintact meat products, and control of *Listeria monocytogenes* in ready-to-eat (RTE) meat products. Since these topics are also broad, the material presented is not comprehensive or a critical literature review, but rather summaries of related major research activities undertaken at the Center for Meat Safety & Quality and Food Safety Cluster of Colorado State University in recent years. It should be noted that in addition to the overall introduction of meat hygiene and safety issues, the initial part of each of the specific topics covered includes an overview of that topic. It is acknowledged that numerous other scientists have conducted important studies related to the topics examined. However, due to space limitations only key pertinent literature reviews of such studies have been cited.

Current challenges and concerns related to consumption of meat products may be divided into those associated with microbial pathogens and into other meat safety issues. Major challenges related to microbial pathogens include foodborne illness outbreaks, associated product recalls, regulatory compliance, and issues related to pathogen control. Other issues are the emergence of pathogens with increased virulence and low infectious doses, pathogen resistance to antibiotics or food-related stresses, cross-contamination of foods other than meat products, as well as water, with enteric pathogens, animal manure disposal issues, and potential for implementation of food safety programs at the farm. In the category of other meat safety concerns we may include food additives, chemical residues, animal identification and traceability issues, the safety and quality of organic and natural products, the need for and development of improved and rapid testing and pathogen detection methodologies, regulatory harmonization issues at the national and international levels, products of food biotechnology or genetically modified organisms (GMO), and intentional bioterrorism concerns. These issues have been presented in some detail in recent publications (Doyle & Erickson, 2006; Sofos, 2008a, 2009a).

Sofos (2008a, 2009a) also indicated that potential reasons for the increasing food safety concerns of recent and future years include changes in animal production, product processing and distribution practices; increased international food trade; consumer expectations for minimally processed and convenient food products; projected increases in worldwide meat consumption; higher numbers of consumers at-risk for infection; emerging pathogens and microbial pathogen changes which may be associated with increased virulence and resistance to control or clinical treatment; advances in microbial detection methodologies; inadequate food-handler and consumer education and training in proper food handling; and, increased interest, awareness and scrutiny of food safety issues by consumers, news media, and activist groups.

The objectives of this paper are to: (i) provide a brief overview of meat hygiene and safety issues and challenges, especially those associated with microbial pathogens and their control; and (ii) summarize selected Colorado State University Center for Meat Safety & Quality and Food Safety Cluster recent research activities on the potential for pathogen biofilm formation in processing and foodservice environments, control of *E. coli* O157:H7 in nonintact meat products, and control of *L. monocytogenes* in RTE meat products.

2. Meat hygiene and safety challenges

Major pathogens that need to be controlled in fresh meat include *Salmonella*, *Campylobacter*, and enterohaemorrhagic *E. coli* O157:H7. Even though progress is being made in their control, some of these pathogens will continue to be of concern well into the future (Bacon & Sofos, 2003). *L. monocytogenes* will also continue to be the number one target for control in RTE meat and poultry products, considering its ubiquitous presence, potential to contaminate products after processing, and the ability to multiply even at cold temperatures (FDA/FSS (Food and Drug Administration/Food Safety & Inspection Service), 2003; ILSI (International Life Sciences Institute) Research Foundation/Risk Science Institute, 2005; Ryser & Marth, 2007; Tompkin, 2002). The main cause of concern for foodborne illness caused by agents introduced into the food at foodservice will remain to be viruses, such as Norovirus, which presently are considered as the biggest cause of foodborne illness in the United States (www.cdc.gov/). Additional pathogens may emerge and become of concern in meat products in the future (Sofos, 2008a) and include non-O157 shigatoxin producing *E. coli* serotypes, *Mycobacterium avium* subsp. *paratuberculosis*, *Escherichia albertii*, *Clostridium difficile*, etc. Emergence of pathogens should not be a surprise as approximately 60–70% of outbreaks and 40–50% of reported cases of foodborne illness are of unknown etiologic agent (www.cdc.gov/). Therefore, progress in detection methodologies and associated progress in knowledge of the ecology of additional pathogens should lead to elucidation of their role in the safety of foods (Sofos, 2008a, 2009a).

3. Improving the safety of meat products

3.1. Pathogen control

As indicated by Sofos (2008a, 2009a), control of meatborne pathogens will continue to be one of our major goals well into the future. The best strategy for improving the safety of meat is by applying proper hygiene and antimicrobial intervention technologies that (Sofos, 2008a, 2009a): (i) reduce contamination on live animals; (ii) minimize access and transfer of microorganisms to carcasses and meat; (iii) reduce, through decontamination, microbial levels on carcasses or meat; (iv) reduce or eliminate, by killing, microbial contamination on products; (v) avoid or minimize cross-contamination; and (vi) inhibit growth of surviving microorganisms (Juneja & Sofos, 2002, 2009; Sofos, 1994, 2002, 2005; Stopforth & Sofos, 2006). Thus, foodborne pathogen control requires application of interventions at pre-harvest, post-harvest, processing, storage, distribution, merchandizing, preparation, foodservice, and consumption.

3.1.1. Pre-harvest pathogen control

Pre-harvest pathogen control should aim at minimizing sources, levels, access and transfer of contamination to the animal (Koutsoumanis & Sofos, 2004; Koutsoumanis, Geornaras & Sofos, 2006; Samelis & Sofos, 2003a,b; Sofos, 2005; Stopforth & Sofos, 2006). Pathogen reduction programs at the farm level contribute to food safety by decreasing the probability of pathogen presence in animals and associated foods and by reducing water and produce contamination, as well as direct animal-to-human pathogen transmission (Sofos, 2008a). Proposed or used on-farm interventions include diet manipulation, use of feed additives or supplements, antibiotics, bacteriophage therapy, administration of vaccines or immunization, competitive exclusion, prebiotics or probiotics, and proper animal management practices such as pen management, clean feed, clean and chlorinated water, and clean and stressless transportation (Huffman, 2002; LeJeune & Wetzel, 2007; Sofos, 2004a,b, 2005; Stopforth & Sofos, 2006). In addition, it is important to apply proper animal manure treatment and disposal procedures in order to limit spreading of pathogens in the environment, water and other food crops. Overall,
however, pathogen control in animals pre-harvest is difficult and there is a lack of widely accepted interventions (Sofos, 2004a,b, 2006; Stopforth & Sofos, 2006).

3.1.2. Pathogen control at slaughter and processing

Control at harvest and processing should be designed to minimize introduction of additional contamination and to reduce or eliminate contamination levels through implementation of decontamination or sanitization procedures, processing treatments for complete or partial destruction of contamination, or antimicrobial interventions for inhibition of microbial growth during subsequent distribution and storage of products (Koutsoumanis et al., 2006; Samelis & Sofos, 2003a,b; Sofos, 2005, 2008a, 2009a; Stopforth & Sofos, 2006). As a result of major foodborne outbreaks, regulatory authorities in the United States have established new inspection regulations which require meat and poultry operations to: (i) establish sanitation standard operating procedures; (ii) operate under the hazard analysis critical control point (HACCP) system; and (iii) meet microbiological performance criteria and standards for E. coli biotype I and Salmonella, as a verification of HACCP (FSIS (Food Safety & Inspection Service), 1996). Another regulation in the United States (FSIS (Food Safety & Inspection Service), 2003) is aimed at controlling L. monocytogenes in RTE meat and poultry products that may be contaminated after processing (during handling for peeling, slicing and packaging), and allow growth of the pathogen during distribution and storage, even at refrigeration temperatures. Although presence of L. monocytogenes in one of two 25-gram samples is still unacceptable (“zero tolerance”), the new regulation directs the industry to select one of three alternatives to control the pathogen: (i) application of a post-lethality treatment (may be an antimicrobial agent or process) that reduces or eliminates L. monocytogenes on the product plus an antimicrobial agent or process that suppresses or limits growth of the pathogen; (ii) application of a post-lethality treatment that reduces or eliminates L. monocytogenes on the product or an antimicrobial agent or process that suppresses or limits growth of the pathogen; or (iii) a combination of verified sanitation procedures and a microbiological testing program for food contact surfaces and holding of products with positive testing results (FSIS (Food Safety & Inspection Service), 2003). Thus, this regulation offers alternatives that the industry can apply in order to resume production when there is a zero tolerance failure, provided that the selected alternative is validated and introduced in their HACCP plan or pre-requisite programs (Sofos, 2008a).

In response to these food safety regulations and in order to enhance product hygiene and safety, the meat industry in the United States employs pathogen reduction interventions (Dorsa, 1997; Huffman, 2002; Sofos, 2005; Sofos & Smith, 1998). They include animal cleaning and removal of hair, removal, by knife-trimming or steam-vacuuming, of visibly soiled carcass spots, washing/spraying/rinsing of carcasses with cold, warm or hot water or chemical solutions, or pressurized steam, followed by chilling of carcasses, and in some instances application of chemical interventions on carcasses as they exit the cooler for cutting and of meat cuts before grading or packaging and shipping (Huffman, 2002; Sofos, 2005; Sofos & Smith, 1998). Pathogen control in processed meat products is achieved through hurdles of a physical, physicochemical or biological nature (ILSI (International Life Sciences Institute) Research Foundation/Risk Science Institute, 2005; Koutsoumanis et al., 2006), including low and high temperature, nonthermal (e.g., irradiation, high pressure) processes, acidity or low pH, reduced water activity or drying, modification of the oxidation/reduction potential ( Eh) through packaging, application of antimicrobial additives, microbial competitors (lactic acid bacteria) or their antimicrobial products (bacteriocins, such as nisin), and packaging (modified atmospheres, active, etc.) treatments. In addition, a variety of novel technologies (CAST (Council of Agricultural Science & Technology), 1996; Koutsoumanis et al., 2006; Ross, Griffiths, Mittal & Deeth, 2003; www.fda.gov/FoodScienceResearch/ResearchAreas/SafePracticesforFoodProcesses/ucm100158.htm) are being evaluated, proposed or in some cases approved, and to some degree used, in the processing and preservation of meat and other food products (Sofos, 2008a). They include pulsed electric fields, ultrasonic waves, oscillating magnetic fields, cell lysis with bacteriophages or enzymes, smart antimicrobial packaging or edible antimicrobial films and various combinations of such treatments or processes (e.g., manothermosonication involving ultrasonic radiation, pressure and heat; irradiation and heat; pressure and heat) (Ross et al., 2003; Sofos, 2008a). Continuous efforts to enhance food safety are needed, as approaches to pathogen control may be complicated with developments such as: changing consumer needs and expectations from their food supply; projected increases in meat consumption worldwide; expanded use of sublethal antimicrobial multiple hurdles in food processing and preservation leading to potential for stress-adaptation and cross-protection of pathogens; and, increasing numbers of consumer groups at-risk for severe foodborne illness (Samelis & Sofos, 2003a,b; Sofos, 2008a, 2009a).

3.1.3. Pathogen control at retail and foodservice

Efforts for pathogen control at the retail, foodservice and consumer level should have the objectives of preventing transfer of contamination among foods and food contact surfaces, cross-contamination or recontamination, and inactivation or inhibition of growth of existing contamination (Lianou & Sofos, 2007; Sofos, 2008a). Proper sanitation, hygiene, cooking and storage are key approaches to avoid food safety problems at this stage of the food chain.

4. Biofilms in meat processing and foodservice environments

4.1. The problem

Persistence of organic soil residues in food processing environments may lead to creation of microbial harborage sites, biofilms and niches, which may serve as sources of cross-contamination. Biofilms consist of bacterial cells encapsulated in an exopolysaccharide matrix which allows them to adhere to each other and to surfaces, and also protects them from adverse conditions (Chmielewski & Frank, 1995; Hood & Zottola, 1995; Sofos, 2009b). Cells form microcolonies or clusters enclosed within the hydrated matrix, and pores or channels throughout the structure allow transport of oxygen, nutrients and waste. Cell matrices form a network which facilitates formation and maintenance of the biofilm structure, and increases the resistance of biofilms to sanitizers. Generally, biofilms may become major problems in foods, food processing equipment, wounds and surgical instruments. Bacteria forming biofilms include pathogens and spoilage types such as Listeria, Salmonella, Campylobacter, E. coli, Pseudomonas and lactic acid producing bacteria; they may be in mixed cultures or one species may dominate (Chmielewski & Frank, 2003; Hood & Zottola, 1995; Sofos, 2009b).

As discussed by Sofos (2009b), biofilms may form in all areas of food processing environments, including floors, walls, pipes and drains. Materials commonly used in food processing, such as stainless steel, aluminum, nylon, teflon, rubber, plastic, Buna-N, glass, etc., may be subject to biofilm formation. Hard to clean and sanitize crevices, in conveyor belts, pasteurizers, gaskets and dead spaces, become hosts of biofilms. Strong attachment of cells on food surfaces and potential biofilm formation may also affect the efficacy of antimicrobial interventions applied to carcasses, meat, produce or other foods to reduce contamination (Chmielewski & Frank, 2003; Hood & Zottola, 1995; Sofos, 2009b). Pathogens such as L. monocytogenes may persist in food plants for months and up to several years (Tompson, 2002).

In order to improve hygiene, control contamination and enhance food safety, it is important to prevent formation or to remove and inactivate biofilms in food processing and foodservice environments.
This is a crucial, but difficult task, because bacterial cells in biofilms may be up to 500 times more resistant to sanitizers than free-floating cells of the same species. In some cases, the concentration of sanitizers and exposure time may have to be increased by 10- to 100-fold in order to be effective against cells in biofilms compared to planktonic cells. The increased resistance of biofilm cells compared to planktonic cells may be associated with the smaller surface area exposed to the sanitizer, and potentially due to expression of sanitizer resistance genes. It is crucial to understand the conditions and mechanisms allowing bacteria to attach and form biofilms on various surfaces, including food and food contact surfaces, in order to develop effective control procedures (Sofos, 2009b). The issue of biofilms in the food industry has been reviewed by numerous authors (Brooks & Flint, 2008; Carpenteret & Cerf, 1993; Kumar & Anand, 1998; McLandsborough, Rodriguez, Pérez-Conesa & Weiss, 2006; Sharma & Anand, 2002; Simões, Simões & Vieira, 2010; Wong, 1998; Zottola & Sasahara, 1994).

4.2. Colorado State University work on biofilms

Selected findings were summarized by Sofos (2009b) as follows: Cell attachment to beef fabrication surfaces varied among nine strains of *E. coli* O157:H7, making strain selection important in biofilm studies (Dourou, Simpson, Belk, et al., 2008). If introduced into the processing environment, *E. coli* O157:H7 could be considered a source of product contamination since it can attach and grow under limited nutrient availability, if temperature permits (Adler et al., 2008). *E. coli* O157:H7 may attach to stainless steel and high density polyethylene (HDPE) food contact surfaces, not only at abusive temperatures (15 °C) but also during cold storage (4 °C), demonstrating the need for the development of effective sanitation programs for various plant environments (Dourou, Simpson, Yoon, et al., 2008). Plastic beef contact surfaces may allow more biofilm formation by *E. coli* O157:H7 than stainless steel (Simpson, Dourou, Belk, et al., 2008). *E. coli* O157:H7 cells allowed to dry on stainless steel before exposure to reduced nutrient, but moist conditions, had a stronger strength of attachment but a slower growth rate than cells that remained hydrated (Adler, Geornaras, Belk, Smith & Sofos, 2009b). If allowed to dry on surfaces, *E. coli* O157:H7 may have an increased strength of attachment making its removal more difficult, thus demonstrating the importance of proper cleaning and sanitizing of equipment surfaces after each use. Beef residues may facilitate attachment, while spoilage bacteria may outgrow *E. coli* O157:H7 in biofilms (Adler et al., 2008). *E. coli* O157:H7 remained detectable on stainless steel for up to four days in mixed organic acid and water carcass decontamination runoff fluids (washings) (Stopforth, Samelis, Sofos, Kendall & Smith, 2003). Contaminated beef fat served as a better means of transfer of *E. coli* O157:H7 cells to beef fabrication contact surfaces than ground beef (Simpson, Dourou, Yoon, et al., 2008). Drying of beef residues on surfaces resulted not only in cell attachment, but also in cell entrapment on the surface (Simpson, Dourou, Belk, et al., 2008). Chemical decontamination of beef carcasses immediately before fabrication should be useful in pathogen control because it decreased attachment and pathogen levels on fabrication equipment surfaces (Simpson, Dourou, Yoon, et al., 2008). No differences were observed in biofilm formation between quorum sensing-positive and -negative strains of *E. coli* O157:H7 (Yoon, Mukherjee, Geornaras, et al., 2008b). Surface material did not influence the fate of biofilm cells exposed to sanitizers (Simpson et al., 2009). When presence of biofilms is suspected or verified, sanitizers should be applied at highest allowable concentrations for extended dwell times. In all cases, however, adequate cleaning of surfaces before sanitation is essential in order to avoid formation or to remove biofilms (Simpson et al., 2009; Sofos, 2009b). Additional studies examined formation and control of *L. monocytogenes* biofilms on food contact surfaces present in foodservice environments or the home. *L. monocytogenes* cells have the ability to adhere to various food contact surfaces used in these environments, including HDPE, polypropylene (PP) and laminate, and if not properly cleaned, they form resistant biofilms (Gupta, Geornaras, Kendall, Medeiros & Sofos, 2009; Parikh, Kendall, Yang, Geornaras, Medeiros, et al., 2009; Parikh, Kendall, Yang, Geornaras & Sofos, 2009; Sofos, 2009b; Yang, Kendall, Medeiros & Sofos, 2009b).

A study (Parikh, Kendall, Yang, Geornaras & Sofos, 2009) examined the persistence of *L. monocytogenes* on HDPE and PP surfaces and compared the effectiveness of three commercial sanitizers (lactic acid-, sodium hypochlorite- and quaternary ammonium-based) and three home-prepared sanitizers (active ingredients: 300 ppm sodium hypochlorite, 5% acetic acid, and 3% hydrogen peroxide) in reducing pathogen levels. Multi-species biofilms containing high levels of *L. monocytogenes* developed and survived for at least 14 days on all surfaces at 25 °C, except for PP at 4 °C. Biofilm survival was greater on rough than smooth HDPE surfaces. All sanitizers were effective in reducing attached *L. monocytogenes* cells, and were more effective on younger than older biofilms. Among sanitizers evaluated, the lactic acid- and quaternary ammonium-based were most effective against developed biofilms (Parikh, Kendall, Yang, Geornaras, Medeiros, et al., 2009). Persistence of *L. monocytogenes* on the same food contact surfaces and survival following treatment with the six common sanitizers/household compounds was again evaluated, but this time, some of the surfaces received nutrients (in the form of a diluted culture broth) during a 21-day storage period to simulate home food preparation and cleaning practices. The results indicated that repeated exposure of food contact surfaces to nutrients, as during use with no cleaning or sanitation, increased the resistance of *L. monocytogenes* biofilms to sanitizers. To reduce such risk, consumers may consider treating surfaces with products such as vinegar when commercial sanitizers are not available (Parikh, Kendall, Yang, Geornaras, Medeiros, et al., 2009; Sofos, 2009b).

Another study (Yang et al., 2009b) also examined persistence of *L. monocytogenes* on smooth and rough surface HDPE cutting board surfaces (up to 21 days at room temperature), and compared the effectiveness of 10 commercial sanitizers against *L. monocytogenes* biofilms. Overall, sanitizer efficacies were higher against biofilms on smooth than on rough surfaces, and against younger (7-day old) than older (21-day old) biofilms on smooth surfaces. A lactic acid-based sanitizer (pH 3.03) was the most effective, while quaternary ammonium-based sanitizers of higher pH (10.42–11.46) were more effective than those of lower pH (6.24–8.70). Sanitation of cutting boards should, thus, be performed with selected sanitizers after each use, or at least daily, to achieve maximum efficacy (Sofos, 2009b; Yang et al., 2009b). Gupta, Geornaras, Kendall, et al. (2009) investigated survival of *L. monocytogenes* on laminate kitchen countertop surfaces and compared the efficacy of four household wiping materials for physical removal of cells from the surfaces. The pathogen survived and was recovered, by wiping, from the surfaces at room temperature (25 °C; 50 and 90% relative humidity) in the presence of food residues (ham homogenate) for at least 96 h; populations recovered at 90% relative humidity were higher than those at 50% relative humidity. Wiping materials were not different (P > 0.05) in removing pathogen cells from the laminate surfaces (Gupta, Geornaras, Kendall, et al., 2009; Sofos, 2009b). Another study (Yang, Kendall, Medeiros & Sofos, 2009a) found that, of products commonly found in households, effectiveness against three pathogens (*Salmonella* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes*) decreased in the order: household bleach (0.0314%) > hydrogen peroxide (3%) > undiluted vinegar > baking soda (50% sodium bicarbonate), while pathogen sensitivity followed the order: *Salmonella* Typhimurium > *E. coli* O157:H7 > *L. monocytogenes*. Sanitizer activity increased at warm temperatures (55 °C) and with longer exposure time (10 min) (Sofos, 2009b). As indicated, it is important to develop and apply effective and
validated biofilm prevention or removal and inactivation procedures for food processing, foodservice and home environments.

5. Safety of nonintact meat products

5.1. The problem

As discussed by Sofos (2009c) and Sofos, Geornaras, Belk and Smith (2008), a major proportion of steaks and roasts derived from muscles of lower tenderness, which in the United States constitute 74% of the beef carcass, may be subjected to mechanical tenderization, moisture enhancement, marination or restructuring, into nonintact products of increased tenderness, juiciness and flavor for use in hotel, restaurant and institutional settings; the total annual servings are estimated at 36 billion in the United States (BIFSCO (Beef Industry Food Safety Council), 2006; NCBA (National Cattlemen’s Beef Association), 2006).

Specifically, nonintact meat products include (Sofos, 2008c) intact meat cuts such as chucks, ribs, tenderloins, striploins, top sirloin butts, and rounds, that are injected with marination, flavoring, moisture-enhancing or tenderizing solutions, or mechanically tenderized by treatment with solid- or hollow-needle injectors or blades, or with cubing, frenching, or pounding devices. In addition, this category includes any comminuted product processed by chopping, grinding, flaking or mincing, as well as manufacturing beef trimmings destined to be processed into formed and shaped items such as gyro (FSIS (Food Safety & Inspection Service), 1999).

An important public health concern associated with these products is the potential translocation or entrapment of pathogen cells, such as E. coli O157:H7, into the interior of the products from: (i) the surface of intact beef cuts to below the surface; and (ii) contaminated meat to previously non-contaminated pieces of meat (e.g., cross-contamination via needle injection and/or recycling of brines). Consumers may perceive nonintact (FSIS (Food Safety & Inspection Service), 2002a,b) and intact products as the same, and in fact, may not know that the product has been moisture-enhanced or mechanically tenderized, and hence may cook them to the same degree of doneness, without considering the potential presence of microbial contamination in the interior of the product (Sofos, 2009c; Sofos et al., 2008). Subsequently, E. coli O157:H7 in the interior of nonintact products may survive cooking and cause illness, especially if the injected ingredients interfere with thermal inactivation or increase the heat resistance of the pathogen (Sofos et al., 2008). The public health risk associated with nonintact meat products in the United States is supported by outbreaks of E. coli O157:H7 illness linked to consumption of such products (FSIS (Food Safety & Inspection Service), 2005, 2007). Thus, even though reports indicate that E. coli O157:H7 contamination on the surface of beef subprimals is low (BIFSCO (Beef Industry Food Safety Council), 2006; NCBA (National Cattlemen’s Beef Association), 2006), translocation of surface contamination to the interior of products appears to be likely. Consequently, in 1999, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) included such products in the category of adulterated if samples are found contaminated with E. coli O157:H7; raw ground beef contaminated with the same pathogen was declared as adulterated in 1994 (FSIS (Food Safety & Inspection Service), 1999).

The safety of nonintact (i.e., non-comminuted) meat products is a more recent concern compared to other meat safety issues covered in this paper, and has been examined by a risk assessment (FSIS (Food Safety & Inspection Service), 2002a,b) and by the United States National Advisory Committee on Microbiological Criteria for Foods (NACMF (National Advisory Committee on Microbiological Criteria for Foods), 2002). Related research was recently summarized by Sofos (2009c) and Sofos et al. (2008). Sofos (2009c) indicated that the risks associated with nonintact meat products depend and may be controlled through implementation of effective carcass decontamination interventions, potential application of approved and effective antimicrobial treatments to subprimals before tenderization (Heller et al., 2007), proper chilling and rotation of injection solutions, potential use of antimicrobials in injection brines, effective sanitation and temperature controls, and proper cooking. It is recommended that manufacturers of such products follow industry recommended best practices (BIFSCO (Beef Industry Food Safety Council), 2006). Processors should develop and implement facility and equipment sanitation standard operating procedures, whereas equipment manufacturers should consider the American Meat Institute 10 Principles of Sanitary Design (AMI (American Meat Institute), 2003). In fact, processors of nonintact meat products should follow sanitation practices similar to those recommended for establishments producing RTE products (NMA (National Meat Association), 1999). The following section summarizes findings of our work related to nonintact meat products.

5.2. Colorado State University work on nonintact meat products

Studies in our laboratory were designed to identify ingredients used in brine solutions (traditional and modified formulations) that may have antimicrobial effects during storage and/or enhance thermal inactivation of E. coli O157:H7 during cooking of moisture-enhanced beef products. Specific objectives of this work (Geornaras, Chorianopoulos, et al., 2009) were to evaluate: (i) quantitative transfer of E. coli O157:H7 during moisture enhancement of beef under different contamination scenarios; (ii) survival of E. coli O157:H7 in brine solutions containing one or more antimicrobial ingredients; (iii) the effect of brine formulation ingredients, alone or in combination with antimicrobial ingredients, on thermal inactivation of E. coli O157:H7 in a ground beef (simulating nonintact products) model system; (iv) survival/growth during frozen, refrigerated, or retail-type storage, and subsequent thermal inactivation during cooking (using different cooking methods) of E. coli O157:H7 in moisture-enhanced products; and (v) thermal inactivation of E. coli O157:H7 in moisture-enhanced steaks of different thicknesses, cooked with different cooking appliances and from different starting temperatures. Major specific studies and findings include (Adler, Geornaras, Belk, Smith & Sofos, 2009a; Adler, Geornaras, Byelashov, et al., 2009; Byelashov, Adler, et al., 2009; Chorianopoulos et al., 2009, 2010; Geornaras, Chorianopoulos, et al., 2009; Gupta, Geornaras, Goodridge, et al., 2009; Gupta, Geornaras, Belk, Smith & Sofos, 2010; Ko, Belk, Smith & Sofos, 2009; Ko et al., 2010; Shen, Adler, Geornaras, et al., 2009; Shen, Geornaras, Belk, Smith & Sofos, 2010a,b).

E. coli O157:H7 contamination was transferred to the interior of beef tissue following moisture enhancement (via needle injection), when the surface of the meat, or brine solution, were contaminated (Gupta, Geornaras, Goodridge, et al., 2009; Chorianopoulos et al. (2009) confirmed that E. coli O157:H7 contamination was transferred from surface-inoculated steaks to subsequently processed non-inoculated steaks, as well as, from the surface to the interior of each steak, through blade tenderization. Adler, Geornaras, Byelashov, et al. (2009) evaluated survival of the pathogen in brine solutions, prepared in a 3% meat homogenate (simulating recirculated brine) or water (simulating freshly prepared brine), and formulated without or with several antimicrobials, during storage (up to 24 or 48 h) at 4 °C or 15 °C. Pathogen populations did not increase or decrease in the basic brine solution (i.e., sodium chloride [NaCl, 5.5%]+sodium tripolyphosphate [STP, 2.75%]), and also when the brine was combined with potassium lactate (22%) and/or sodium diacetate (1.65%). However, addition of sodium metasilicate (2.2%) or cetylpyridinium chloride (CPC, 5.5%) to the brine resulted in immediate pathogen reductions of 1.4 to >2.6 log CFU/ml, and non-detectable (<1.3 log CFU/ml) levels during storage (4 °C or 15 °C) (Adler, Geornaras, Byelashov, et al., 2009). In a companion study (Byelashov, Adler, et al., 2009), NaCl (0.5%) + STP (0.25%) + CPC (0.5%) reduced E. coli O157:H7 counts in moisture-enhanced (110% of initial weight) meat samples by approximately 1 log CFU/g during storage (4 °C, 24 h), and enhanced...
thermal destruction of the pathogen, compared to NaCl + STP (without antimicrobials), when samples were cooked to 65 °C (simulating medium-rare doneness of beef) (Byelashov, Adler, et al., 2009). Other antimicrobials (used in the brines of Adler, Geornaras, Byelashov, et al. (2009)) and in this study; i.e., potassium lactate, 2%; sodium diacetate, 0.15%; potassium lactate [2%] + sodium diacetate [0.15%]; lactic acid, 0.3%; acetic acid, 0.3%; citric acid, 0.3%; nisin, 0.0015% or pediocin, 1000 AU/g + EDTA, 20 mM; sodium metasilicate, 0.2%; hops beta acids, 0.0005%) added to the basic (NaCl + STP) brine formulation did not enhance killing, nor did they protect the pathogen from heat inactivation, compared to the water control (Byelashov, Adler, et al., 2009). In another study (Ko et al., 2009), immediate bactericidal effects (>3.5 log CFU/ml reductions) of \( E. \) coli O157:H7 were obtained in a beef extract containing NaCl (0.5%) + STP (0.25%) and thyme oil (0.25% and 0.5%) or grapefruit seed extract (0.5% and 1.0%), alone or in combination with CPC (0.04%) or sodium diacetate (0.25%).

\( E. \) coli O157:H7 counts in restructured steaks and roasts moisture-enhanced with NaCl (0.5%) + STP (0.25%) + CPC (0.2%) were lower (by 0.7–1.2 [steaks] or 0.7–2.4 [roasts] log CFU/g) than those in samples moisture-enhanced with NaCl + STP + lactic acid (0.3%) or sodium metasilicate (0.2%) (Chorianopoulos et al., 2010; Gupta et al., 2010; Ko et al., 2010). Overall, pathogen inactivation in steaks cooked by different cooking methods to an internal temperature of 60 °C (simulating rare degree of doneness) increased in order of: pan-broiling < roasting < double pan-broiling (Chorianopoulos et al., 2010; Ko et al., 2010). Out of 16 roasts that were cooked to 60 or 55 °C (rare and very-rare degrees of doneness, respectively), 37.5 and 50.0%, respectively, had undetectable (<0.5 log CFU/g) levels of the pathogen in all of the tested subsamples (three [top, middle and bottom] core [2.3 cm diameter] subsamples each from side and center subsections) analyzed from each roast. Recovery of high numbers (>2.1 log CFU/g) of the pathogen from some of the cooked roasts appeared to be correlated with a short cooking time to reach the target temperature (55 °C or 60 °C) combined with a low final temperature (Gupta et al., 2010).

In general, greater inactivation of \( E. \) coli O157:H7 was obtained in 4.0 cm than 1.5 or 2.5 cm thick moisture-enhanced (NaCl [0.45%] + STP [0.23%]) restructured steaks during cooking (65 °C internal temperature) by pan-broiling, double pan-broiling and roasting using different cooking appliances (Presto® electric skillet, Sanyo® indoor barbecue grill, George Foreman® grill, Oster® toaster oven, and Magic Chef® standard kitchen oven) (Shen, Adler, Geornaras, et al., 2009). Thermal destruction of \( E. \) coli O157:H7 increased in order of: double pan-broiling < pan-broiling < roasting, with no differences obtained between the different cooking appliances used for the same cooking method (Shen, Adler, Geornaras, et al., 2009). In a related study (Shen et al., 2010a), thermal destruction of \( E. \) coli O157:H7 was compared in partially thawed (−2.5 °C) moisture-enhanced restructured steaks cooked to 65 °C using different cooking appliances (Presto® electric skillet, Sanyo® grill, Oster® toaster oven, and Magic Chef® kitchen oven) set at different starting temperatures. Appliance starting temperatures of 204–260 °C, regardless of appliance, resulted in greater pathogen reductions (3.3–5.5 log CFU/g) than those obtained at a starting temperature of 149 °C (1.5–2.4 log CFU/g). In general, the higher the starting temperature, the shorter the time needed to reach 65 °C (204–260 °C, 27–35 min; 149 °C, 35–60 min) (Shen et al., 2010a). Thermal (65 °C) inactivation of stress-adapted and unstressed \( E. \) coli O157:H7 cells in moisture-enhanced restructured steaks showed that cold and desiccation stress-adapted cells had higher pathogen reductions (3.0–4.5 and 2.6–3.9 log CFU/g, respectively) while acid stress-adapted cells had lower reductions (1.3–1.9 log CFU/g), compared to unstressed cells (2.1–2.7 log CFU/g). Reductions of heat and starvation stress-adapted cells were similar to those of unstressed cells (Shen et al., 2010b). Another study (Adler, Geornaras, Belk, et al., 2009a) investigated thermal inactivation of \( E. \) coli O157:H7 at different depths of contamination of needle-tenderized steaks cooked with different appliances. Numbers of pathogen survivors increased as the depth of contamination (0–12 mm of a 24 mm steak) of nonintact steaks increased, when samples were cooked (60 °C internal temperature) by pan-broiling, whereas, no differences in survivors were obtained at the different depths of contamination when steaks were cooked by roasting (Adler, Geornaras, Belk, et al., 2009a).

Results of additional studies (Mukherjee et al., 2008, 2009; Yoon, Mukherjee, & Sofos, 2007; Yoon, Mukherjee, Belk, et al., 2008; Yoon, Mukherjee, Geornaras, et al., 2008a; Yoon, Mukherjee, et al., 2009) have indicated that marination, tenderization and meat-binding (restructuring) formulations for nonintact beef products may be improved with organic acids such as lactic, citric, and acetic acid, which may increase thermal inactivation of internalized \( E. \) coli O157:H7; inclusion of low levels of NaCl or phosphate in the formulations may have a protective effect on the pathogen, but it maintains product pH and improves cooking yields. Overall, the data generated should be useful for development and/or improvement of formulations for brining, marination, tenderization and restructuring of beef products for control of \( E. \) coli O157:H7 in nonintact meat products. Furthermore, the data should be useful in updating risk assessments on nonintact beef products, and for development of proper cooking procedures for nonintact products to be used by the industry and consumers (Sofos, 2009c; Sofos et al., 2008).

6. Control of \( L. \) monocytogenes in RTE meat products

6.1. The problem

\( L. \) monocytogenes is widely distributed in nature and is able to survive and proliferate under adverse environmental conditions, including refrigeration temperatures, as indicated previously. Human illness due to \( L. \) monocytogenes occurs rarely; however, when it occurs it can be life threatening (20–30% fatality rate in the United States) or it can have serious clinical manifestations in susceptible population groups, including the elderly, fetuses, neonates and immunocompromised individuals (Farber & Peterkin, 1991; Gandhi & Chikindas, 2007; Ryser & Marth, 2007). RTE meat and poultry products contaminated with \( L. \) monocytogenes have been associated with major fatal outbreaks, as well as large volume food product recalls (Conly & Johnston, 2008; Farber & Peterkin, 1991; Sofos, Skandamis, Stopforth & Bacon, 2003). These products may be contaminated with the pathogen through raw materials, the processing environment, and at retail, foodservice or in the home (Lianou & Sofos, 2007; Sofos, 2008b). Processing of RTE meat and poultry products involves lethality treatments such as thermal, fermentation/drying and ultra-high pressure, which if done properly, should eliminate expected levels of the pathogen. However, contamination may also be introduced on products when exposed to the environment after processing during peeling, slicing and repackaging (Kathariou, 2002; Tompkin, 2002). Other opportunities for introduction of contamination exist during slicing and packaging at retail or delicatessen operations, or during handling at foodservice and in the home (Lianou & Sofos, 2007). Good hygiene and manufacturing practices, appropriate cleaning, sanitation programs, and temperature control, required for prevention or inhibition of growth of the pathogen, are critical for its control post-processing, including the retail/foodservice/home sector. However, a concern exists for products of long shelf-life that allow proliferation of the pathogen. Therefore, there is a need for development and application of control measures for the pathogen in such circumstances, since verification of absence of contamination is impossible. Antimicrobial interventions applied during processing, as required by regulation (FSIS (Food Safety & Inspection Service), 2003), should be able to control contamination introduced at any link of the chain and during the product shelf-life. In addition to research with antimicrobials applied during processing and packaging, we have also examined the fate of contamination introduced at various stages during
the shelf-life of the product and effects of potential interventions that may be applied by foodservice and consumers. An overview of the issues related to the above as well as results of such studies follows.

6.2. Colorado State University work on control of *L. monocytogenes* in RTE meat products

To investigate contamination patterns of *L. monocytogenes* in RTE meat processing environments, a two-year longitudinal study (Williams et al., 2008; Williams, Nightingale, et al., 2009; Williams, Roof, et al., 2009) was conducted in six small or very small RTE meat processing plants in Colorado, Kansas, and Nebraska. A total of 1744 samples that included environmental sponge samples collected from non-food contact surfaces (i.e., drains, floors, sinks, door handles, cart wheels and equipment surfaces), food contact surface samples (i.e., tables, knives and cutting boards), and finished RTE food product samples in some of the plants, were analyzed for *L. monocytogenes* presence. Overall, prevalence of the pathogen ranged from 1.67 to 10.80% and 0.85 to 8.73% across the different plants during year-1 and year-2 of sampling, respectively. Molecular subtyping of isolates suggested that the pathogen may persistently colonize RTE meat processing environments, therefore emphasizing the importance of control strategies (Williams et al., 2008; Williams, Nightingale, et al., 2009; Williams, Roof, et al., 2009).

A study (Lianou, Stopforth, Yoon, Wiedmann & Sofos, 2006) found that growth differences (i.e., lag phase durations and growth rates) among 25 strains of *L. monocytogenes* were less notable at 30 °C than at 4 °C. No clear trends were evident within serotypes, nor among strains of different origin, catalase reaction or source of isolation within a single outbreak. Strain variation was also observed in acid and heat resistance. The findings of this study should be useful in strain selection for evaluation of antimicrobial alternatives, and for completion of risk assessments (Lianou et al., 2006). Based on the findings of Lianou et al. (2006), studies described below used multiple strain composites for inoculation of RTE meat products in order to provide a worst case scenario.

Numerous studies examined control of *L. monocytogenes* post-processing contamination in RTE meat and poultry products through inclusion of antimicrobials in the formulation (e.g., sodium/potassium lactate, sodium acetate/diacetate, benzoate, sorbate, glucono-delta-lactone, etc.) or external application as dipping or spraying solutions (e.g., organic acids, bacteriocins, essential oils, etc.), with or without short exposure to hot water of packaged products (Barmaplia et al., 2004, 2005; Bedie et al., 2001; Byelashov, Daskalov, et al., 2008; Byelashov, Kendell, Belk, Scanga & Sofos, 2008; Durán & Sofos, 2005, 2006; Durán, Geornaras & Sofos, 2008a; Geornaras et al., 2005; Geornaras et al., 2006a, b; Geornaras, Kendall, Scanga & Sofos, 2007; Samelis et al., 2001, 2002, 2005; Shen & Sofos, 2008; Shen, Geornaras, Kendall & Sofos, 2009a; Sofos et al., 2005; Yoon et al., 2005; Yoon, Kendall, et al., 2006; Yoon, Kendall, et al., 2009; Yoon, Skandamis, Kendall, Smith & Sofos, 2006; Yoon, Geornaras, Durán & Sofos, 2007). In brief, findings of such studies are described below. The most effective treatments were lactate–diacetate (L/D) combinations in product formulations, which are used extensively by the United States industry as it moves from FSIS (Food Safety and Inspection Service) (2003) regulation alternative (iii) to alternative (ii) or (i). In addition, effective antimicrobials applied externally on the product surfaces included organic acids (lactic, acetic, and citric), benzoate, a mixture of lactic acid and sodium lauryl sulfate, and, Nisin® followed by acetic acid, lactic acid or benzoate.

Antimicrobials (malic acid [MA; 0.032%] + sodium citrate [SC; 0.06%] + sodium acetate [SA; 0.12%]) added at low concentrations to the formulation of frankfurters, with and without application of a post-packaging heat treatment (93 ± 2 °C, 5 or 15 s), were evaluated for control of post-processing inoculated *L. monocytogenes* (Geornaras et al., 2007). The post-packaging heat treatments reduced initial pathogen populations by 1.2–1.4 log CFU/cm² (5 s) and 1.8–2.0 log CFU/cm² (15 s). Compared to non-treated samples, post-packaging heat treatments extended the lag phase of *L. monocytogenes* on control (no antimicrobials) and product formulated with MA + SC + SA. Inclusion of MA + SC + SA in the formulation, in combination with the initial reductions resulting from the heat treatment (5 or 15 s), allowed <1.0 log CFU/cm² increases of the pathogen in 19 or 25 days at 7 °C (Geornaras et al., 2007).

Research has shown that organic acids (acetic, lactic) are inhibitory against *L. monocytogenes* on RTE meat products when applied as dipping solutions (Barmaplia et al., 2004; Geornaras et al., 2005; Geornaras et al., 2006a, b; Samelis et al., 2001). Lactoferrin or activated lactoferrin used individually as formulation ingredients or surface treatments, respectively, were less effective than lactic or acetic acid, and L/D as antilisterial agents in ham, bologna, turkey breast and frankfurters (Sofos et al., 2005). Since most of the research has evaluated volume-to-volume (v/v) concentrations, and found acetic acid as the most effective, a study by Durán and Sofos (2006) compared the antilisterial effects of low equal molar concentrations (0.083 M) of acetic (0.5% v/v, pH 2.90), lactic (0.75% v/v, pH 2.30) and citric (1.6% v/v, pH 2.05) acid, and their combination (0.17% v/v acetic, 0.25% v/v lactic, 0.53% v/v citric acids, pH 2.24) in bologna. The acid treatments inhibited growth of *L. monocytogenes* as follows: citric→combination→lactic→acetic (Durán & Sofos, 2006).

Shen and Sofos (2008) evaluated the antilisterial activity of hops beta acids (HBA) alone or in combination with other known antimicrobials in a culture broth medium. HBA (1.0–5.0 µg/ml) inhibited growth of *L. monocytogenes*; inhibition was more pronounced at higher concentrations and at the lower storage temperature (4 °C). The antilisterial activity of HBA (0.5–3.0 µg/ml) was also enhanced when combined with sodium diacetate, acetic acid or potassium lactate, achieving complete inhibition at 4 °C (35 days) when 3.0 µg/ml HBA was used in combination with each of the above antimicrobials (Shen & Sofos, 2008). Another study (Shen, Geornaras, et al., 2009a) evaluated HBA as dipping solutions (0.03–0.1%) against *L. monocytogenes*-inoculated frankfurters. Dipping in HBA solutions caused immediate pathogen reductions of 1.3–1.6 log CFU/cm², whereas distilled water reduced counts by 1.0 log CFU/cm². Pathogen growth was completely suppressed until 30–50 days (4 °C) or 20–28 days (10 °C) on frankfurters dipped in HBA solutions. HBA may be considered for use to improve the microbial safety of RTE meat products, provided that future studies on sensory qualities show no adverse effects and that their use is economically feasible (Shen, Geornaras, et al., 2009a). A related study (Durán, Geornaras & Sofos, 2008a) investigated the antilisterial effects of carvacrol, acetic acid, and their combinations as dipping solutions for inoculated bologna slices. The treatments suppressed growth as follows: 0.4% acetic<5 mM carvacrol<10 mM carvacrol = 5 mM carvacrol + 0.4% acetic<10 mM carvacrol + 0.4% acetic = 5 mM carvacrol + 1.0% acetic<1.0% acetic<10 mM carvacrol + 1.0% acetic (Durán, Geornaras & Sofos, 2008a).

As previously indicated, federal regulations require RTE meat and poultry processors to control *L. monocytogenes* by using intervention alternatives including antimicrobials that reduce post-processing contamination by at least 1 log cycle (FSIS (Food Safety & Inspection Service), 2003, 2006). If the treatment achieves ≥2 log reduction, the plant is subject to less frequent microbial testing. A study (Byelashov, Daskalov, et al., 2008) evaluated the effect of lactic acid solution concentration and temperature on *L. monocytogenes* counts of inoculated frankfurters in order to identify parameters (concentration, temperature and time) achieving 1 and 2 log-unit immediate reductions. Distilled water, at any temperature, and lactic acid applied at 4 °C reduced pathogen numbers by approximately 1 log cycle. The desired 2-log reduction was achieved by 3% lactic acid applied at 25 °C for 120 s or by 1% lactic acid applied at 55 °C for 60 s, while the effectiveness of lactic acid increased with solution temperature. The regression equation for *L. monocytogenes* reduction developed may allow processors to vary conditions of treatment with lactic acid to
achieve a 1 or 2 log-unit reduction of the pathogen and comply with regulations (Byelashov, Daskalov, et al., 2008).

Spraying (10 s, 20 bar, 23 ± 2 °C) frankfurters with lactic acid (LA, 5%) or sodium laurel sulfate (SLS, 1%) after inoculation reduced L. monocytogenes numbers by 1.8 and 2.0 log CFU/cm², respectively, while water removed only 1.3 log CFU/cm² (Byelashov, Kendall, et al., 2008). A mixture of LA and SLS (LA/SLS) reduced pathogen populations by 1.8 log CFU/cm² when applied before and 2.8 log CFU/cm² when applied after inoculation. During storage (4 °C) of vacuum-packaged product, pathogen counts were suppressed by LA or LA/SLS, while SLS did not have a bacteriostatic effect when used alone. Thus, spraying of frankfurters with LA/SLS may be considered as an antilisterial alternative for RTE meat products (Byelashov, Kendall, et al., 2008).

Bacterial cell membranes act as selective barriers, but may be affected by stresses such as antimicrobials (Davidson, Sofo & Bransen, 2005). A study (Durán, Geornaras & Sofo, 2008b) examined the effects of acetic (0.4%), citric (0.3%), lactic (0.4%) and octanoic (0.04%) acids, sodium diacetate (0.1%), potassium lactate (0.6%), bufferedsodium-citrate (0.4%), nisin (0.2%), pediocin (0.2%), carvacrol (0.4%), eugenol (0.4%), thymol (0.4%) and monolaurin (0.02%) on L. monocytogenes cells using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Under SEM and TEM, cells exposed to acids and salts appeared elongated and thinner than control cells. Cells exposed to bacteriocins showed cell-division but not separation; some dissolution of cell membrane was observed. Under SEM, cells exposed to essential oils and fatty acids/esters had noticeable valleys and under SEM and TEM some cells appeared stunted. No variation was observed among strains (Durán, Geornaras & Sofo, 2008b). A related study (Durán, Geornaras, Engle & Sofo, 2008) examined cell membranes for changes in fatty acids by gas-chromatography when exposed to the same antimicrobials. Exposure to acids, salts and bacteriocins stimulated greater membrane fluidity as expressed by an increase in short-chained/branched fatty acid content. Cultures treated with essential oils and fatty acids/esters showed an increase in 20:0 fatty acid content, which also denotes reduced fluidity. The effects of antimicrobials on membrane fatty acid composition varied depending on antimicrobial and L. monocytogenes strain. This information could be used to determine more efficient sequences or combinations of antimicrobials in multiple hurdle systems for control of the pathogen (Durán, Geornaras, Engle, et al., 2008).

Data on the growth potential of L. monocytogenes on commercial RTE meat and poultry products are needed for improvement or re-evaluation of current risk assessments (FAO/WHO (Food and Agriculture Organization & World Health Organization), 2004; FDA/FSIS (Food and Drug Administration/Food Safety & Inspection Service), 2003) of the pathogen in these products, or for development of new risk assessments. Relative to this, the growth potential (lag phase duration and growth rate) of L. monocytogenes was investigated in 26 commercial products from various companies (formulated with or without antimicrobials, L/D; sliced ham, uncurled and cured turkey breast, roast beef, pastrami, bologna, and beef and turkey frankfurters), during vacuum-packaged storage at 4, 7, and 12 °C for up to 90 days (Byelashov, Carlson, et al., 2009; Byelashov, Geornaras, et al., 2009; Geornaras, Le Marc, Durán, et al., 2008; Geornaras, Le Marc, Yoon, et al., 2008; Geornaras, Grosulescu, et al., 2009; Grosulescu, et al., 2009; Simpson, Geornaras, et al., 2008). Among ham products from company A (product X) and B (products Y and Z) formulated without antimicrobials, growth rates of the pathogen were lower when samples were stored at 4 °C (0.19–0.26 log CFU/cm²/day) than at 12 °C (0.67–0.95 log CFU/cm²/day), as expected; however, in all cases, growth to maximum levels of 6.5–7.9 log CFU/cm² was obtained. When L/D was present in ham formulations, the lag phase of L. monocytogenes was extended; lag times at 4 °C were up to 29.2, 5.2 and 22.2 days on products X, Y and Z, respectively. Growth rates of the pathogen on all products with L/D were 0.06–0.14 (4 °C), 0.06–0.17 (7 °C) and 0.17–0.38 (12 °C) log CFU/cm²/day (Geornaras, Grosulescu, et al., 2009). In two additional products, namely pastrami and roast beef, pathogen growth rates were similar for the products formulated without L/D (Byelashov, Geornaras, et al., 2009). However, in products with L/D stored at 4 and 7 °C, growth rates on pastrami were higher than those on roast beef. The longest lag phase (≥ 16 days) and the slowest growth rates (0–0.04 log CFU/cm²/day) were observed on roast beef formulated with L/D and stored at 4 °C. The fastest growth rate, 0.84–1.09 log CFU/cm²/day, was observed on roast beef without L/D and stored at 12 °C (Byelashov, Geornaras, et al., 2009). These data should be valuable to risk assessors in reducing uncertainties in predictive models used in developing or updating quantitative risk assessments of L. monocytogenes in RTE meat and poultry products.

Mathematical modeling may be useful in predicting the fate of L. monocytogenes in RTE meat products under various conditions of product storage and distribution, and can also be useful in determining safety-based product shelf-life under different conditions, assuming that the product is contaminated. The objective of several studies (Geornaras, Le Marc, Durán, et al., 2008; Geornaras, Le Marc, Yoon, et al., 2008; Le Marc, Geornaras, Barmpalia-Davis, et al., 2008; Le Marc, Geornaras, Carlson, et al., 2008; Le Marc, Geornaras, Baranyi & Sofo, 2009) was to develop models to predict L. monocytogenes populations in various RTE meat products as a function of product type, antimicrobials, storage temperature and time. L. monocytogenes data were first fitted (Baranyi & Roberts, 1994) to calculate lag phase durations (day) and exponential growth rates (log CFU/cm²/day) for each set of conditions. Rates and lag phases were then fitted with a polynomial equation as a function of temperature. Models predicting the fate of L. monocytogenes were then developed using the predicted values as a function of various variables. Predictive models were developed for the growth of L. monocytogenes on commercial frankfurters, bologna, cured and uncured turkey breast, and roast beef (Geornaras, Le Marc, Durán, et al., 2008; Geornaras, Le Marc, Yoon, et al., 2008; Le Marc, Geornaras, Carlson et al., 2008; Le Marc et al., 2009). For uncured turkey breast, the growth rate of L. monocytogenes was reduced by 60–68% in the presence of L/D; greater reductions (68–81%) were observed on cured turkey breast. The model describes accurately the effect of temperature on the growth rate of the pathogen (\(R^2_{adj} = 0.98\)) (Geornaras, Le Marc, Durán, et al., 2008). For roast beef, the developed model quantified accurately the effects of temperature and antimicrobials (1.5% potassium lactate and 0.05% sodium diacetate) on the kinetics of L. monocytogenes on this product (Le Marc, Geornaras, Carlson et al., 2008). In a related study (Yoon, Kendall, et al., 2009), models were developed for the prediction of lactic acid concentration, dipping time and storage temperature combinations determining growth/no growth interface boundaries, preventing growth, or allowing selected levels of growth of L. monocytogenes on frankfurters and bologna slices. The models were developed with data from real foods and their validation was done with published L. monocytogenes data. The results showed successful prediction of the response of the pathogen in 90% of the tested cases. These models may be useful in selecting appropriate lactic acid concentrations and dipping times to control L. monocytogenes on RTE meat products (Yoon, Kendall, et al., 2009). In general, models developed may be useful in efforts to select storage conditions and safety-based shelf-life for control of L. monocytogenes on products evaluated, may be applicable in the development of models for other products, conditions or pathogens, and may be used in new or updated risk assessments.

Although dry/semi-dry fermented sausages are characterized as low risk for listeriosis (FDA/FSIS (Food and Drug Administration/Food Safety & Inspection Service), 2003), data are lacking relative to the fate of post-processing contamination during storage of these products. Results (Simpson, Geornaras, et al., 2008) indicated that pathogen
levels decreased during storage of inoculated salami slices at rates that increased with storage temperature; acid-adapted inocula decreased more rapidly at 4 °C than those of other inocula, but survived longer than other inocula at 25 °C. A product-habituated inoculum survived the longest at 4 °C, but died faster than other inocula at 25 °C; at 12 °C, all inocula decreased similarly. Overall, all tested inocula decreased below detection (−0.4 log CFU/cm²) between 27 and 90 days of storage, depending on storage temperature and inoculum type (Simpson, Geornaras, et al., 2008). A similar study (Byelashov, Carlson, et al., 2009) was conducted with pepperoni slices. Overall, levels of the pathogen decreased continuously during storage, but the rate of death varied also with temperature of storage and type of inoculum (Byelashov, Carlson, et al., 2009). The results of these studies may also be useful in risk assessments and in helping processors select proper storage conditions for salami and pepperoni.

Lianou, Geornaras, Kendall, Scanga and Sofos (2007) evaluated growth of L. monocytogenes on uncured turkey breast under conditions simulating home storage (aerobic, 7 °C, 12 days) of product contaminated during slicing at the processing facility (soon after processing) versus at retail/home. Overall, the fate of the pathogen depended on whether the product was formulated with L/D, and on the contamination scenario. Processing plant-contamination of product without antimicrobials and retail/home-contamination of product formulated with L/D were found to be the worst and best case scenario, respectively, relative to pathogen levels encountered during subsequent home storage of product at 7 °C. Irrespective of contamination scenario, 12 days of home storage of sliced uncured turkey breast could result in pathogen levels of 7.3–7.7 log CFU/cm² in product without antimicrobials (Lianou, Geornaras, Kendall, Scanga, et al., 2007). A similar study (Lianou, Geornaras, Kendall, Belk, et al., 2007) with cured ham slices had similar conclusions, but growth occurred at slower rates than on the uncured turkey breast slices. These findings emphasize the importance of controlling L. monocytogenes throughout the food chain, and can be useful in establishing risk-based consume-by date labels for RTE meat products.

Recommendations by the USDA-FSIS indicate that frankfurters may be safely stored at home at 4 °C for 7 days in open, and 14 days in vacuum-sealed, packages, while the Food and Drug Administration (FDA) Food Code recommends that retail and foodservice operations should not store RTE foods that are not vacuum-sealed for longer than 4 days at 7 °C. A study (Byelashov, Simpson, et al., 2008) was conducted to validate these recommendations. Under the conditions tested, pathogen counts remained relatively constant in samples formulated with L/D, while in product without antimicrobials, counts reached 7.0 log CFU/cm² within 60 days of vacuum-sealed storage (4 °C). At day-4 of home storage, counts increased by <1.0 log CFU/cm² on frankfurters formulated without L/D, regardless of storage conditions. Fastest growth under USDA-FSIS recommended storage conditions (4 °C) occurred in samples stored for 20 days prior to home storage; the increases were 2.2 and 2.4 log CFU/cm² for samples stored for 7 (open packages) and 14 (vacuum-sealed packages) days, respectively. Thus, it may be advisable to re-evaluate the USDA-FSIS safe storage time limits for frankfurters formulated without antimicrobials (Byelashov, Simpson, et al., 2008).

Although surveys have shown that consumers regularly store unopened frankfurter packages in home freezers, little information is available regarding the fate of L. monocytogenes during home storage after thawing. When available, instructions recommend thawing in the refrigerator and discourage countertop thawing, while instructions for microwave defrosting are not always given on package labels. A study (Simpson Beauchamp et al., 2010) examined effects of antimicrobials (L/D), storage time, freezing, thawing method and subsequent home storage on L. monocytogenes on frankfurters. In general, the fate of the pathogen during aerobic storage, following thawing, was not influenced by freezing or by thawing method (Simpson Beauchamp et al., 2010).

Limited data are available on the safety of frankfurters or other RTE meat products reheated in household microwave ovens, even though their use is recommended by certain food processors. A study (Rodríguez-Marval et al., 2009) was done to evaluate manufacturer guidelines for reheating frankfurters, and to optimize recommendations (time/power-level combinations) for safe reheating of such products before consumption. Exposure to high (1100 W) microwave power for 75 s reduced pathogen levels to below detection (<−0.4 log CFU/cm²) on frankfurters formulated with L/D, even after 54 (4 °C) and 7 (7 °C) days of vacuum-packaged and aerobic storage, respectively. On frankfurters without L/D, where pathogen numbers on non-reheated (control) product increased (1.5 to 7.2 log CFU/cm²) as storage under vacuum and aerobic conditions progressed, reductions of up to 5.9 log CFU/cm² were obtained following microwave oven reheating at high power for 75 s. Depending on the treatment (i.e., product formulation and time/power-level combination), viable L. monocytogenes counts detected in the water used to reheat the frankfurters were <−2.4−5.5 log CFU/ml (Rodríguez-Marval et al., 2009). A companion study (Rodríguez-Marval, Kendall, Belk & Sofos, 2010) evaluated the effectiveness of different time and water-temperature combinations in destroying L. monocytogenes contamination on stove-top water-reheated frankfurters. The 80 °C (60, 120 s) and 94 °C (30, 60 s) treatments reduced counts on frankfurters formulated with L/D (initial levels were 0.6−0.9 log CFU/cm²) to/or below detection (<−0.4 log CFU/cm²). Average counts on non-reheated frankfurters without antimicrobials were the highest (4.5 log CFU/cm²) on 60-day old vacuum-packaged (4 °C) product stored aerobically (7 °C) for 7 and 14 days, and heat treatments reduced counts by 0.7 (30 s/80 °C) to >4.0 (120 s/80 °C and 60 s/94 °C) log CFU/cm². No survivors were detected in the heated water after any treatment (detection limit, <−2.5 log CFU/ml). While low levels of L. monocytogenes on frankfurters may be inactivated with short exposure to hot water, increased contamination that may occur as the product ages, needs longer times and/or higher temperatures for inactivation (Rodríguez-Marval et al., 2010).

Consumers show a preference in the use of natural, instead of synthetic, antimicrobials in their foods. Olive oil, lemon juice and vinegar have been reported to exhibit antimicrobial activities in mayonnaise and on vegetables. A study (Shen, Geornaras, Kendall & Sofos, 2009b) evaluated the antilisterial effects of salad dressings, oil and lemon juice or vinegar, without or with prior microwave oven heating, on frankfurters during simulated home storage. Dipping in salad dressings and in the combination of oil and lemon juice or vinegar caused significant (P<0.05) reductions of L. monocytogenes, which were greater than dipping in distilled water. Reductions increased with product storage (7 °C) from 0.5−0.9 (day-0) to 1.2−2.1 (day-14) log CFU/cm², as levels of contamination increased. More reduction of the pathogen was observed when treated after product microwave oven heating; reductions ranged from 1.2−1.9 (day-0) to 2.2−3.3 (day-14) log CFU/cm² (Shen, Geornaras, et al., 2009b). A related study (Shen, Geornaras, Kendall & Sofos, 2009c) found similar results with cured ham and uncured turkey breast slices (1.5×1.5×1.5 cm). Salad dressings and oil with lemon juice or vinegar may potentially contribute to control of L. monocytogenes on RTE meat products in the home environment.

The ability for gastrointestinal survival of L. monocytogenes is considered as an element of virulence. Studies evaluated survival among 13 L. monocytogenes strains in broth (Barmpalia-Davis et al., 2008a), and of a 10-strain composite on stored frankfurters (Barmpalia-Davis, Geornaras, Kendall & Sofos, 2009) or bologna and salami slices (Barmpalia-Davis, Geornaras, Kendall & Sofos, 2008b), during a simulated dynamic gastrointestinal challenge (gastric, for 120 min; intestinal, for 240 min; 37 °C) following exposure to artificial saliva. Additional studies evaluated survival of L. monocytogenes during exposure of stored frankfurters (Stopforth et al., 2005) and bologna slices (Formato et al., 2007) to a simulated gastric fluid (pH
1.0). Results suggested that L. monocytogenes levels on a food may affect cell numbers in the gastrointestinal tract more than differences in acid resistance among strains. Increased fat content had a protective effect against gastric destruction of L. monocytogenes, especially in fresher product. However, since the effect of fat was observed mainly at later stages of gastric exposure, it did not influence numbers of viable cells reaching the intestine. Prolonged storage may result in increased or decreased L. monocytogenes numbers during digestion depending on whether the product allows (bologna) or depresses (salami) growth of the pathogen (Barmpalia-Davis et al., 2008b).

In general, however, gastric acid resistance was associated with high levels of contamination in older product (Formato et al., 2007; Stopforth et al., 2005).

The 2003 FDA/FSIS quantitative risk assessment estimated that contaminated deli meats are responsible for 90% of human listeriosis cases in the United States (FDA/FSIS (Food and Drug Administration/Food Safety & Inspection Service), 2003). This risk assessment, however, did not distinguish between different types of deli meats and only considered post-retail growth of the pathogen. In order to provide an improved quantitative risk assessment for L. monocytogenes in deli meats, a revised risk assessment was developed (Pradhan, Ivanek, Gröhn, Geornaras, et al., 2009) that modeled: (i) the risk for three types of deli meats (i.e., ham, turkey, and roast beef); and (ii) L. monocytogenes contamination and growth from production to consumption while taking into consideration deli meat type-specific lag phase durations and exponential growth rates. The model was also used to assess how reformulation of the deli meats with growth inhibitors (i.e., L/D) would impact the number of human listeriosis cases. The results showed that: (i) the three types of deli meats differ in their ability to support L. monocytogenes growth and their estimated per annum risk to human health; thus, they should be separated into different categories in risk assessments; and (ii) reformulation of deli meats with growth inhibitors reduce listeriosis cases by an estimated 2.5- to 7.8-fold; however, even with reformulation, deli meats would still cause a considerable number of illnesses (Pradhan, Ivanek, Gröhn, Geornaras, et al., 2009). A companion study (Pradhan, Ivanek, Gröhn, Bukowski, et al., 2009) estimated the relative risk of listeriosis from ham and turkey formulated without and with growth inhibitors, under two scenarios: (i) in prepackaged products with contamination originating only from the manufacturer (at a frequency of 0.4%, based on reported data); and (ii) in retail-sliced products with contamination originating only from retail (at a frequency of 2.3%, based on reported data). Reformulation of products with growth inhibitors was estimated to reduce human listeriosis cases by 2.8- (ham) and 9- (turkey) fold, when contamination originated at the manufacturer, and by 1.9- (ham) and 2.8- (turkey) fold, when contamination originated at retail (Pradhan, Ivanek, Gröhn, Bukowski, et al., 2009). Risk assessment models, like the ones described here, will be critical for evaluating and identifying different control approaches so as to achieve the greatest public health impact (Pradhan, Ivanek, Gröhn, Bukowski, et al., 2009; Pradhan, Ivanek, Gröhn, Geornaras, et al., 2009).

7. Conclusions

In summary, hygiene, microbial hazards, and associated issues will continue to be major challenges to meat safety well into the future. It is important to realize that management of meat safety risks should be based on an integrated effort and approach that applies to all sectors, from the producer through the processor, distributor, packer, retailer, foodservice worker and consumer. We should also keep in mind that most foodborne illness cases are due to mishandling of foods in ways we know we should avoid, while animal-borne pathogens introduced into the environment also lead to illness associated with consumption of water or other foods. Thus, consumer education and environmental pollution issues should be major targets in our efforts to improve meat and food safety. The results of studies addressing some major current meat hygiene and safety issues, namely biofilm formation, safety of nonintact meat products, and control of L. monocytogenes in RTE meat and poultry products, presented here, should be useful in developing pathogen control strategies at processing, foodservice and in the home.

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References


