Nonintact Whole Muscle Food Safety: The Problem and Research Needs

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The Problem

Nonintact beef products include intact meat cuts such as chucks, ribs, tenderloins, striploins, top sirloin butts, and rounds, exposed to or injected with marination, flavoring, 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 that has been processed by chopping, grinding, flaking, or mincing, as well as manufacturing beef trimmings destined to be processed into formed and shaped items such as gyros (USDA-FSIS, 1999). A microbiological safety concern associated with these processes is that they may lead to contamination of interior parts of the products through transfer of cells from the surface to the interior, through cross-contamination, or by mixing of trimming or cuts to form larger items. A major proportion of steaks and roasts derived from muscles of lower tenderness may be subjected to mechanical tenderization or marination or restructuring, or both, 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 (BIFSCO, 2005, 2006).

Needle or blade-tenderized, moisture-enhanced, and restructured or formed meat products may be perceived by users as intact steaks or roasts, whereas in actuality they are classified together with ground beef. Thus, there is a concern that if internally contaminated products are unintentionally or intentionally undercooked, the pathogens may survive and result in human illness. Another concern is that injection solution ingredients may interfere with thermal inactivation of pathogens by increasing their heat resistance. In addition to undercooked ground beef, transmission of *Escherichia coli* O157:H7 has also been associated with consumption of products like beef roasts and nonintact beef steaks (Rodrigue et al., 1995; USDA-FSIS, 2003, 2004, 2005, 2007; Laine et al., 2005; Rangel et al., 2005; CDC, 2007). Generally, such outbreaks have been attributed to inadequate cooking (USDA-FSIS, 2005, 2007) or to products cooked directly from the frozen state without prior thawing (Laine et al., 2005). These outbreaks were responsible for several confirmed cases of *E. coli* O157:H7 infection and major product recalls (http://www.fsis.usda.gov/Fsis_Recalls/index.asp).

Following the notable outbreak of foodborne illness associated with consumption of undercooked ground beef contaminated with *E. coli* O157:H7 in 1992 to 1993, the United States Department of Agriculture Food Safety and Inspection Service (**USDA-FSIS**) declared in 1994 raw ground beef was contaminated with *E. coli* O157:H7 as adulterated under the Federal Meat Inspection Act. In 1999, USDA-FSIS classified all raw nonintact beef products described above as adulterated, if contaminated with *E. coli* O157:H7 (USDA-FSIS, 1999).

Control and Best Practices

The risks associated with blade tenderization, needle injection, and other technologies used in 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, proper chilling and rotation of injection solutions, potential use of antimicrobials in injection brines, effective sanitation and temperature controls, and proper cooking. Manufacturers should consider and follow industry recommended controls and best practices (http://www.bifsco.org/BestPractices.aspx) addressing raw material control and supplier selection, facilities and equipment design and sanitation, temperature control, process controls, lotting and traceability, interventions and antimicrobial agents, packaging and labeling, integrated approach to control, and hazard analysis critical control point principles. Processors should develop and implement facility and equipment sanitation standard operating

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procedures, whereas equipment manufacturers should consider the American Meat Institute 10 Principles of Sanitary Design (AMI, 2003; http://www.meatami.com/ht/a/GetDocumentAction/i/7281; http://www.meatami.com/ht/a/GetDocumentAction/i/1232; http://www.meatami.com/ht/a/GetDocumentAction/i/11006; http://www.meatami.com/ht/d/ArticleDetails/i/3175). It is recommended that processors of mechanically tenderized and enhanced products should follow sanitation practices similar to those recommended for establishments producing ready-to-eat products, as described in the Guidelines for Developing Good Manufacturing Practices, Standard Operating Procedures, and Environmental Sampling/Testing Recommendations in Ready-to-Eat Products (NMA, 1999).

Concerning microbiological sampling and testing of raw materials and products, it may include *E. coli* O157:H7, *Salmonella*, aerobic plate counts, coliforms, and *E. coli.* Results of raw material testing may be useful in hazard analysis, whereas a goal of testing should be to track supplier and production trends over time. Finished product testing may be useful in process and best practices validation and verification, but it should not be relied upon to declare a batch or lot of product as safe. Microbiological testing is also useful in the verification of cleaning and sanitation effectiveness and to ensure absence of *E. coli* O157:H7 niches or harborage sites on equipment or in the plant environment.

Research Needs

General

Research needs should address data gaps after a thorough evaluation of existing knowledge. Studies should then be designed to provide information needed to update, validate, and implement recommended controls and best practices. Input from industry and regulatory authorities should also be considered for optimally coordinated research, because the objective is to enhance the safety of nonintact products (NACMCF, 2002; USDA-FSIS, 2002a, b; BIFSCO, 2005, 2006). In addition to considering existing information, it is important to standardize methods and study protocols by giving specific consideration to product manufacturing methods, storage and distribution conditions, cooking procedures, sample collection and handling procedures, bacterial strain selection for testing, analytical methods, etc. Study designs should be developed in ways that facilitate use of generated data in predictive modeling. Development of product-specific models will allow prediction of risk for pathogen survival in nonintact products produced using current or novel tenderizing, marination, and enhancement formulations or techniques and yielding products of desired tenderness and flavor. Generated knowledge should be used to develop industry educational material and extension fact sheets for use in outreach education activities for industry personnel as well as for foodservice and consumers for proper product production, storage, handling, and cooking.

Research needs may be grouped into those related to raw materials, product manufacturing, ingredients and antimicrobial agents, and cooking. As indicated above, specific studies to address these and other needs should be designed based on existing knowledge, highlights of which are also presented in the following paragraphs.

Raw Materials

Research dealing with raw materials of nonintact meat products should:

- Develop, compare, and validate additional intervention approaches for more efficient reduction of contamination on carcasses and meat during harvest, fabrication, and processing.
- Collect qualitative and quantitative baseline data for *E. coli* O157:H7 and other pathogens of concern or appropriate indicator organisms, such as coliforms and *E. coli* biotype I, present in raw materials before needle or blade tenderization, enhancement, or restructuring.
- Evaluate the efficacy of antimicrobial interventions applied during harvest, fabrication, and on primal or subprimal cuts on pathogen survival, potential for growth, and on thermal resistance in nonintact products.

Reasons for only a limited number of outbreaks of *E. coli* O157:H7 associated with mechanically tenderized beef may be the low incidence of the pathogen in raw materials (Kennedy et al., 2006; Heller et al., 2007) and the concentration of the internalized contamination near the surface of the product (Gill and McGinnis, 2005). According to a study by Heller et al. (2007), only 0.2% of surface-sponge samples of 1,014 subprimals from 6 beef processing plants taken over a 5-wk period were positive for *E. coli* O157:H7 at <0.375 cfu/cm^2.

Processing

Research needs associated with nonintact product manufacturing should be designed to:

- Evaluate the effects of types of equipment, number of passes through the tenderizer, sanitation of equipment, quantity throughput, and temperature of processing room, primal/subprimals, and brines or enhancement solutions for production of nonintact products.
- Compare effects of needle/blade tenderizing equipment (e.g., hollow needle, solid needle, double-edge blade) and use the findings to establish proper operating practices.
- Determine differences in contamination potential among various types of nonintact products such

as needle- or blade-tenderized, pumped or otherwise enhanced, marinated, or formed.

- Evaluate the extent, distribution, numbers, and depth of bacterial cell translocation and internalization following mechanical and chemical enhancement.
- Validate the efficacy of sanitation protocols for removal of *E. coli* O157:H7 from blades/needles and equipment used to produce nonintact products.
- Determine the effect of brine recirculation on bacterial populations and pathogen prevalence.
- Compare pathogen behavior, growth, and survival between intact and nonintact products, including ground beef, of various formulations, processing conditions, distribution, and storage temperature and preparation protocols.
- Consider modifications in processing protocols to enhance pathogen control.

Although limited, published data indicate that surface-contaminating bacteria, including pathogens such as *E. coli* O157:H7 and *Salmonella*, may be translocated or entrapped from the surface into the interior of meat products when exposed to mechanical tenderization and enhancing or restructuring processes. According to a study by Sporing (1999) at Kansas State University, approximately 3 to 4% of *E. coli* O157:H7 inoculated on the surface of intact steaks was transferred to the interior during single-pass blade tenderization. The level of contamination transfer was approximately 3 log units when the surface contamination of loins was 7 log units. Mechanical tenderization of beef striploins did not change surface tissue levels of contamination with aerobes which were 2.8 log cfu/cm^2, whereas deep tissue contamination levels of tenderized samples were 2.0 log cfu/25 g (Gill et al., 2005b). Bohaychuk and Greer (2003) found initial counts of psychrotrophic bacteria in moisture-enhanced pork loins approximately 2 log units higher than those of noninjected controls. Deep tissue contamination may increase during blade tenderization with higher surface contamination levels (Gill and McGinnis, 2005). Thus, good sanitation and proper hygiene are important in minimizing internalized contamination of nonintact products.

Evidence exists that the concentration of cells translocated into the interior of meat decreases with increasing depth of blade penetration (Sporing, 1999). Another study also found that translocation of surface inoculated *Salmonella* to the interior of blade-tenderized and needle-injected pork loins decreased progressively with depth of penetration of the blades or needles (Thippareddi et al., 2000). Thus, most internalized cells should be deposited near the surface during single-pass blade tenderization, whereas only a few cells apparently remain on the blades for translocation to deeper tissue. In addition, multiple-pass tenderization may not result in significantly higher pathogen cell translocation from the surface into the interior of the meat (Sporing, 1999). Gill and McGinnis (2005) also reported that in beef tenderized by thin blade piercing, deep tissue contamination with total aerobes decreased with increasing depth and did not change with repeated piercing; recovery of contamination was similar after 1 or 8 incisions. Deep tissue contamination may vary with type of tenderizing equipment, whereas proper blade design and low surface contamination of meat may minimize deep tissue contamination (Gill and McGinnis, 2005). Heller et al. (2007) found that inoculated *E. coli* O157 was isolated from a larger proportion of injected than blade-tenderized beef samples.

Gill et al. (2005a) determined that brine injection of pork for 30 or 60 min allowed increases of aerobic bacteria in brine of more than 1 log unit to more than 4.5 log cfu/mL, whereas coliform counts remained at <2 log cfu/mL. Injection did not change numbers of bacteria on the meat surface, whereas numbers of aerobes and coliforms recovered from injected deep tissues were 2.1 log cfu/g and 1 log cfu/25 g, respectively. Greer et al. (2004) also reported that bacterial counts of recirculating brines increased after 2.5 h of moisture-enhanced pork production. Contamination levels of injected loins remained higher than those of controls during a 5-wk storage period at 2 or 5°C in vacuum packages (Bohaychuk and Greer, 2003).

As reported by Gill et al. (2008), injection of beef with brine after mechanical tenderization may increase deep tissue contamination by 1,000-fold compared with injecting without or before mechanical tenderizing. Thus, combining mechanical tenderization with injection may increase risks, whereas injection after mechanical tenderization should be avoided (Gill et al., 2008).

Ingredients and Antimicrobials

Studies are also needed to evaluate effects of nonmeat ingredients and antimicrobials on pathogen survival during processing, storage, and cooking of nonintact products. Specific objectives include:

- Examine effects of physical (e.g., ultraviolet light), chemical (e.g., ozonated water), and biological antimicrobial agents and combination interventions on the microbiological quality of recirculating brines, enhancement solutions, and products.
- Study brine formulations (e.g., salts, phosphates, flavoring agents, hydrocolloids such as carrageenan, proteins, etc.) for effects on microbial contamination during processing and frozen, refrigerated, or retail storage and potential protective or increased lethality effects during cooking of nonintact products.

- Evaluate antimicrobials (e.g., lactate, diacetate, acetate, lactic acid, citric acid, acidified sodium chlorite, peracetic acid, acidified calcium chlorite, cetylpyridinium chloride, sodium metasilicate, nisin, pediocin, spice extracts, and essential oils) introduced in products for effects on pathogen survival during processing, storage, and cooking.
- Conduct studies to develop novel/modified marinades or enhancement/marination strategies including marination times and stage/sequence of ingredient addition by immersion or injection and evaluate survival/growth of *E. coli* O157:H7 during storage and cooking.
- Evaluate survival/growth during frozen, refrigerated, or retail-type storage of *E. coli* O157:H7 in products formulated or moisture-enhanced with ingredients shown to enhance thermal destruction.
- Monitor the effect of stress-adaptation of *E. coli* O157:H7 on survival/growth during storage and evaluate subsequent heat tolerance in nonintact products (Samelis and Sofos, 2003).

Results of studies have indicated that addition of antimicrobials (e.g., sodium lactate and sodium lactate plus sodium diacetate) to enhancement solutions may control contamination in enhanced products (Wicklund et al., 2006). Ransom et al. (2003) reported that lactic acid and acidified sodium chlorite were the most effective pathogen decontamination solutions currently approved for commercial use when tested by immersion of *E. coli* O157:H7-inoculated beef. Other compounds tested included acetic acid, acidified chlorine, cetylpyridinium chloride, lactoferricin B, and peroxyacetic acid. Overall, cetylpyridinium chloride was most effective and reduced bacterial populations by 2.1 to 4.8 log cfu/cm^2, whereas reductions by other compounds were in the range of 0.3 to 1.8 log cfu/g. Djenane et al. (2003) reported that spraying of beef trimmings destined for restructured steaks with 1.5% lactic acid solution inhibited growth of spoilage bacteria such as *Pseudomonas* and *Brochothrix thermosphacta.* Heller et al. (2007) compared the effectiveness of surface trimming, hot water, lactic acid, or activated lactoferrin plus lactic acid treatments for reducing levels of *E. coli* O157:H7 on inoculated surfaces of beef cuts before blade tenderization or moisture enhancement. Average reductions of surface contamination, following treatment with antimicrobials, was 0.93 to 1.10 log cfu/100 cm^2. Internal levels of contamination were higher in untreated samples. Thus, interventions applied before mechanical tenderization or enhancement may reduce surface contamination and its transfer into the tissue. However, additional work is needed to determine the effect of these and other ingredients on the subsequent thermotolerance of *E. coli* O157:H7 and other pathogens during cooking.

We have evaluated the effects of restructuring, marination, flavoring, and tenderizing ingredients (e.g., potassium lactate, sodium lactate, calcium lactate, calcium ascorbate, calcium chloride, sodium chloride, sodium tripolyphosphate, etc.) on cooking (i.e., 60 or 65°C simulating rare and medium rare doneness) destruction of *E. coli* O157:H7, internalized in a ground beef model system (Yoon et al., 2007; Mukherjee et al., 2008a,b). Marination and tenderization 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 sodium chloride/phosphate in the formulations may have a protective effect on the pathogen, but it maintains product pH and improves cooking yields. C. F. Gill, L. F. Moza, and S. Barbut (Agriculture and Agri-Food Canada, personal communication) has unpublished data indicating that brines containing sodium chloride and sodium tripolyphosphate resulted in cell injury during cooking of steaks inoculated with *E. coli* but not *Listeria innocua*, whereas inclusion of soy protein or emulsified sunflower oil in the brine did not protect the cells from injury or inactivation.

It is important to consider the potential effect of prior exposure of *E. coli* O157:H7 to processing conditions on the thermotolerance of the organism during cooking. The destruction of *E. coli* O157:H7 during heating of nonintact steaks may be altered if meat is subjected to marination procedures. For instance, previous work (Yen et al., 1991, 1992) found that pathogens were protected from thermal inactivation in the presence of salt and other curing agents at certain heat-processing temperatures. Traditional marinades applied on fresh beef destined for jerky production may have a protective effect on *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* during drying (60°C) compared with nonmarinated beef (Calicioglu et al., 2002a,b, 2003). In contrast, exposure of beef to acetic acid or Tween 20 before marination enhanced the inactivation of *E. coli* O157:H7 during drying (Calicioglu et al., 2002a,b, 2003). Thus, it may be useful to evaluate the thermotolerance of the pathogen originating in environments or conditions representative of fresh beef processing or marination procedures that could potentially create sublethal stresses. The application of such stresses may render the pathogen more resistant to secondary stresses during actual processing or offer cross-protection to other processing stresses (Samelis and Sofos, 2003).

Cooking

Because proper cooking renders intact as well as nonintact products safe, it is important to develop effective cooking protocols for various products. Research studies should be designed to:

- Collect additional data on the extent of inactivation of internalized cells in terms of prevalence, numbers, and depth.
- Compare heat transfer rates, pathogen thermotolerance, and extent of inactivation in

products injected with various levels of brines and of variable fat contents.

- Compare effects of various cooking methods (grilling, broiling, frying) and equipment on pathogen inactivation.
- Compare pathogen inactivation in intact, ground, and various nonintact products cooked to the same degree of doneness.
- Determine integrated resistance and lethality characteristics (e.g., D and z values) of various *E. coli* O157:H7 strains and other pathogens and identify strains of various resistance characteristics.
- Compare thermal inactivation of pathogens in nonintact steaks and roasts of different sizes and thickness.

As indicated, cells embedded into muscle tissues could be protected from thermal destruction, especially if consumers or foodservice, intentionally or unintentionally, undercook the product. However, studies examining the effectiveness of heating on killing *E. coli* O157:H7 that has been translocated into the interior of meat tissue by mechanical tenderization, injection, or restructuring are limited (USDA-FSIS, 2002a,b; Mukherjee et al., 2008a). The comparative risk assessment between nonintact and intact beef steaks conducted by USDA-FSIS indicated that oven broiling to ≥60°C would result in safe blade-tenderized beef steaks (USDA-FSIS, 2002a,b). According to Gill and McGinnis (2004), a medium rare state of cooking is adequate for safety of mechanically tenderized beef that has no excessive (1 to 2 log units) deep tissue contamination. Gill et al. (2005a) concluded that cooking of injected moisture-enhanced pork to a medium rare condition of doneness should be adequate for safety, because a cooking temperature of 61°C allowed recovery of only 1.0 log cfu of aerobic bacteria/25 g from deep tissues, whereas cooking to 70°C resulted in no recovery of bacteria. Another study found that cooking mechanically tenderized striploins to medium rare or well done allowed recovery of aerobes (initially present at 2 log cfu/25 g) only from 2 of 25 samples cooked to each state, suggesting that such cooking should result in safe products (Gill et al., 2005b). C. O. Gill, L. F. Moza, and S. Barbut (Agriculture and Agri-Food Canada, personal communication) have unpublished data indicating that cooking steaks enhanced with brines containing sodium chloride and sodium tripolyphosphate, with or without soy protein or emulsified sunflower oil, to >60 to 65°C inactivated inoculated *E. coli* and *L. innocua*; cooking to 65°C inactivated more than 7 log units of cells in the steaks. According to the Food and Drug Administration Food Code (FDA, 2005), nonintact beef products should be cooked to 68.3°C (155°F).

It was reported by Sporing (1999) that thicker steaks (3.2 cm compared with 1.3 cm) had higher reductions of the pathogen when cooked to the same internal temperature. According to Thippareddi et al. (2000), *Salmonella* lethality at 71.1°C (160°F) was higher in nonintact pork loin chops of 2.5 cm thickness compared with 1.25 cm thickness. Because the time needed to achieve a target internal temperature increases with thickness, thicker cuts are subjected to heat for longer cooking times, resulting in higher microbial inactivation levels.

Sporing (1999) determined that broiling was more effective than grilling and frying in inactivation of contamination in blade-tenderized beef regardless of steak thickness or temperature; cooking effectiveness on pathogen inactivation increased in the order broiling > grilling > frying. Our studies have also shown that among cooking methods, broiling to the internal temperature of 65°C was most effective compared with grilling and frying. In general, the effectiveness of cooking methods in decreasing surviving counts of *E. coli* O157:H7 was in the order of broiling > frying ≥ grilling; cooking of frozen products from their frozen state was less effective compared with products stored at 4 or 12°C (Yoon et al., 2007; Mukherjee et al., 2008a,b). Potential explanations for this are as follows: the high temperature of the heating element used in broiling may be more efficient in inactivating bacterial cells on the surface of steaks than grilling and frying, which involve less contact with a flame and even distribution of heat on a skillet, respectively, and contamination in the interior may be inactivated better by broiling than other methods, because when the internal temperature is reached the surface of the product has reached significantly higher temperature, allowing the temperature to continue to rise even after removal from the broiler, and therefore, broiled steaks likely reached internal temperatures higher than desired. In general, available information indicates that the higher the cooking temperature and the thicker the steaks, the greater the lethality (Luchansky et al., 2005).

Summary

It can be summarized that even though at low rates, restructuring, mechanical tenderization, and injection of flavoring, brining, and tenderizing ingredients could potentially, even though infrequently and at low levels, internalize foodborne pathogens from the surface to the interior of products like beef steaks and roasts. If such contaminated products are considered or mistaken as intact and intentionally or accidentally undercooked by consumers during cooking, then the internalized pathogen cells might survive and pose a safety concern to consumers. It should be emphasized that this concern exists only when the product is undercooked. The risk of pathogen survival could be higher if chemical ingredients used in marination, tenderization, moisture enhancement, or restructuring formulations were protective against thermal inactivation of pathogens. In contrast, other ingredients or additives may enhance thermal inactivation of pathogens. Therefore, there is a need for additional research, as presented above, to generate information to be used in

updates of risk assessments and for development of improved best industry practices for safe production of such products.

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