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Cooking Inactivation of Shiga Toxin Producing *Escherichia coli* (STEC) in Non-intact Beefs--a mini-Review

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Abstract

Escherichia coli O157:H7 and Non-O157 shiga toxin producing *E. coli* (STEC) serogroups O26, O45, O103, O111, O121, or O145 are both considered adulterated in non-intact beefs. Forty to fifty-eight percent of U.S. consumers prefer to order beef steaks of medium rare to rare status. From 2000 to 2007, undercooked non-intact beef products have been involved in several outbreaks in the United States due to contamination with *E. coli* O157:H7.Traditional cooking practices of non-intact beef steaks contaminated with Non-O157 STEC may result in the same food safety risks as shown in *E. coli* O157:H7 through consumption of undercooked contaminated products. This mini-review focuses on the research advances in recent 10 years to evaluate the effectiveness of cooking inactivation of *E. coli* O157:H7 and Non-O157 STEC contaminated in ground beef, mechanical tenderized beef, and moisture enhanced non-intact beef.

Introduction

Non-intact beef products include ground beef, mechanically or chemically tenderized beef cuts, restructured entrees, and meat products that have been injected with brining solutions for enhancement of flavor and/or tenderness [1]. *Escherichia coli* O157:H7 or Non O157 Shiga toxin-producing *E. coli* (STEC) may be translocated from the meat surface to internal tissue by mechanical tenderization or needle injection of solutions, or entrapped in the tissue during restructuring. These processing procedures could protect the pathogens from lethal heating effects, especially if the products are undercooked [2,3].

E. coli O157:H7 or Non-O157 STEC can generate shiga-like toxin and cause severe hemolytic uremic syndrome in infected human bodies, and 10 cells of infection may cause a healthy person dead [4]. Since 1999, *E. coli* O157:H7 has been considered as an adulterant of raw non-intact beef products [1]. On November 2011, U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) announced that, as of June 2012, non-intact beef products would also be considered adulterated if they were contaminated with Non-O157 STEC serogroups O26, O45, O103, O111, O121, or O145 [5]. In an early report from Centers for Disease Control and Prevention (CDC), 62,000 cases of symptomatic *E. coli* O157:H7 were estimated to occur annually in the United States, resulting in approximately 1,800 hospitalizations and 52 deaths [6]. However, CDC's new data [7] generated in 2011, revealed that *E. coli* O157:H7 and Non-O157 STEC caused annual foodborne illnesses ranging from 73,000 to 97,000 and 37,000 to 169,000, respectively, which increased dramatically since their report [6] of 1999.

Most recently, USDA-FSIS's "Risk Profile of Pathogenic Non-O157 STEC" states that traditional cooking of non-intact steaks contaminated with Non-O157 STEC may result in the same food safety risks as shown in *E. coli* O157:H7 through consumption of undercooked contaminated products [8]. A recent study reported that, Non-O157 STEC with 9 serogroups was isolated from 300 out of 4133 ground beefs collected from 18 commercial ground beef produces [9]. This high isolation rate indicates that non-intact beefs, if contaminated or undercooked, could be a source of foodborne illness associated with Non-O157 STEC. Recent estimates indicate that Non-O157 STEC is responsible for 112,752 foodborne illnesses in the U.S. annually [7]. According to the survey, 40-58% of U.S. consumers ordered beef steaks at medium rare (60 to 62.8°C) to rare (54.4 to 57.2°C), which could be a potential threat to public health [10].

Thus, comprehensive studies of demonstrating *E. coli* O157:H7 and Non-O157 STEC lethality during common cooking practices for non-intact beef products lead a great understanding and meaningful comparative risk assessment of non-intact beef safety. This mini-review discusses the regulatory cooking recommendations and research progress related to thermal inactivation of STEC in non-intact beefs.

Regulatory cooking recommendations

The USDA-FSIS indicated that ground beef cooked to an internal temperature of at least 160°F (71.1°C) [11], and non-intact blade-tenderized beef steaks oven-broiled to an internal temperature of 140°F (60°C) or above would not present a great risk to the consumers [2,3]. Recently, they suggested that the microbiological safety of mechanically tenderized beef steaks could be assured by cooking to an internal temperature of 71°C, or cooking to internal temperature of 63°C with rest time of 3 min [12]. They also recommended that the starting temperature of the cooking oven should be at 350°F (176°C) or no lower than 325°F (162.8°C) when cooking corned beef [13]. The North Carolina State's Division of Environmental Health in the Department of Environment and Natural Resources stated that cooking all muscle beef such as steaks and roasts to an internal temperature of 155°F (68.3°C), as measured by a food thermometer at home, will eliminate the bacteria inside the meat [14]. In 2002, a

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comparative risk assessment for non-intact and intact beef steaks conducted by USDA-FSIS [2,3] indicated that there were insufficient data to determine whether the traditional cooking methods are adequate to destroy *E. coli* O157:H7 translocated during the blade tenderization process. Because proper cooking renders foods microbial safe, it is important to develop effective cooking protocols for non-intact beef products.

Research Progress of Thermal Inactivation of *E. coli* O157:H7 and Non-O157 STEC

Previous studies showed clearly that the lethality of STECs during and after cooking depends on multiple factors including cooked internal temperatures, cooking appliances, products' thickness, ingredients and the location of the bacteria within the meat. Besides, *E. coli* O157:H7 and Non-O157 STEC behave similarly when exposed to thermal treatments. The efficacy of thermal inactivation of STECs in non-intact beefs relies on cooked internal target temperatures. An early research from Sporing [15] showed that oven-broiling 3.2 cm thick beef steaks to the internal temperature of 60 to 76.7°C resulted in a 6.5 log reduction of *E. coli* O157:H7. Luchansky *et al.* [16] reported that cooking blade-tenderized beef subprimal steaks to internal temperature ranging from 48.8 to 60°C on a commercial open-flame gas grill resulted in 2.6 to 4.6 log CFU/g reductions of surfaced inoculated *E. coli* O157:H7. Recently, Luchansky et al. [17] reported that cooking brine-injected steaks on a commercial open-flame gas grill to internal temperatures of 37.8°C (100°F) to 71.1°C (160°F) achieved reductions of 0.3 to 4.1log CFU/g of *E. coli* O157:H7 and 0.5 to 3.6 log CFU/g of Non-O157 STEC among different brine formulation or internal temperatures. In a related study, they also found that cooking refrigerated, frozen, or freeze-thawed ground beef patties on commercial gas or electric grills to internal temperatures of 71.1 and 76.6°C reduced 5.1-7.0 log CFU/g of *E. coli* O157:H7 and Non-O157 STEC [18].

Non-intact beefs are typically prepared in commercial restaurants or consumer homes using a variety of cooking methods including broiling, grilling, frying and microwaving [19,15,2,3]. The survey of food intakes by individuals conducted by the USDA indicated that approximately 30% of consumers grill or broil steaks, while 40% of them fry steaks [20]. According to the guidelines of cooking by the American Meat Science Association, cooking methods include roasting, broiling and pan-broiling. Roasting is a method transmitting heat to the meat by convection in a closed preheated oven; broiling cooks directly through radiant heat from one direction; and pan-broiling is used for cooking thin patties by direct heat of conduction [21]. The terminologies of the cooking methods were also described by other names in different research studies, which are shown in Table 1.

Various cooking methods affected thermal inactivation efficacy of STEC in non-intact beefs. In a study to compare a double-side grill with a single side grill simulating consumer-style, Rhee et al. [22] revealed that *E. coli* O157:H7 reductions increased as one-turnover-single-sided grill (4.7 log CFU/g) <multi-turnover-single-sided grill (5.6 log CFU/g) < double-sided grill (6.9 log CFU/g) when ground beef was cooked to internal temperature of 71.1°C. Studies [15,23,24,25] from various research institutions suggested that broiling was the best cooking method regarding thermal inactivation of *E. coli*O157:H7 in non-intact beefs. Sporing [15] reported approximately 3 to 5, 4 to 6 and 5 to 6 log CFU/g reductions of *E. coli* O157:H7 on beef steaks cooked to the internal temperature of 65.6°C using electric skillet frying, gas grilling and oven broiling, respectively; they also found that cooking effectiveness on pathogen inactivation increased in the order of broiling > grilling > frying. Ortega-Valenzuela et al. [23] reported that broiling was more effective in eliminating *E. coli* O157:H7 compared with gas grilling when the same internal temperature was reached. Similar results were also obtained by Mukherjee *et al.* [24]. Moreover, Yoon et al. [25] confirmed that broiling was more effective in reducing *E. coli* O157:H7 than grilling and pan-frying in tenderized ground beef patties when cooked to 60 or 65°C.The possible explanation is that broiling causes more even distribution of heat surrounding the samples, takes longer time to reach the final internal temperature, and allows the temperature of the product near the surface to increase more significantly than grilling or frying [15]. In a recent study conducted by the author, thermal inactivation of *E. coli* O157:H7 in salt and sodium-tripolyphosphate moisture enhanced reconstructed beefs increased in the order of double pan-broiling ≤ pan-broiling<roasting when the internal temperature reached 65°C [26]. The author's another study found that cooking appliance with high starting temperatures of 204-260°C resulted in greater reductions (3.3-5.5 log CFU/g) than those obtained at low starting temperature of 149°C (1.5-2.4 log CFU/g) [27]. In the same project, Adler *et al.* [28] found that *E. coli* O157:H7 survivals caused by pan broiling increased with increasing depth of the contamination, whereas roasting in a kitchen oven showed similar pathogen survivals regardless of the depth.

Table 1: The conundrum of terminologies of cooking methods of various studies.

Heat transferring	What is stated by other studies	What is stated by AMSA Appliances
Conduction	Grilling (23; 24; 25)	Double pan-broiling George Foreman® grill (26) (26)
Conduction	Frying (15; 23; 24; 25)	Pan-broiling Presto® electric skillet (26; 27; 34)Sanyo® grill (26;27;34)
Convection	Broiling (15; 23; 24; 25)	Roasting Oster® toaster oven (26; 27)Magic Chef ® kitchen oven (26;27)

In addition to the cooking methods, the thickness of non-intact beef is another factor that influences the heat transfer through the product and consequently the elimination rates of pathogens inside the steaks. Sporing [15] reported that thick steaks (3.2 cm) compared to thin ones (1.3 cm) had more reduction of *E. coli* O157:H7 when cooked to the same internal temperature by broiling in a standard kitchen oven. The study also found that the thick (4.0 cm) moisture enhanced non-intact beefs resulted in greater inactivation of *E. coli* O157:H7 than their thinner (1.5 cm or 2.5 cm) counterparts when cooked to an internal temperature of 65°C [26]. In a similar study, Adler *et al.* [28] observed that pan-broiling or roasting blade-tenderized beef steaks with a thickness of 2.4 cm resulted in an additional 1 log CFU/g reduction of *E. coli* O157:H7 compared to the 1.2 cm thick samples. This might be attributed to overheating of the thicker products due to the longer cooking time that was taken to cook them to the same internal temperature. However, Luchansky's study [29] found that regardless of temperature or thickness, cooking 2.54 and 3.81 cm thick mechanical tenderized steaks on a commercial open-flame gas grill to internal temperatures of 48.9-71.1°C reached

2.0-4.1 log (*E. coli* O157:H7) and 1.5-4.5 log (Non O157-STEC) reductions, respectively. They pointed out that *E. coli* O157:H7 and Non O157-STEC behaved similarly during heat processing, and some unexpected survivors were due to uneven heating of the products [29].

The above major factors influencing thermal inaction of the pathogen in non-intact beefs were concluded in table 2. Additional studies showed that other factors including beef ingredients, stress adapted STEC cells, cooked beef searing time and turning over times influenced STEC reduction during thermal process. Four studies conducted by Muherjee and Yoon [30,31,32,33] indicated that lethality rate of internalized *E. coli* O157:H7 in marinated, tenderized, and reconstructed beef patties increased with adding organic acids (lactic, citric and acetic acid) in beef mixture. Muherjee *et al.* [30] found that potassium lactate protected *E. coli* O157:H7 in beefs during thermal processing. Their study showed that addition of cetylpyridiniumchlorite (CPC) to the brine solution of moisture enhanced reconstructed beefs resulted in the lowest number of *E. coli* O157:H7 survivors when the samples were cooked to 65°C [34]. The same study also found that acid stress-adapted cells were resistant to heat treatments, however, cold or desiccation stress-adapted cells were more sensitive to heat as compared with unstress-adapted E. coli O157:H7 cells [34]. In a study of investigating searing time of cooked non-intact beef, Porto-Fett *et al.* [35] suggested that cooking beef prime rib to internal temperatures of 48.9, 60.0, or 71.1˚C with following hold at 60.0˚C for ≥ 8 h could achieve a 5.0-log reduction of *E. coli* O157:H7. In another study of investigating the effect of steaks' turning over times for STEC control, Gill *et al.* [36] observed that turning over mechanically tenderized steaks more than twice during grilling to 63˚C killed all contaminated *E. coli* O157:H7, which achieved better pathogen inactivation effect than those cooking to 71˚C with only once turning over.

Other technologies regarding inactivation of *E. coli* O157:H7 and Non-O157 STEC

In addition to thermal treatments, other technologies including application of surface trimming, commercial antimicrobials and probiotics (e.g. lactic acid bacteria) during non-intact beef processing were reported to effectively reduce *E. coli* O157:H7and Non-O157 STEC populations. In a study of evaluating the efficacy of water-wash with beef surface trimming to inactivate *E. coli* O157:H7 on vacuum packaged beef trimmings, Lemmons *et al.* [37] reported that full surface trimming without or with water-wash reduced the pathogen populations below detective limit. A study conducted by Echeverry *et al.* [38] found that spraying lactic acid bacteria, acidified sodium chlorite and lactic acid on mechanically tenderized and brine enhanced beef reduced internalized *E. coli* O157:H7 by 1.2 to >2.2 log, and 0.8 to 3.0 log, respectively. In a similar study, Echeverry *et al.* [39] reported that lactic acid bacteria and lactic acid reduced internal *E. coli* O157:H7 up to 3.0 log and acidified sodium chlorite reduced the pathogen ranged from 1.4 to 2.3 login mechanically tenderized and brine enhanced beef under a simulated purveyor setting. A series studies of Adler *et al.* [40], Byelashov *et al.* [41], and Ko *et al.* [42] found that adding cetylpyridinium chloride (0.5-5.5%) in non-intact beef bring solutions showed immediate or additional reduction of *E. coli* O157:H7 by 1 to > 3.5 log/unit. In the same research group, Geornaras *et al.* [43] reported that acidified sodium chlorite, peroxyacetic acid, or sodium metasilicate reduced *E. coli* O157:H7 or Non-O157 STEC on non-intact beef trimmings by 0.7 to 1.0, 0.6 to 1.0, and 1.3 to 1.5 log CFU/cm^2, respectively. In a related study, Fouladkhah *et al.* [44] found that dipping beef trimmings in 5% lactic acid of 25 or 55˚C reduced *E. coli* O157:H7 and Non-O157 STEC by 0.5 to 0.9 and 1.0 to 1.4 log CFU/cm^2, respectively. Wolf *et al.* [45] also reported that lactic acid treatment reduced *E. coli* O157:H7 on beef trimmings by 0.91 to 1.41 and Non-O157 STEC by 0.48 to 0.82 log CFU/cm^2. A most recent study of Jadeja and Hung [46] suggested that washing beef trimming in electrolyzed oxidative water with free chlorine of 50 ppm reduced *E. coli* O157:H7 and Non-O157 STEC by 0.44 to 1.54log CFU/cm^2.These studies clearly showed that applying commercial antimicrobials during non-intact beef processing reached averagely 0.5 to 3.0 log reduction of *E. coli* O157:H7 or Non-O157 STEC, which is lower than those of thermal treatments.

Table 2: Major thermal processing factors influencing cooking inactivation of pathogens in non-intact beefs.

Factors	Influence	References
Cooked internal temperatures	The higher, the more reduction	[15-18]
Cooking methods	Broiling (roasting) is the best	[15,23-26]
Thickness of beef	The thicker, the more reduction	[15,26,28]

In conclusion, research results generated from these studies quantified the efficacies of cooking intervention as post-harvest STEC control practices applied to non-intact beef preparation at retail, foodservices, and home. They provided useful information regarding developing educational material and extension fact sheets, such as a cooking guidance manual about proper production and storage of non-intact beef for industry personnel, foodservice, and consumers, and choosing appropriate cooking approaches and degree of doneness when cooking beef products. These findings should also be useful for U.S. Department of Agriculture, Food Safety and Inspection Service USDA-FSIS to update risk assessments of *E. coli* O157 and Non-O157 STEC on non-intact and intact beef products.

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