Research Note

Thermal Inactivation of *Escherichia coli* O157:H7 and Non-O157 Shiga Toxin–Producing *Escherichia coli* Cells in Mechanically Tenderized Veal†

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ABSTRACT

Preflattened veal cutlets (ca. 71.5 g, ca. 0.32 cm thick) were surface inoculated with ca. 6.8 log CFU/g of a multistrain cocktail of *Escherichia coli* O157:H7 (ECOH) or a cocktail made of single strains of serogroups O26, O45, O103, O104, O111, O121, and O145 of Shiga toxin–producing *E. coli* (STEC) cells and then were mechanically tenderized by passing once through a “Sir Steak” tenderizer. For each cooking time, in each of at least three trials, three inoculated and tenderized cutlets, with and without breading, were individually cooked in 15 or 30 ml of canola oil for 0.0, 0.75, 1.0, 1.25, 1.5, 1.75, or 2.25 min per side on an electric skillet set at 191.5°C. The temperatures of the meat and of the skillet were monitored and recorded using a type J thermocouple. Regardless of the breading or volume of oil used to cook the meat, the longer the cooking times, the higher was the internal temperature of the meat, along with a greater reduction of both ECOH and STEC. The average final internal temperature of the meat at the approximate geometric center ranged from 56.8 to 93.1°C. Microbiological reductions of ca. 2.0 to 6.7 log CFU/g and ca. 2.6 to 6.2 log CFU/g were achieved for ECOH and STEC, respectively. Our data also revealed no differences in thermal inactivation of ECOH relative to the volume of oil used to cook nonbreaded cutlets. However, when cooking breaded cutlets, the use of more (30 ml) compared with less (15 ml) cooking oil resulted in greater reductions in pathogen numbers. To deliver about a 5.0-log reduction of ECOH and STEC, and to achieve the recommended internal temperature of 71.1°C, it was necessary to cook mechanically tenderized veal cutlets for at least 1.5 min per side on a preheated electric skillet set at 191.5°C and containing 15 ml of cooking oil. These data also established that cooking times and temperatures effective for inactivating serotype O157:H7 strains of *E. coli* in tenderized veal are equally effective against the additional six non-O157 Shiga toxin–producing strains investigated herein.

Over the past 30 years, illnesses caused by serotype O157:H7 strains of Shiga toxin–producing *Escherichia coli* (ECOH) have been linked to undercooked and/or underprocessed beef products, including at least six outbreaks likely involving nonintact meat products (4). The U.S. Centers for Disease Control and Prevention (CDC) (8, 21) estimates that there are about 73,000 to 97,000 and 37,000 to 169,000 illnesses each year attributed to ECOH and non-O157 Shiga toxin–producing *E. coli* (STEC), respectively. Data compiled by the CDC also established that about 70 to 83% of illnesses confirmed to be caused by STEC are associated with serogroup O26, O45, O103, O111, O121, and O145 strains (6, 7). In addition to ECOH, strains of the abovementioned STEC serogroups (also known as “The Big Six”) are also considered adulterants if present in raw ground beef, raw intact beef and other products intended for manufacture of ground beef or nonintact beef, and nonintact beef (26). Nonintact beef would include raw beef products that are enhanced by blade tenderization or enzymatic-chemical injection and related processes. Such processes, commonly used to enhance the quality of meat, may also have a significant public health impact due to the internalization and distribution of pathogens into the deeper regions of the meat, wherein these pathogens may be protected and possibly survive to a greater extent during subsequent cooking (16).

The market for veal is considerably smaller than the market for beef, but the former still generates some $1.5 billion in sales each year in the United States alone (2). Although there have been no reported foodborne illnesses due to veal in the United States, veal products were implicated in several recalls recently due to possible contamination with ECOH and STEC. For example, in 2009, meat markets in Ohio recalled ca. 800 lb (ca. 363 kg)
of veal due to possible contamination with ECOH (12). In
2013, a California company recalled ca. 1,260 lb (ca. 571 kg) of boneless veal trimmings due to a positive ECOH sample (3). And, as recently as 9 August 2013, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) announced a recall of ca. 12,600 lb (ca. 5,715 kg) of boneless veal products due to potential contamination with serotype O157:H7, O145, and O45 strains of E. coli (29). Given the latest recalls associated with veal (3, 12, 28, 29) and, ostensibly, the greater likelihood of recovering ECOH and STEC from veal trim than beef trim, additional studies are warranted to validate cooking practices for preparing veal. Thus, we evaluated the effect of cooking times, application of breading, and the volume of cooking oil used on the subsequent thermal inactivation of Shiga toxin–producing E. coli in tenderized veal cutlets.

The USDA-FSIS verifies that establishments control relevant physical and microbial hazards in regulated meat, poultry, and processed egg products. In 2012, the USDA-FSIS began verification testing for six serogroups of non-O157 STEC in domestic and imported beef manufacturing trimmings from cattle slaughtered on or after 4 June 2012. Verification sampling results collected to date by the USDA-FSIS suggest that there is a greater prevalence of STEC in veal products than in other beef products. More specifically, data provided by the USDA-FSIS, as part of their testing of raw ground beef component samples in federal plants as of 14 July 2013, found 0 (0%) and 3 (0.78%) of 383 positive for ECOH and STEC in beef, respectively, whereas 3 (6.12%) of 49 and 1 (7.69%) of 13 were positive for ECOH and STEC in veal, respectively (27). Although far fewer veal samples were tested compared with beef samples, these findings raise the question as to what are the most significant risk factors associated with veal production and postharvest processing, as well as retail, food service, and consumer handling of veal, that are associated with STEC contamination. To address these concerns, we validated the effect of mechanical tenderization and cooking of veal cutlets on the comparative thermal inactivation of ECOH and STEC.

MATERIALS AND METHODS

Bacterial strains. The five rifampin-resistant (100 µg/ml) derivatives of ECOH ([i] USDA-FSIS 380-94 [meat isolate], [ii] ATCC 43888 [human isolate, CDC B6914-MS-1], [iii] ATCC 43889 [human isolate, CDC B1409-C1], [iv] ATCC 43890 [human isolate, CDC C984], and [v] USDA-FSIS 45756 [meat isolate]) and seven rifampin-resistant derivatives of non-O157:H7 STEC strains ([i] H30 [serotype O26:H11], [ii] JB1-95 [serotype O111:H-], [iii] CDC 96-3285 [serotype O45:H2], [iv] CDC 90-3128 [serotype O103:H2], [v] O104:H4, [vi] CDC 97-3068 [serotype O121:H19], and [vii] 83-75 [serotype O145:NM]) used in this study were confirmed, cultured, and/or maintained as described previously (13, 15, 16). STEC O104:H4 was the cause of a large foodborne outbreak of hemorrhagic colitis and hemolytic uremic syndrome in Germany in 2011. This strain or serotype was a hybrid of STEC and enterohaemorrhagic E. coli pathotypes that carries genes for Shiga toxin 2 (str2) and enterohaemorrhagic fimbrial adhesins but that lacks intimin (eae) and enterohemolysin (ehxA). Thus, we also evaluated an O104 strain of STEC because, at the time this study was initiated, there was general interest as to whether or not strains of this serotype would behave similar to STEC.

Inoculation and cooking of veal. Freshly processed and preflattened veal was kindly provided by a cooperating producer (Nagle Veal and Quality Meats, San Bernardino, CA) and was stored for up to 3 days at 4°C, or at −20°C for up to 3 months, prior to use. The meat was stored, inoculated, tenderized, cooked, and sampled essentially as described (1,16) and as requested by our collaborating producer. In brief, after separately inoculating the surface of each cutlet with multistrain cocktails of ECOH or STEC (ca. 6.8 log CFU/g), the preflattened veal cutlets (ca. 71.5 g, ca. 0.32 cm thick) were passed once through a “Sir Steak” tenderizer (model Pro-9, Biro, Marblehead, OH). One set of cutlets was battered and breaded just prior to cooking by coating both sides of the meat with all-purpose enriched presifted flour (America’s Choice, OnPoint Inc., Montvale, NJ), immersing the flour-coated cutlets into pasteurized liquid whole eggs (EggBeaters, ConAgra Foods Inc., Omaha, NE) for ca. 20 s, then covering both sides of the cutlet with flavored bread crumbs (Centro, Centro Fine Foods Inc., Thorofare, NJ). Prior to cooking, 15 or 30 ml (i.e., low and high volume, respectively) of canola oil (America’s Choice) was preheated to ca. 191.5°C (376°F) on an aluminum nonstick skillet (Kitchen Essential, model no. CR1211PS, Calphalon, Toledo, OH) by placement of the skillet on a ceramic hot plate (Isovolt 110-100-49SH, Fisher Scientific, Pittsburgh, PA). Next, each cutlet was separately cooked for a target time of 0.0, 0.75, 1.0, 1.25, 1.5, 1.75, or 2.25 min per side. These cooking times were selected based on cooking instructions provided by the producer and as detailed in the USDA-FSIS cooking guidelines for veal (28). The surface temperature of the skillet and the internal temperature at the approximate geometric center of each cutlet were continuously monitored using calibrated stainless steel type J thermocouples (Omega Engineering Inc., Stamford, CT) that were individually connected to an eight-channel thermocouple data logger (model OM-CP-OCTTEMP, Omega Engineering). When the target cooking time was achieved, each cutlet was flipped with the aid of a sterile stainless steel spatula and was cooked for the same target time with the opposite face of the meat in contact with the skillet surface. Next, cutlets were removed from the skillet and were aseptically transferred into separate sterile filter stomacher bags (type XX-C003, Microbiology International, Frederick, MD), which were immediately submerged into an ice-water bath. Each cutlet was analyzed within 30 min of being placed on ice.

Enumeration of Shiga toxin–producing E. coli from veal. Survivors were recovered from both heated and nonheated (i.e., controls) veal cutlets essentially as described (19). In brief, either 75 ml (nonbreaded cutlets) or 100 ml (breaded cutlets) of sterile 0.1% peptone water (BD, Sparks, MD) was added to the filter bags, and the contents were macerated for ca. 2 min (Stomacher 400, Seward, Cincinnati, OH). Portions of the resulting macerate, with and without subsequent dilution in sterile 0.1% peptone water, were surface plated in duplicate onto sorbitol MacConkey (BD) plus rifampin (100 µg/ml; Sigma Chemical Company, St. Louis, MO) agar plates for enumeration of ECOH and STEC. Only colonies resistant to rifampin that were also colorless or pink were enumerated as ECOH or STEC, respectively. When pathogen levels decreased to below the detection limit (i.e., ≤0.04 to ≤0.16 log CFU/g) by direct plating, these samples were enriched as described (13). The detection limit was determined as follows: one CFU count multiplied by the volume of the macerated veal sample.
plated multiplied by the volume of the diluent used to macerate the veal sample divided by the weight of the veal sample after cooking.

Data analyses. The CFU per gram was converted to log CFU per gram before data analyses. Log reductions were calculated as the difference of the log populations. Means and standard deviations were calculated from individual sets of data for each trial at each of the cooking times tested using triplicate samples. Analysis of variance was used to determine the effects and interactions on the log reduction values of ECOH and STEC related to pathogen type, cooking time, oil volume, application of breading, and/or combinations thereof (P < 0.05) using the Bonferroni least significant difference method (version 9.3, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Serotype O157:H7 strains of Shiga toxin–producing E. coli have been recognized as a foodborne pathogen since the early 1980s (4) and were deemed an adulterant if present in raw ground beef and in nonintact beef in 1994 and 1999, respectively (24, 25). As reported by Samadpour and colleagues (20), 5 (63%) of 8 ground veal samples from grocery stores in the Seattle, WA, area tested positive for stx1 and/or stx2 genes, whereas 14 (32%) of 60 ground beef samples tested positive for one and/or both stx genes. Cook et al. (10) isolated E. coli from 87% of 153 veal samples, obtained from milk-fed animals after the animals were harvested, that were purchased at retail establishments in Ontario, Canada. Although these authors did not determine whether any of the retained isolates from positive samples produced a Shiga toxin, they did report that 274 (70%) of 392 of the retained isolates were resistant to one or more of 16 antimicrobials tested. In a related study by Cook et al. (9), E. coli was recovered from 387 (88%) of 438 of veal samples, harvested from grain-fed animals, that were purchased at retail establishments in Ontario, Canada. Among veal samples tested by the USDA-FSIS from June to September of 2012, 3 (27.3%) of 11 were confirmed positive for Shiga toxin–producing E. coli, compared with 5 (0.69%) of 729 for beef trim samples. Although considerably fewer veal samples were tested, this difference in recovery of confirmed positives from veal samples compared with that from beef raises the question of whether consumption of veal, particularly if tenderized, poses a greater risk to public health than beef.

The beef industry and the public health community must remain vigilant to prevent ground beef and veal adulteration, especially because nonintact beef is a commonly consumed food and because undercooked nonintact meat remains an important cause of illness due to E. coli O157:H7 infection (5, 6, 27, 29). Although adequate data are not available, one might assume that the prevalence and levels of STEC in ground beef would be similar to those found for ECOH, and one might also assume that the prevalence and levels of these pathogen types would be similar in veal to those found in beef. Based on the limited data collected thus far, in reality this may not be the case; therefore, further research is necessary to substantiate this hypothesis.

As expected, the longer the cooking times at 191.5°C, the higher the internal cutlet temperatures and, in turn, the greater the lethality toward both ECOH and STEC. More specifically, the average final internal temperature of breaded and nonbreaded veal cutlets cooked in 15 or 30 ml of oil at 191.5°C is shown in Table 1. When nonbreaded veal cutlets were cooked for 0.75 to 2.25 min at 191.5°C in 15 ml of canola oil, it was possible to achieve reductions ranging from ca. 2.0 to 6.7 log CFU/g and from ca. 2.6 to 6.2 log CFU/g for ECOH and STEC, respectively (Fig. 1). The range in log reductions and deviations in the internal temperature of the veal for each cooking time is directly attributable to the effect of increasing cooking time and to the thinness of the preflattened and tenderized cutlets, respectively. These data compare favorably with earlier studies wherein ECOH and STEC behaved similarly regarding translocation and/or thermal inactivation in beef (11, 14–17). More specifically, in previous studies we reported reductions of ca. 0.5 to 4.5 log CFU/g for ECOH and STEC in nonintact steaks (ca. 2.54 cm thick) cooked on a commercial gas grill to 37.8 to 71.1°C (100 to 160°F) (14, 15). In related studies, we cooked inoculated ground beef patties to 60.0 to 76.7°C and reported average total reductions of ca. 3.0 to ≥7.0 log CFU/g for ECOH and from ca. 2.5 to ≥6.4 for STEC (16). Also relevant to the present study, when simulated nonintact beef steaks (ca. 1.5 to 4.0 cm thick; with ca. 10% total added water) were pan

### TABLE 1. Average final internal temperature of nonbreaded and breaded veal cutlets

<table>
<thead>
<tr>
<th>Cooking time (min)</th>
<th>15 ml of oil</th>
<th>30 ml of oil</th>
<th>15 ml of oil</th>
<th>30 ml of oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonbreaded</td>
<td>Breaded</td>
<td>Nonbreaded</td>
<td>Breaded</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>56.8 ± 14.0</td>
<td>63.7 ± 18.2</td>
<td>65.0 ± 14.5</td>
<td>62.8 ± 13.0</td>
</tr>
<tr>
<td>1.0</td>
<td>59.1 ± 17.7</td>
<td>69.7 ± 12.1</td>
<td>ND</td>
<td>73.2 ± 14.4</td>
</tr>
<tr>
<td>1.25</td>
<td>67.4 ± 15.1</td>
<td>75.2 ± 10.5</td>
<td>80.1 ± 11.3</td>
<td>80.7 ± 19.5</td>
</tr>
<tr>
<td>1.5</td>
<td>70.8 ± 16.4</td>
<td>81.5 ± 11.2</td>
<td>80.2 ± 7.6</td>
<td>77.8 ± 9.2</td>
</tr>
<tr>
<td>1.75</td>
<td>68.5 ± 16.4</td>
<td>ND</td>
<td>85.4 ± 7.7</td>
<td>91.4 ± 12.3</td>
</tr>
<tr>
<td>2.25</td>
<td>79.4 ± 11.5</td>
<td>93.1 ± 11.5</td>
<td>88.3 ± 5.2</td>
<td>87.2 ± 10.0</td>
</tr>
</tbody>
</table>

a Cutlets cooked in 15 or 30 ml of canola oil on an aluminum nonstick surface skillet maintained at 191.5°C (N = 3 trials, n = 3 replicates per trial). ND, not determined.
Because similar reductions were obtained for ECOH and STEC in nonbreaded veal cutlets that were cooked in 15 ml of canola oil, we also addressed the potential effect of the volume of oil used to cook the meat and/or the effect of breading on the thermal inactivation of ECOH in tenderized veal cutlets (Table 2). Our results confirmed that the volume of oil used for cooking nonbreaded cutlets did not \((P \geq 0.05)\) affect thermal inactivation of ECOH; pathogen reductions ranging from ca. 2.0 to 6.7 or 2.3 and 6.4 log CFU/g were achieved when cutlets were cooked in 15 or 30 ml of oil, respectively. In contrast, the volume of cooking oil used resulted in a significant \((P \leq 0.05)\) effect on thermal inactivation of ECOH in breaded cutlets. In general, cooking with more rather than less oil resulted in greater lethality toward ECOH in breaded veal cutlets. In breaded cutlets cooked in 15 ml of oil, pathogen numbers decreased by ca. 1.1 to 3.5 log CFU/g, whereas in breaded cutlets cooked in 30 ml of oil, pathogen numbers decreased by ca. 2.6 to 6.4 log CFU/g. These results may be attributed to a more rapid and extensive heat distribution inside of the meat due to cooking the cutlets in a greater volume of oil (i.e., higher thermal conductivity). The use of more oil may also have allowed for a better heat exchange or contact between the meat and the oil (i.e., heat source). With respect to breading, when nonbreaded or breaded cutlets were cooked in 15 ml of oil, a significant \((P \leq 0.05)\) effect of breading on thermal inactivation of ECOH was observed; however, no effect \((P \geq 0.05)\) of breading was observed when the meat was cooked in 30 ml of oil. Coating the cutlets with batter and breading may alter the heat transfer rate and/or serve as a thermal insulator, which in turn may allow for more cells to survive during cooking. It is well known that inclusion of solutes such as sugars (i.e., carbohydrates) and salts in food matrices reduces the water activity of food and, consequently, increases the thermal resistance of foodborne pathogens. For example, Osaili et al. \((18)\) suggested that the breading ingredients used to coat pork patties may have a significant effect on thermal inactivation of \(E. \) coli O157:H7, because the batter or breading was formulated mostly by probably carbohydrates and salts, such as sodium.

### TABLE 2 Recovery of ECOH from mechanically tenderized veal cutlets following cooking

<table>
<thead>
<tr>
<th>Cooking time (min)</th>
<th>15 ml of oil</th>
<th>30 ml of oil</th>
<th>15 ml of oil</th>
<th>30 ml of oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.88 ± 0.14 A</td>
<td>6.48 ± 0.13 A</td>
<td>6.05 ± 0.24 A</td>
<td>6.43 ± 0.03 A</td>
</tr>
<tr>
<td>0.75</td>
<td>4.93 ± 0.57 A</td>
<td>4.16 ± 0.34 A</td>
<td>ND</td>
<td>4.70 ± 0.17 A</td>
</tr>
<tr>
<td>1.0</td>
<td>3.77 ± 0.50 AB</td>
<td>3.04 ± 0.55 B</td>
<td>4.96 ± 0.20 A</td>
<td>3.87 ± 0.61 AB</td>
</tr>
<tr>
<td>1.25</td>
<td>2.88 ± 0.69 AB</td>
<td>2.08 ± 1.66 B</td>
<td>4.71 ± 0.12 A</td>
<td>2.25 ± 0.65 B</td>
</tr>
<tr>
<td>1.5</td>
<td>1.43 ± 0.96 B</td>
<td>1.48 ± 1.07 B</td>
<td>4.10 ± 0.64 A</td>
<td>2.55 ± 0.56 B</td>
</tr>
<tr>
<td>1.75</td>
<td>1.16 ± 0.73 B</td>
<td>ND</td>
<td>4.01 ± 0.20 A</td>
<td>0.26 ± 0.30 B</td>
</tr>
<tr>
<td>2.25</td>
<td>0.13 ± 0.19 B</td>
<td>0.10 ± 0.12 B</td>
<td>2.57 ± 0.83 A</td>
<td>0.05 ± 0.08 B</td>
</tr>
</tbody>
</table>

\(^a\) Cutlets were cooked for 0 to 2.25 min on an aluminum nonstick surface skillet maintained at 191.5°C \((N = 3 \) trials, \(n = 3 \) replicates per trial). The experimental matrix for ECOH (oil + breading) was composed of one inoculation level \(\times\) one pathogen type \(\times\) one cooking temperature \(\times\) seven cooking time points \(\times\) two oil volumes \(\times\) two veal coating (with and without breading) \(\times\) three cutlets per each cooking time point \(\times\) three trials, for a total of 252 cutlets tested. For a given volume of oil, means with the same uppercase letter are not significantly \((P \geq 0.05)\) different by Bonferroni least significant difference test. ND, not determined.

\(^b\) For breaded cutlets, means with the same lowercase letter are not significantly \((P \geq 0.05)\) different by Bonferroni least significant difference test.
chloride and sodium phosphate. The USDA-FSIS currently recommends that 0.32-cm-thick veal cutlets be cooked for 3 to 4 min to a minimum internal temperature of 62.8°C (145°F) followed by a rest time of 3 min and that ground veal be cooked to a minimum internal temperature of 71.1°C (160°F) (28). Our data validate the effectiveness of cooking cutlets for 1.5 min per side on a commercial, flat-surface, electric skillet set at ca. 191.5°C for achieving the recommended internal instantaneous target temperature of 71.1°C and for delivering a ≥5-log reduction of Shiga toxin–producing E. coli that may be present on a veal cutlet (and that may then likely be distributed throughout the cutlet following blade tenderization). Further studies are in progress to elaborate the type and amount of breading used and the type and volume of cooking oil used on thermal inactivation of ECOH and STEC in veal cutlets of varying thickness and enhancement.

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