

ESCHERICHIA COLI O157:H7 RISK ASSESSMENT FOR PRODUCTION AND COOKING OF RESTRUCTURED BEEF STEAKS

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Summary

Distribution of *Escherichia coli* O157:H7 in restructured beef from artificially inoculated meat pieces and destruction of *E. coli* O157:H7 in restructured beef steaks prepared from artificially inoculated meat was evaluated following broiling and grilling. In Study I, *longissimus dorsi* trimmings were inoculated with fluorescently marked *E. coli* O157:H7 cells to microscopically identify bacterial distribution throughout restructured steak cross-sections. *E. coli* O157:H7 fluorescent density was observed along the glue lines where meat pieces were enzymatically attached. Study II quantified the level of *E. coli* O157:H7 throughout the entire thickness of restructured beef. Cross-sectional slices of core samples from the steaks showed that bacterial contamination was evenly distributed (ca. 10^6 CFU/g). Study III determined the extent of *E. coli* O157:H7 reduction achieved during cooking. Beef trimmings were inoculated to a level of 10^7 CFU/g and used to prepare restructured beef chubs. Restructured steaks of three thicknesses (0.5, 1.0, and 1.5 inches) were sliced from the chubs and cooked to one of six target internal temperatures (120, 130, 140, 150, 160, or 170°F) by commercial gas grill or oven broiler. Broiling was more effective than grilling, although *E. coli* O157:H7 survival decreased as endpoint temperatures increased incrementally. To achieve an adequate level of safety confidence, restructured steaks should be cooked in a manner similar to ground beef; to an internal temperature of at least 160°F.

(Key Words: *E. coli* O157:H7, Restructured Beef Steaks, Cooking.)

Introduction

Meat restructuring using cold set binding technologies like fibrinogen and thrombin enzymes provide quality, economic, nutritional, and marketing advantages in meat processing. Food poisoning outbreaks with *Escherichia coli* O157:H7 have frequently been attributed to ground beef consumed after cooking that was insufficient to destroy pathogens that were translocated from the surface to the meat interior. Restructured steaks may be perceived as more like intact muscle steaks than as ground beef. Because of this perception, restructured steaks may be cooked at temperatures inadequate to destroy surface microbial contamination that may have been carried to the interior of the restructured product. The objectives of our research were to determine the extent of *E. coli* O157:H7 translocation in restructured beef and to determine adequate cooking protocols to eliminate *E. coli* O157:H7 from their interior.

Experimental Procedures

Study I:

Five strains of *Escherichia coli* O157:H7 (USDA-FSIS 011-82, ATCC 43888, 43889, 43890, and Eh 7-4) were grown and marked using a fluorescent probe, live-cell nucleic acid stain. *Longissimus dorsi* beef trimmings were mist inoculated (ca. $7 \log_{10}$ CFU/g) and allowed to attach at 39°F for 1 hr. Thawed proportional parts of fibrinogen and thrombin enzymes, were mixed with the *longissimus dorsi* trimmings for 90 sec and stuffed into perforated casings using an Aligned Grain System™. The formed meat was hung at 41°F for 10 hr for setting the meat pieces, and frozen for 1.5 hr to form a

surface crust to facilitate sample collection. A sterile stainless steel coring device coupled to an electric drill was used to core the sample along the width of the cylindrical meat piece. The cores were fixed in a mixture of paraformaldehyde (2%) and glutaraldehyde (0.2%) in neutral buffer solution (pH 7) for 6 hr, sectioned and visualized under a confocal laser microscope.

Study II:

A strain of *E. coli* O157:H7 (USDA-FSIS 011-82, rifampicin resistant) was mist inoculated (ca. $7 \log_{10}$ CFU/g) onto the surface of *longissimus dorsi* beef trimmings. The restructuring procedure from Study I was followed. The formed beef roll (crust frozen) was sampled as previously described at three different locations about 4 inches apart. The meat cores were aseptically sliced into cross-sectional strips of 0.8, 0.4, 0.4, 0.4, and 0.8 inches. Each cross-sectional strip was plated onto TSA-rif and the *E. coli* O157:H7 population was reported.

Study III:

The inoculation and restructuring procedures from Study I were followed. The cylindrical restructured beef chub (2.8" inch diameter) was sliced into steaks of 0.5, 1.0, and 1.5 inch thickness. Steaks were randomly assigned to six target internal temperature groups (120, 130, 140, 150, 160, and 170°F) and were cooked under a typical kitchen oven broiling element set at 500°F, or cooked using a commercial gas grill. Steaks were flipped upon reaching the midpoint between original and final temperature. Internal temperatures were constantly monitored using a type T thermocouple threaded through the steak edge to the steak's geometric center. After reaching the target internal temperature, steaks were removed from the grill or broiler, placed in heat resistant bags and immersed in an ice bath. Steaks were blended in a sterile food processor and a 25 g sample stomached in peptone water to provide a 1:5 w/v dilution. Surviving *E. coli* O157:H7 populations were enumerated on MacConkey Sorbitol Agar (MSA) and Phenol Red Sorbitol Agar (PRSA). PRSA agar provided an estimate of the level of thermally injured cells that may not have been detected on MSA agar. Samples testing

negative by direct plating were qualitatively evaluated following a modified USDA method. Presumptive *E. coli* O157:H7 colonies were confirmed biochemically and serologically. Three replications were performed for each study.

Results and Discussion

Study I:

Confocal laser scanning microscopy revealed a high level of *E. coli* O157:H7 contamination along cross-sections of the restructured beef chub. The fluorescent bacteria present midway through the cylindrical restructured meat piece shows that the restructuring process translocates surface contamination into the interior.

Study II:

E. coli O157:H7 was uniformly distributed ($P > 0.05$) across the diameter (5 individual cross sections) of the restructured beef chub, indicating translocation of surface inoculated *E. coli* O157:H7 into the interior of the chubs during restructuring. Section 3, corresponding to the geometric center of the core, revealed a mean bacterial population of $6.16 \log_{10}$ CFU/g. This section would be the coldest point during cooking.

Study III:

Broiling steaks from restructured beef trimmings to endpoint temperatures of 120, 130, 140, 150, 160, and 170°F resulted in *E. coli* O157:H7 reductions of 1.03, 1.94, 2.70, 4.32, 6.27, and 6.08 log CFU/g, respectively, when plated on PRSA (1 log = 90%, 4 log = 99.99% reduction). Greater reductions were obtained when a selective medium (MSA) was used for plating, indicating sub-lethal injury to *E. coli* O157:H7 cells during cooking. Cooking of thicker steaks (1.0 and 1.5 inches) resulted in consistently larger reductions in *E. coli* O157:H7 compared to the 0.5 inch steaks. This probably was due to thicker steaks requiring longer cooking times. The post-cook temperature rise was higher for 1.5-inch thick steaks (9 to 22°F) compared to steaks 1.0 and 0.5 inch thick (3 to 8°F and 7 to 12°F for 0.5 and 1.0 inch thick steaks, respectively). The additional microbial destruction in thicker steaks due to longer cook times and higher post-cook

temperature rise results in a safer steak, compared to thinner steaks.

Cooking restructured steaks on a gas grill to endpoint temperatures of 120, 130, 140, 150, 160, and 170°F resulted in *E. coli* O157:H7 reductions of 0.87, 1.16, 1.25, 2.62, 3.73, and 4.51 log CFU/g, respectively, when plated on PRSA. These reductions were greater when a selective medium (MSA) was used for plating the samples, indicating a significant number of injured but not killed *E. coli* O157:H7 cells. These reductions were lower than the reductions attained using oven broiling. This was probably due to the commercial grill using higher temperatures and shorter cooking times to reach target temperatures.

Initial studies indicated that some restructured steaks curled during cooking, probably due to different fiber alignment than normal steaks. Use of cook weights (1 lb steel plates with a handle) minimized but did not eliminate the curling. Using commercial grill systems can result in cold spots on steaks due to curling, and thus reduce microbial destruction. In addition, when cooked on a grill, the top surface of the steak is exposed to near ambient temperatures. On the contrary, in oven broiling, the temperature is more

consistent and uniform because steaks are heated from the top (broiling element). The surface of the holding grill is close to the oven temperature, resulting in larger reductions in *E. coli* O157:H7 populations.

Grilling thicker steaks (1.0 and 1.5 inches) resulted in larger reductions of *E. coli* O157:H7 compared 0.5 inch thick steaks, probably due to the longer cook times and higher post-cook temperature rise. As observed in oven broiling, the post-cook temperature rise was greater in steaks of 1.0 and 1.5 inches (1 to 16°F and 6 to 19°F).

This study incorporated the use of an ice bath to rapidly halt destruction of bacteria after steaks were removed from the broiler, to provide more accurate information concerning microbial destruction achieved at the identified target internal temperatures. However, even after transfer to the ice bath, internal temperature of the steaks continued to rise above the target temperatures by as much as 4 to 29°F. In food service applications, the finished product would not be cooled. Therefore, internal temperatures would likely rise to higher endpoints and be maintained longer, thereby increasing margin of safety in pathogen destruction.