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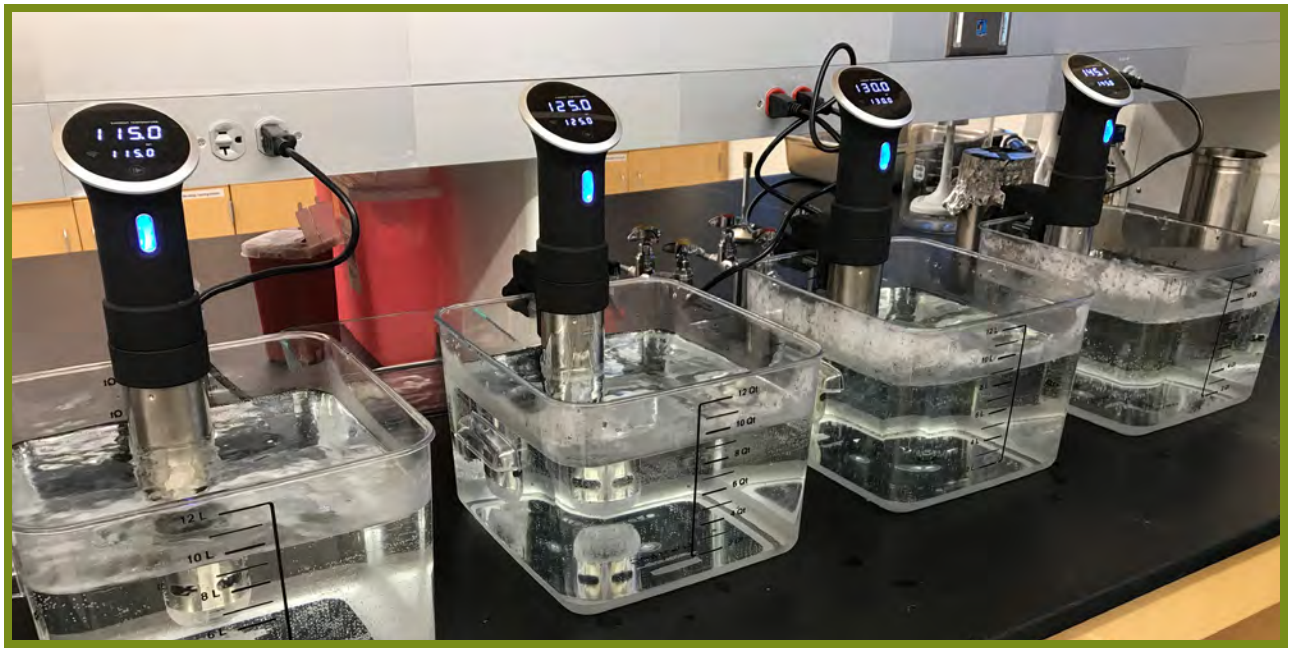

Fate of *Escherichia coli* in Nonintact Beef Steaks during Sous-Vide Cooking at Different Holding Time and Temperature Combinations

ABSTRACT

Sous-vide cooking has increased in popularity due to ease of use, but some manufacturer recommendations for sous-vide cooking of nonintact meat products include potentially unsafe time and temperature combinations. This experiment was designed to address these concerns by validating a 5-log reduction of *Escherichia coli* in sous-vide cooked beef steaks. Beef *semitendinosus* steaks were inoculated externally and internally with *E. coli* ATCC 25922 via immersion and use of a pin pad, respectively. Individual steaks were vacuum packaged and cooked in sous-vide water baths held at 46, 51, 54, and 62°C. A 5-log reduction was reached at 51°C, 54°C, and 62°C after 258, 64.5, and 2.25 min, respectively ($P < 0.01$). At 46°C, cooking achieved a final 1.07-log reduction ($P < 0.01$) after 420 min. These results confirm the utility of U.S. Department of Agriculture, Food Safety and Inspection Service guidelines (Appendix A) and raise concerns about the safety of sous-vide meat cooked at ≤ 46°C. Further experimentation is needed with pathogenic *E. coli* strains during sous-vide cooking of steaks using time and temperature combinations at and below recommended Appendix A values.

INTRODUCTION

Sous-vide cooking is a method that involves sealing food in vacuum pouches and holding those pouches in a hot water bath at a fixed temperature. Because the temperature can be controlled more precisely in a water bath than on a stove or grill, sous-vide cooking allows for a precise level of doneness to be achieved in all types of cooked food products from custard to eggs, meat, and even vegetables *(1, 13, 14)*. The method has been used in high-end restaurants throughout the world since the 1970s and has become increasingly common in restaurants and foodservice applications in the last 20 to 30 years due to its ability to precisely cook large quantities of food product with very little monitoring *(5)*. Recently, the technique has become more popular among home users due to affordability and availability of sous-vide cooking units starting in the 2010s *(1, 5, 14)*.

Shiga toxin-producing *Escherichia coli* strains, including *E. coli* O157:H7, are a serious health hazard to people consuming

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raw or undercooked beef products. Cattle serve as a natural reservoir for pathogenic *E. coli*, and beef surfaces may become contaminated with these pathogens during slaughter and fabrication *(2, 4, 6, 8, 9)*. Although this contamination is a surface concern in intact beef products, in nonintact beef products such as an injection-marinated or blade-tenderized steaks or roasts, pathogens can migrate from the surface of the meat to the otherwise sterile interior during processing. Thus, these products must reach an appropriate internal temperature during cooking to ensure that they are safe to consume. Meat processing establishments are required by the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) to implement hazard analysis critical control point (HACCP) plans with preventive measures to produce meat products that are safe for human consumption *(15)*. The USDA-FSIS document "Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products," commonly known as Appendix A *(16)*, provides scientifically validated guidance for the control of Salmonella, is a cornerstone for the production of cooked meat products, and is commonly referenced in HACCP plans for the prevention of pathogenic *E. coli* adulteration of cooked beef products. Although this resource is widely used in industry, home cooks are less likely to have this information.

Sous-vide cooking of meat commonly involves a final high-heat finishing step such as searing or broiling to develop color and flavor on the product surface *(1)*, and this step is typically sufficient for destroying pathogens on the surface of intact meat products. However, this process may be insufficient for the safe cooking of nonintact products in the home because the final searing or broiling step may not result in a sufficient or even appreciable increase in the internal temperature of the product. Risk exists because some popular chefs and manufacturers of sous-vide cooking equipment may not adequately distinguish between intact and nonintact products and may recommend cooking nonintact meat products to final temperatures that are potentially not safe for consumption. Some published sources recommend cooking ground beef to internal temperatures as low as 46°C *(7, 12)*.

One popular chef that works in partnership with a sous-vide equipment manufacturer recommends that consumers purchase whole muscle cuts of beef and grind those cuts at home to decrease the risk associated with sous-vide cooking ground beef at low temperatures *(12)*. A food safety risk similar to this example could occur in a domestic setting with products that are blade tenderized or marinated for an extended period of time. Recommendations such as this may lead to consumption of potentially unsafe beef products due to the use of in-home cooking methods that are not adequately validated for microbial safety. For cooking nonintact beef products in the home, use of proper internal cooking temperatures for a sufficient amount of time during the sous-vide step is the safest and most practical method for destroying any vegetative pathogens that may exist in the product.

Limited research has been conducted on the quality of sous-vide cooked meat products *(3, 10)*. Our research group evaluated the quality of beef steaks treated with combinations of high-pressure processing and sous-vide cooking *(13)*. However, few projects have included evaluation of the safety of individual sous-vide thermal processing procedures, especially for cooking time and temperature combinations below those recommended in Appendix A. Briggs et al. *(5)* stated that lack of food safety guidance for sous-vide cooked products in restaurants is a serious concern and that food safety validation of individual recipes for safety is necessary.

A novel experiment utilizing low temperature sous-vide cooking of nonintact beef was conducted to determine whether holding time and temperature combinations recommended by the USDA-FSIS in Appendix A could be used for safe sous-vide cooking of nonintact beef products and whether holding time and temperature parameters outside the USDA-FSIS recommendations could potentially be used to safely sous-vide cook beef products in a domestic setting.

MATERIALS AND METHODS

Inoculum preparation

Frozen stocks of *E. coli* ATCC 25922 biotype 1 (Manassas, VA; a common laboratory quality control strain and pathogen surrogate) were thawed, streaked for isolation on tryptic soy agar, (Becton Dickinson, and Co., Franklin Lake, NJ) and incubated statically for 24 h at 37°C to obtain cultures in the late exponential phase of growth. For each replication, a single colony was inoculated into 10 mL of tryptic soy broth (TSB; Becton, Dickinson, and Company, Franklin Lake, NJ) and incubated statically for 24 h at 37°C. The 10-mL culture was added to 990 mL of fresh TSB and incubated statically under the same conditions. On the day of meat inoculation, two 1-L bottles of culture were combined in a sterile metal tub to facilitate meat immersion.

Steak inoculation and sous-vide cooking

Beef *semitendinosus* muscles (IMPS 171C) were received from a local meat packing facility. Steaks of 2.4 cm thickness were cut perpendicular to the long axis of the entire muscle, vacuum packaged, and stored frozen until use. For each replication, steaks were thawed (48 h, 4°C) prior to use and exposed to UV light for 15 min on each side to reduce any latent surface contamination. To emulate the creation of the contamination of a nonintact product (e.g., blade tenderized steaks), steaks were internally inoculated with 2 L of *E. coli* ATCC 25922 liquid inoculum (prepared as above) grown to at least 8.5 log CFU/mL. Each steak was placed in the inoculum and pressed with a pin pad inserted five times into each side of each steak to ensure adequate migration of bacteria to the interior of the steak. After inoculation, steaks were air dried (30 min, 23°C), individually vacuumed sealed (3 mil; Clarity, Koch Supplies, Riverside, MO), and then transferred to preheated sous-vide water baths (Anova

TABLE 1. Time and holding temperature combinations for sous-vide cooking

46°C	51°C	54°C	62°C
150 min	150 min	64.5 min	2.25 min
420 min	193.5 min	86 min	3 min
	258 min	107.5 min	3.75 min
	322.5 min		

Precision Cooker, Anova Applied Electronics, Inc., San Francisco, CA) for cooking. Duplicate steak samples were taken from raw inoculated steaks (prior to cooking) and from steaks at each hold time and temperature combination *(Table 1)*. The median sampling times for 54 and 62°C were taken directly from the USDA-FSIS Appendix A 5-log reduction table, and the other times were ±25% of the median time. The median sampling time for 51°C was extrapolated from the thermal death curve used for the 5-log reduction table. The 46°C sampling times represented potential worst-case scenarios for sous-vide cooking at low temperatures and were based on recommendations found in commonly distributed sous-vide recipes. Steaks were submerged in the sous-vide water bath and cooked according to the previously stated times. For each temperature treatment, the internal temperature was monitored in one steak that had been fitted with a Type T thermocouple and TC-08 Data Logger (Omega Engineering Inc., Norwalk, CT) and sealed into a bag with an air-tight sealed septum. Come-up times for each cooking treatment were approximately 80 min for 46°C, 70 min for 51°C, 50 min for 54°C, and 45 min for 62°C. After samples were removed from the water bath, they were immediately submerged in an ice-water bath and allowed to cool for at least 15 min. After cooling, steaks were removed from the individual vacuum pouches, and core samples (25 g) were cut from each steak with flame-sterilized core cutters and knives. Each core sample was then homogenized in 100 g of buffered peptone water (Becton, Dickinson, and Company, Franklin Lake, NJ) in a benchtop stomacher (Stomacher 400, Seward, London, UK). Homogenates were then serially diluted and plated onto EC Peal plates (Charm Sciences, Lawrence, MA) according to the manufacturer's instructions. *E. coli* colonies were enumerated after 24 h at 35°C according to the manufacturer's guidelines.

Statistical analysis

The experiment was conducted as three independent replications with six total steaks for each sampling time and temperature combination. Mean *E. coli* levels were reported as log CFU per gram. Reductions were determined by subtracting levels at given sampling times from the levels in the raw sample. Data were analyzed using a general linearized model with consecutive contrasts within temperature treatments ($\alpha = 0.05$) with the Statistical Analysis Software (SAS 9.4, SAS Institute, Cary, NC). To maximize statistical power, comparisons of *E. coli* levels between temperatures were not analyzed.

RESULTS AND DISCUSSION

Temperatures of 51, 54, and 62°C all achieved 5-log reductions of *E. coli* during sous-vide cooking of the nonintact steaks, with reductions of 5.80, 6.62, and 6.83 log CFU/g achieved at 258, 64.5, and 2.25 min, respectively *(Table 2)*. Reductions at the longest cook times for 51, 54, and 62°C treatments were all >6 log CFU/g. In the samples cooked at 46°C, *E. coli* levels after 150 min of holding were not significantly different from the levels in the raw inoculated samples, and even after 420 min of holding, only a 1.07-log reduction was achieved.

The safety of potentially hazardous foods cooked via sous vide has become a concern as the popularity of sous-vide cooking has grown in both foodservice and domestic settings. The finding of the present study revealed that the time and temperature combinations recommended by some sous-vide equipment manufacturers and popular chefs for nonintact products may not be safe. Studies have been conducted on other potential avenues of pathogen control. Juneja et al. *(11)* evaluated the thermal death times of *E. coli* O157:H7 in sous-vide cooked ground beef with and without natural antimicrobial extracts. Although the tea and apple extracts blended into the ground beef samples acted as antimicrobials and were able to decrease the temperature needed to inactivate *E. coli* O157:H7 compared with the controls without extracts, the lowest temperature tested was 55°C, which is much higher than the temperatures suggested in many recipes. Thus, the evaluation of nonintact meat sous-vide cooked to final temperatures <54°C was investigated in the present study.

Beef steaks internally inoculated with the nonpathogenic *E. coli* ATCC 25922 were cooked to final temperatures below those recommended in the USDA-FSIS Appendix A to address concerns about potentially unsafe sous-vide cooking recommendations for nonintact meat. Hold time and temperature combinations taken from the Appendix A 5-log reduction table were sufficient to achieve >5-log reductions of

TABLE 2. Levels of *E. coli* during sous-vide cooking (n = 6)

Holding time (min)	Mean ± SE *E. coli* (log CFU/g)	*P*-value[a]	Total reduction (log/CFU/g)
46°C holding temperature			
Raw steak	7.41 ± 0.13		n/a
150	7.37 ± 0.07	0.88	0.04
420	6.33 ± 0.28	<0.01	1.07
51°C holding temperature			
Raw steak	7.02 ± 0.15		n/a
150.0	3.88 ± 0.28	<0.01	3.14
193.5	2.21 ± 0.31	<0.01	4.81
258.0	1.22 ± 0.20	<0.01	5.80
322.5	0.39 ± <0.0	<0.01	6.63
54°C holding temperature			
Raw steak	7.13 ± 0.12		n/a
64.5	0.51 ± 0.07	<0.01	6.62
86.0	0.47 ± 0.05	0.89	6.66
107.5	1.01 ± 0.42	0.08	6.12
62°C holding temperature			
Raw steak	7.25 ± 0.10		n/a
2.25	0.42 ± 0.03	<0.01	6.83
3.00	0.42 ± 0.03	1	6.83
3.75	0.58 ± 0.19	0.31	6.67

[a]Statistical comparisons were made between *E. coli* levels from one sampling time and the preceding sampling time.

E. coli needed to demonstrate the safety of cooked nonintact beef products. Holding at 54°C (130°F) and 62°C (145°F) resulted in >6-log reductions at all sampling times. Although some numerical differences were noted between *E. coli* levels in samples cooked at 54 and 62°C, those differences were not significant ($P > 0.05$) and are indicative of sampling variation.

In the present study, holding beef steaks at 51°C (125°F) resulted in a steady decline in *E. coli* levels over 322.5 min of cooking, and a >5-log reduction was first noted at this temperature at 258 min. This time was extrapolated from the Appendix A 5-log reduction table using a line of best fit for temperatures <54°C. Sampling after 322.5 min of holding revealed a final 6.63-log reduction, indicating the potential for safely sous-vide cooking nonintact beef products at this temperature. Because extrapolated cooking times at 46°C (115°F) were >60 h and would have limited practical applications, sampling times of 150 and 420 min were chosen to represent a maximum recommended cooking time at temperatures <54°C (12) and a potential all-day sous-vide cooking scenario for consumers cooking at home, respectively. Samples taken at 150 min of holding did not have a significant reduction compared with the raw sample ($P = 0.88$), and samples taken at 420 min had only a 1.07-log reduction ($P < 0.01$). These data raise significant concerns regarding several of the sous-vide cooking recommendations made by equipment manufacturers and chefs. Although holding beyond 420 min at 54°C could have resulted in greater reductions of the *E. coli* used in this experiment, other strains of *E. coli* and other vegetative pathogens potentially present in beef could grow under these conditions as could spoilage organisms that might render the product unpalatable, even if safe.

Although the results of these experiments support the Appendix A holding time and temperature combinations during sous-vide cooking and the potential for safe sous-vide cooking at 54°C, this research is not intended to replace or supplement USDA-FSIS guidance. To fully validate sous-vide cooking, further research is needed to determine the fate of various pathogenic *E. coli* strains and other relevant aerobic and anaerobic pathogens during sous-vide cooking.

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In Memory

Eugene Frey

We extend our deepest sympathy to the family of Eugene Frey who recently passed away. Mr. Frey was a member of the Association since 1994. IAFP will always have sincere gratitude for his contribution to the Association and the profession.