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Quality Change and Thermal Inactivation of Escherichia coli O157:H7 in Non-Intact Beef and Veal Patties by Double Pan-Broiling

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QUALITY CHANGE AND THERMAL INACTIVATION OF ESCHERICHIA COLI
O157:H7 IN NON-INTACT BEEF AND VEAL PATTIES BY DOUBLE PAN-
BROILING

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

the Degree Bachelor of Science with

Honors College Graduate Distinction at Western Kentucky University

By

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2014

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ABSTRACT

Escherichia coli O157:H7 (ECHO) may translocate from meat surfaces to internal tissues during grinding processes. This study evaluated the inactivation of ECHO in ground beef and veal by cooking to various internal temperatures (55°C, 62.5°C, 71.1°C, and 76°C). Grounded beef/veal were inoculated with 6 log ECHO and prepared into patties, stored aerobically (4°C, 4 -days) before double-pan-broiling to the internal temperatures mentioned above with a three-minute rest. Samples' color were monitored, which changed significantly during storage and cooking. Pathogen concentration was measured by plating the homogenized sample on TSA and McConkey agar. The pathogen population was below detectable limit when samples were cooked to/above 71.1°C with a three-minute rest time, suggesting that said internal temperature reduces *E. coli* amount to an acceptable limit for human consumption. These findings will be useful to the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) for risk assessments on non-intact beef/veal products.

Keywords: *Escherichia coli* O157:H7, Non-intact, color, Beef, Veal, Thermal inactivation

Dedicated to
My Mentors, Family and Friends

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CHAPTER 1

INTRODUCTION

Escherichia coli O157:H7 and Non-O157:H7 Shiga Toxin producing *Escherichia coli* (STEC) can generate shiga toxins that can cause severe hemolytic uremic syndrome in infected human bodies with as few as 10 cells (Doyle et al, 1997). *E. coli* O157:H7 has been seen as an adulterant of raw non-intact beef products since 1999. The United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS, 2011) defines non-intact beef products as products that have gone through treatments like grinding, restructuring or mechanical tenderization processes such as cubing, needling and pounding devices. These non-intact beef products have been involved in several *E. coli* O157:H7 infection outbreaks in the United States since 2000. During non-intact beef production processes, pathogen cells like *E. coli* O157:H7 on the meat surface may be translocated and trapped in sterile internal tissues, protecting themselves from thermal destruction if the meat is undercooked. A recent survey showed that 40-58% of U.S. consumers ordered beef steaks at medium rare (60-62.8°C) to rare (54.4-57.2°C), which could potentially put them at risk to an *E. coli* infection and threaten public health. Although the effectiveness of cooking inactivation of *E. coli* O157:H7 contaminated in moisture enhanced non-intact beef has been documented in three studies (Shen et al., 2010a; 2010b; 2011), limited information is available regarding the thermal inactivation of *E. coli* O157:H7 strains on veal products.

Veal, originated from Europe, is the meat from 16-18 weeks old calves. In the past 10 years, 25% of American households purchased veal products in restaurant or retail stores at least once every three months (available at: <http://www.beef.org/udocs/Beef%20Bytes%20Veal%20Trends.pdf>). Different veal cuts, such as cutlet, coin, rib, breast, and shank, are most popular to restaurant consumers due to their unique tenderness and flavor. Moreover, nutrition of veal products match the dietary guidelines recommended by the American Heart Association, the American Dietetic Association, and the USDA.

Although veal has not been implicated in *E. coli* outbreaks in the U.S., since 2009 there have been multiple recalls on veal products amounting to 14,600 lbs. (ca. 6649 kg) due to possible *E. coli* O157:H7 and STECs contamination (Luchansky et al. 2014). According to the USDA-FSIS, there is a greater prevalence of STEC in veal products than in other beef products. For example, as of 14 July 2013, the USDA-FSIS in their testing of raw ground beef component samples in federal meat-processing factories discovered 0 (0%) of 383 samples positive for ECOH, and 3 (0.78%) of 383 samples positive for STEC in beef; whereas 3 (6.12%) of 49 samples were positive for ECOH and 1 (7.69%) of 13 samples were positive for STEC in veal (USDA-FSIS, 2013). The difference of confirmed STEC positives from veal compared to that from beef is striking and raises the question of whether consumption of veal poses a greater risk to public health than beef.

Beef and veal products' safety is important to the industry and consumer, but consumers tend to identify the products' quality based on appearance. Cornforth and Jayasingh (2004) stated that color is one of the most important characteristics when it

comes to consumers purchasing decisions, even though color is sometimes poorly related to the meat quality. Fresh beef or veal meat is often displayed in Styrofoam trays and covered with PVC oxygen permeable films, which allows the quick desirable red color development due to the quick pigment oxygenation. However, discoloration often occurs within 1 week of shelf time. At this moment, the number of studies that focus on the quality change of veal products during processing, storage and cooking, in terms of factors like water activity, pH, moisture, fat content and color is very limited.

The objective of this study is to investigate the quality variances, including color variation in non-intact coarse ground beef and veal during aerobic storage and the cooking process, and evaluate the thermal inactivation of *E. coli* O157:H7 in coarse ground beef and veal patties. We hypothesize that beef and veal patties have similar tendency in quality change throughout storage and cooking, and similar increases in inactivation of ECOH and STEC when heated to higher internal temperature.

CHAPTER 2

MATERIALS AND METHODS

Inoculum preparation

E. coli O157:H7 strains ATCC43895, ATCC43888, ATCC43889 (Shiga toxin negative, kindly provided by Mr. Beth Whittam, Michigan State University, East Lansing, MI) were cultured and subcultured individually in 10 mL tropic soy broth (TSB) at 35°C for 24 hours. The three cultures were then mixed and centrifuged (Eppendorf model 5810R, Brinkmann Instruments Inc., Westbury, NY) at 4,629×g for 15 min at 4°C. The harvested cells were washed twice with 10 ml of phosphate buffered saline (PBS), centrifuged as previously described, and re-suspended in 30 ml of fresh PBS. The washed inoculum was serially diluted in PBS to obtain an inoculation level of ~6 log CFU/g when 40 ml of inoculum was added to 2 kg of coarse-ground beef or veal.

Preparation of non-intact ground beef and veal patties

Fresh beef knuckles and veal round top were purchased from a local meat retailer for each replicate. The meat was manually cut into trimmings and then coarse grounded in a meat grinder (Gander Mountain #5 Electric Meat Grinder, Saint Paul, MN) (Figure 2.1). Ground meat was then mixed with 40 mL of the aforementioned *E. coli* O157:H7 inoculum cocktail in a bowl-lift stand mixer (Kitchen Aid Professional 600, Benton

Harbor, MI) at medium speed for two minutes (Fig. 2.2) to ensure even distribution of the inoculum into the sample, which simulates *E. coli* O157:H7 contamination during non-intact beef or veal products preparation. A manual hamburger patty maker (Mainstays 6 ounce patty maker, Walmart, Bentonville, AR) was then used to make beef or veal patties with 170-180 g of grounded meat (Fig. 2.3). The beef/veal patties were packaged aerobically in foam trays (20×25 cm, Pactiv, Lake Forest, IL) and covered using air-permeable plastic film (Omni-film, Pliant Corporation, OH), and stored at 4.0°C for four days.



Figure 2.1: Meat obtained from local stores were coarse grounded by the meat grinder (Gander Mountain #5 Electric Meat Grinder, Saint Paul, MN).



*Figure 2.2: The grounded meat is then homogenized with *E. coli* O157:H7 inoculum cocktail in a bowl-lift stand mixer (Kitchen Aid Professional 600, Benton Harbor, MI).*



Figure 2.3: The mixed meat samples were then made patty with a manual burger press. (Mainstays 6 ounce patty maker, Walmart, Bentonville, AR)

Cooking beef or veal patty samples

After the four-day storage, the patties were taken out from their packages, weighed and double pan-broiled in a Farberware griller (Farberware 4-in-1 Grill, Fairfield, CA) with set-up temperature of 177°C (or 350°F) to the internal temperatures of 55°C, 62.5°C, 71.1°C, and 76°C followed by a three-minute rest. Double-broiling, also known as contact grilling, is when the food (usually meat, especially burger patties, chicken, and steaks) is cooked on both sides simultaneously by applying two cooking surfaces, from both the bottom and the top, greatly reducing the cooking time. A type-K thermocouple was attached to the geometric center of the patty to monitor the internal temperature throughout the cooking with using PicoLog (Pico Technology Ltd, Cambridge, UK), a real time data recording software. The samples were then cooled down to room temperature followed by conducting analysis of qualities including cooking losses, color, pH, water activity, moisture and fat content.



Figure 2.4: The aerobically packaged patty samples were then stored in 4°C for five days



Figure 2.5: Patty samples were cooked by double pan-broiling (Farberware 4-in-1 Grill, Fairfield, CA).

Color measurement

Objective color measurements of non-intact beef or veal patties were determined at each day of storage, after cooking to 55°C, 62.5°C, 71.1°C, or 76°C (internal and external parts) using a portable spectrophotometer (HunterLab Miniscan EZ, Reston, VA) with full spectral data being obtained as L* (lightness), a* (redness), and b* (yellowness), along with reflectance data. An average value for L*, a*, and b* was determined from the mean of three random readings on the surface from 3 pieces of each treatments used for color analysis.

Physical, chemical and microbiological analyses

Cooking losses were determined by measuring the difference in patties' weight before cooking, and then after cooking when samples were cooled to room temperature. The pH of the meat homogenate was measured after microbial analysis using a digital pH meter (Fisher Scientific, Fair Lawn, NY). Water activity (a_w) indicates the availability of water for bacterial growth. The water activity of uncooked and cooked samples were measured using an AquaLab water activity meter (model series 3, Decagon Devices Inc., Pullman, WA). All samples were tested for fat and moisture content at the meat science lab of University of Illinois at Urbana– Champaign. For microbiological analysis, the individual uncooked or cooked beef or veal samples were transferred into a Whirl-Pak filter bag (1627 ml, 19 × 30 cm, Nasco, Modesto, CA) with 1:1 weight's worth of nutrition broth and homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2

minutes. Serial tenfold dilutions of each sample, in PBS, were surface-plated onto tryptic soy agar (Acumedia, Lansing, MI) supplemented with 0.1% sodium pyruvate (Fisher Scientific, Fair Lawn, NY; TSAP) and Mcconkey agar (Acumedia, Lansing, MI) for enumeration of total bacterial populations and *E. coli* O157:H7, respectively. Colonies were counted manually after incubation at 35°C for 48 hours.

Statistical analysis

The experiment was repeated twice with two to three samples in each replicate. Quality parameters and microbial populations (log CFU/g) were analyzed with one-way ANOVA of IBM SPSS Statistics 21. All comparisons were performed with alpha = 0.05.

CHAPTER 3

RESULTS AND DISCUSSION

Table 3.1: Summary of the quality control for beef patty samples.

| Beef | Before cooking | After heating to (°C) | | | |
|------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | | 55 | 62.5 | 71.1 | 76 |
| Cooking loss (%) | - | 17.83 ± 5.56 _a | 24.17 ± 2.71 _b | 26.67 ± 2.50 _b | 29.00 ± 1.55 _b |
| pH | 5.60 ± 0.07 _a | 5.98 ± 0.11 _b | 6.07 ± 0.16 _b | 6.08 ± 0.15 _b | 6.09 ± 0.15 _b |
| Aw | 0.992 ± 0.001 _a | 0.990 ± 0.003 _a | 0.991 ± 0.005 _a | 0.990 ± 0.003 _a | 0.987 ± 0.003 _a |
| Moisture (%) | 70.53 ± 0.55 _a | 66.63 ± 1.85 _b | 64.36 ± 1.23 _{bc} | 63.04 ± 1.25 _c | 62.60 ± 1.18 _c |
| Fat | 6.46 ± 0.77 _a | 8.62 ± 0.65 _b | 9.34 ± 0.44 _b | 9.45 ± 0.55 _b | 8.96 ± 0.60 _b |

Table 3.2: Summary of the quality control for veal patty samples.

| Veal | Before cooking | After heating to (°C) | | | |
|------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | | 55 | 62.5 | 71.1 | 76 |
| Cooking loss (%) | - | 19.00 ± 3.56 _a | 20.75 ± 5.50 _a | 28.25 ± 0.96 _b | 29.00 ± 0.82 _b |
| pH | 5.53 ± 0.01 _a | 5.78 ± 0.02 _b | 5.74 ± 0.08 _b | 5.73 ± 0.08 _b | 5.74 ± 0.08 _b |
| Aw | 0.991 ± 0.005 _a | 0.989 ± 0.003 _a | 0.988 ± 0.002 _a | 0.987 ± 0.002 _a | 0.988 ± 0.001 _a |
| Moisture (%) | 76.02 ± 0.36 _a | 71.19 ± 0.51 _b | 69.57 ± 2.01 _{bc} | 67.95 ± 0.49 _{cd} | 67.07 ± 0.89 _d |
| Fat | 2.19 ± 0.25 _a | 2.79 ± 0.54 _a | 3.00 ± 0.44 _a | 3.02 ± 0.46 _a | 2.92 ± 0.17 _a |

* Means with different letter in the same row are significantly (P < 0.05) different.

Cooking curve and cooking losses

The initial geometric center temperature of uncooked beef and veal patties ranged from 3.6°C to 4.8°C and 4.1°C to 8.9°C, respectively. Cooking of beef samples by double pan-broiling required 330, 360, 430 and 460 seconds to reach the internal center temperatures of 55, 62.5, 71.1 and 76°C, respectively (Figure A1). In veal samples, it took 300, 330, 360, and 420 seconds to achieve internal temperatures of 55, 62.5, 71.1 and 76°C, respectively (Figure A2). The slightly shorter cooking time required by veal samples to reach the same internal temperatures compared to beef is possibly due to the relative lower muscle fiber content, allowing heat to transfer and penetrate the veal patties more efficiently. It is also possible that shorter cooking time could be due to the higher moisture content in veal. As more moisture converts into steam, which has higher energy, the cooking rate of the veal patties could increase by cooking the patty from inside. As expected, during the 3-minute resting time, in both beef and veal samples, the geometric center temperatures continued to increase between 61°C to 65.9°C, 68.4°C to 71.6°C, and 72°C to 78.2°C when cooking samples to 55, 62.5, 71.1°C, respectively (Figure A1 and A2). When cooking beef and veal samples to 76°C, the temperature ranged from 74.6°C to 78.5°C and 72.6°C to 78°C, respectively (Figure A1 and A2).

Cooking caused weight losses ranging from 17.83% to 29% in non-intact beef and 19% to 29% in non-intact veal samples (Table 3.1 and 3.2). In beef samples, double pan-broiling to internal temperatures of 71.1°C and 76°C resulted in higher cooking losses (28.25-29%) than those from cooked to 55°C and 62.5°C (19-20.75%) (Table 3.1). In veal samples, cooking to internal temperatures of 65°C to 76°C resulted in higher cooking losses (24.16-29%) than those from cooked to 55°C (17.83%) (Table 3.2). As

expected, the higher cooked internal temperature resulted higher cooking losses due to the prolonged cooking time causing extra moisture loss via evaporation and the release of excess juice inside the meat samples.

pH, water activity, moisture and fat

The pH of uncooked beef and veal patties were 5.60 (Table 3.1) and 5.53 (Table 3.2). Double pan-broiling caused significant increase in beef and veal patties' pH, resulted in pH values ranging from 5.98 to 6.09 and 5.73 to 5.78, respectively (Table 3.1 and 3.2), which agree with previous studies of Berry (1998) and Trout (1992). The increase in pH for cooked meat is due to the reduction of free acidic groups as meat temperature increased during heating process (Lawrie and Ledward, 2006). However, no significant differences of cooked samples' pH were observed when beef or veal patties cooked to various internal target temperatures (55°C to 76°C). Only slight pH increase from 5.98 to 6.09 was detected in beef samples after cooking to 55°C to 76°C.

While moisture content describes the ratio of mass of water to the mass of the sample, the water activity is the partial vapor pressure of pure water, which indicates the availability of water for bacterial growth. The water activity of fresh beef and veal patties were 0.992 and 0.991. In both beef and veal samples, the water activity did not change significantly after cooking to various internal temperatures. These results are in agreement with previous study of Yoon et al. (2011), who reported that cooking non-intact ground beef to internal temperatures of 60°C and 65°C resulted in water activities of 0.981 to 0.982 compared to the uncooked samples' value of 0.982 to 0.984. The initial moisture of beef and veal samples were 70.53% and 76.02%. In both beef and veal

samples, the moisture content significantly decreased as cooked internal temperature increased from 55°C to 76°C (Table 3.1 and 3.2). When cooking beef or veal patties to 71.1°C or 76°C significantly decreased moisture contents to approximately 63% (beef) and 67% (veal) compared to the 66% (beef) and 71% (veal) in samples cooked to 55 °C (Table 3.1 and 3.2). Previous studies of Trout et al. (1992) and Shen et al. (2010) also reported that the moisture content of ground beef patties and moisture enhanced reconstructed beef patties was lower after cooking. The decrease of moisture content of beef and veal is likely due to the loss of water during cooking/heating process (Shen et al., 2010).

The fat content of fresh beef and veal patties was 6.46% and 2.19% (Table 3.1 and 3.2). Cooked beef samples had a significant ($P < 0.05$) increased fat content of 8.62% to 9.45% irrespectively of cooked internal temperatures (Table 3.1 and 3.2). Trout et al. (1998), Yoon et al. (2011) and Shen et al. (2010) all reported that cooking low fat ground beef or non-intact beefs increased fat content due to the moisture loss. A slightly ($P = 0.074, > 0.05$) increased fat content ranging from 2.79% to 3.09% was tested in cooked veal samples compared to the uncooked ones.

Color variation during storage and cooking

The fresh prepared beef patties' a^* , b^* , and L^* value was 34.75, 25.89 and 44.94, respectively (Table A3 and A4). Compared to beef samples, a lower ($P < 0.05$) a^* and b^* value of 26.98 and 22.63, and a higher L^* value of 59.83 was detected in fresh veal patties (Table A3 and A4). The less red and lighter color is expected in veal samples because veal is the meat of bovine animal aged 8 months or less containing less

myoglobin contents than those of beefs. During the aerobic storage, in general, the a^* , b^* , and L^* values were significantly decreased from 34.75 to 15.26, 25.85 to 14.39, and 44.94 to 39.85 in beef patties, and decreased from 26.98 to 12.76, 22.63 to 16.53, and 59.82 to 57.87 in veal samples. These results agree with previous study of Troutt et al. (1992), who found that a^* , b^* , and L^* value decreased as display time increased from 0 to 3 days. Madhavi and Carpenter (1993), also reported that the discoloration occurs within 7 days of oxygen permeable film packaged beef muscles. During PVC film storage, the oxymyoglobin reacted with oxygen forming metmyoglobin causing the less red color of beef or veal samples.

As expected, in general, a^* and b^* values of cooked beef samples' internal color decreased (less red and yellow) (Table A13 and A14) whereas the L^* value increased as the internal end-point temperature increased (Table A15). For a^* and b^* value, a lower value of 13.95 (a^* , less red) and 17.93 (b^* , less yellow) was detected in beef samples cooked to 76°C compared to the 28.05 (a^*) and 24.41 (b^*) of samples cooked to 55°C (Table A13 and A14). However, beef samples cooked to 62.5°C or 71.1°C had similar a^* values of 19.34 to 21.75, and cooked to 55°C or 62.5°C resulted similar b^* values of 23.51 to 24.41 (Table A13 and A14). For L^* value, cooking beef samples to 62.5, 71.1 and 76 °C resulted a higher value of 53.66 to 54.19 compared to the value of 50.24 in samples cooked to 55°C (Table A15). Hague et al. (1994) reported that increasing end-point cooking temperature from 55°C to 77 °C decreased a^* and b^* values of ground beef patties from 14.6 to 11.0 and 18.4 to 15.9, respectively, and increased L^* value from 50.9 to 52.2. The variances of internal cooked color is attributed to the denaturation of

myoglobin in ground beef patties as internal end-point temperatures rose from 55°C to 76 °C (Hunt et al., 1999).

Limited studies reported the internal color variation in ground veal cooked to different end-point temperatures. A study shown similar color variation tendency was detected in veal samples as compared to those of beef. In cooked veal patties, cooking to end-point temperature of 71.1°C and 76°C resulted in lower ($P<0.05$) a^* of 11.21 to 12.2 and b^* of 15.56 to 16.25 than those cooked to 55°C and 62.5°C with a^* of 16.24 to 17.18 and b^* of 18.48 to 18.84. However, there is no difference ($P>0.05$) of L^* values, ranging from 70.09 to 72.96, among veal samples cooked to 55°C to 76 °C.

Cooking inactivation of bacterial populations

The initial inoculum level of *E. coli* O157:H7 in uncooked coarse ground beef and veal samples were 6.4 and 6.6 log CFU/g, and no significant changes in *E. coli* O157:H7 populations were observed following 4-day PVC film aerobic storage at 4°C, which is consistent with previous study of Yoon et al. (2011). The total bacterial population counts on TSA were similar to those observed on Mcconkey agar indicating that the major colonies found on TSA were inoculated *E. coli* O157:H7. As expected, double pan-broiling beef and veal samples to 71.1°C (medium degree doneness) and 76°C (well-done doneness) with 3 minute rest time decreased overall pathogen populations below detection limit (>6 log CFU/g) (Figure A19). The recent study of Luchansky et al. (2013) reported that cooking refrigerated ground beef patties to internal temperature of 71.1°C and 76.6°C reduced 5.1-7.0 log CFU/g of *E. coli* O157:H7. This result also verified that

cooking ground beef to an internal temperature of at least 71.1°C will not present great risk to consumers (USDA-FSIS, 2001).

Conclusion

Since food safety is a major concern in the U.S., to reduce the possibility of food-borne outbreaks due to *E. coli* O157:H7 contamination, the USDA-FSIS recommended cooking non-intact beefs and veal products to internal temperature of 63 °C with at least a 3 minute rest time. To the best of our knowledge, no research publication detected the impact of rest time on the thermal inactivation activity of *E. coli* O157:H7 on non-intact beef, and very limited studies focus on veal products. Luchansky et al. (2014) most recently found that cooking breaded or un-breaded veal cutlets for 1.5 minutes per side on an electronic skillet set up 191.5 °C achieved internal temperature of 71.1 °C and >5.0 log reduction. In this study, cooking beef or veal samples to 55°C and 62.5°C with a 3 minute rest time reduced 4.0-5.5 and 5.7->6.6 log CFU/g, respectively. It is also interesting to find that *E. coli* O157:H7 cells are more ($P<0.05$) sensitive to heat in veal samples compared to beef, which might be explained by the relative lack of muscles in veal allowing more efficient heat transfer, and the higher moisture that produces a stronger steaming effect inside the veal patties. Our study demonstrated that cooking coarse ground beef or veal to internal end-point temperature of 62.5 °C with a 3 minute rest achieves >5.0 log reduction of *E. coli* O157:H7 cells.

In conclusion, higher internal temperature causes increasing inactivation of ECOH and STEC, and veal and beef patties presents similar change in quality in terms of quality change throughout storage and cooking, which supports the hypothesis. Results

from this study covers various aspects of beef and veal quality changes during grinding, storage and cooking process, which will be beneficial to intact and non-intact beef or veal preparation at multiple points including retail, foodservices, and at home. It also verified that cooking coarse ground beef or veal to internal end-point temperature of 62.5 °C with a 3-minute rest will not generate great food safety risk. This information will be useful by USDA-FSIS to develop risk assessments of *E. coli* O157 on non-intact and intact beef or veal products.

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APPENDIX

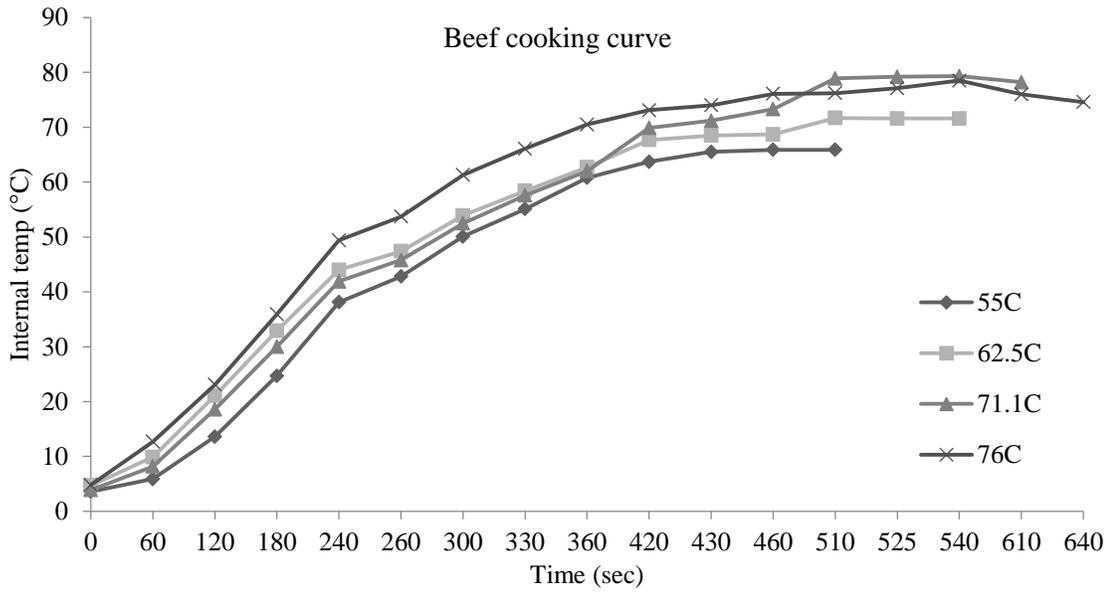


Figure A1: Cooking curve for beef patties.

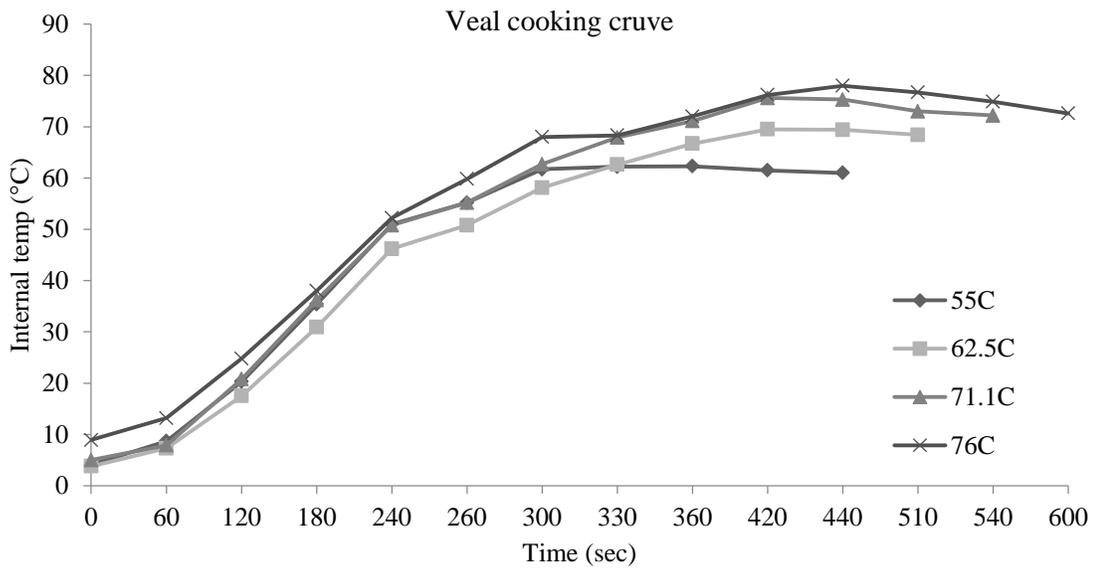


Figure A2: Cooking curve for veal patties

Table A3: Color variance during storage.

| | Beef | | | | | Veal | | | | |
|----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 |
| L* | 46.32±2.11 | 42.06±2.75 | 40.20±2.37 | 43.70±2.71 | 39.85±2.05 | 59.96±1.64 | 59.16±1.55 | 58.15±2.44 | 58.26±1.65 | 58.48±1.64 |
| a* | 34.90±2.27 | 22.92±2.61 | 19.71±2.26 | 15.40±1.61 | 15.27±1.02 | 26.90±0.94 | 22.41±1.38 | 16.97±1.49 | 14.39±1.04 | 12.77±0.60 |
| b* | 25.86±2.07 | 17.91±1.62 | 17.14±1.48 | 14.40±1.60 | 16.93±1.49 | 22.61±0.95 | 20.07±0.96 | 17.99±0.95 | 17.30±0.96 | 16.53±0.83 |

Table A4: Internal Color variance before and after cooking to various internal temperature.

| | After cooking to | | | | | | | | | | | |
|------|------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| | 55°C | | | 62.5°C | | | 71.1°C | | | 76°C | | |
| | L* | a* | b* | L* | a* | b* | L* | a* | b* | L* | a* | b* |
| Beef | 50.32±3.18 | 13.46±1.66 | 18.45±2.21 | 46.87±1.79 | 12.50±0.97 | 17.33±1.03 | 47.08±1.57 | 12.63±1.28 | 17.45±1.04 | 46.67±1.59 | 11.86±0.75 | 16.96±1.04 |
| Veal | 68.98±2.13 | 10.76±1.59 | 19.58±2.69 | 68.40±1.08 | 11.15±0.99 | 21.53±2.78 | 66.45±1.85 | 10.58±0.64 | 19.59±1.84 | 68.45±1.55 | 10.03±0.53 | 18.71±2.09 |

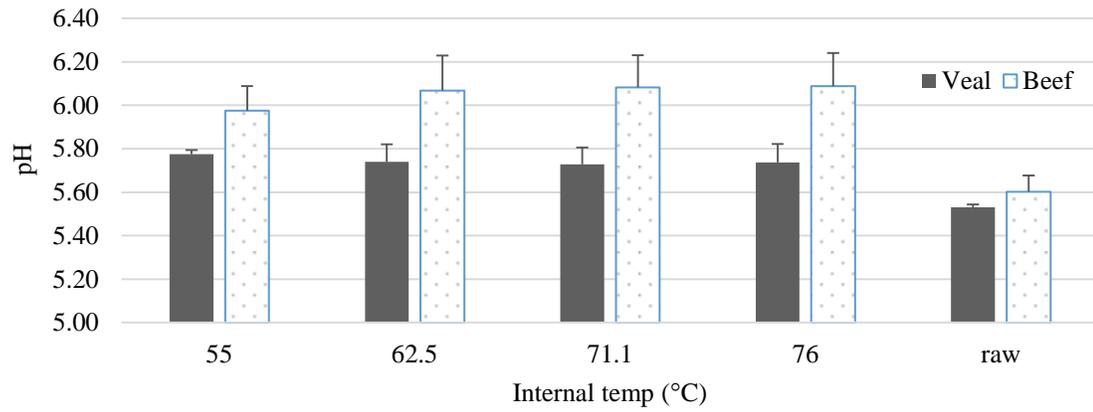


Figure A5: Comparison of pH values for beef and veal patties under different treatments

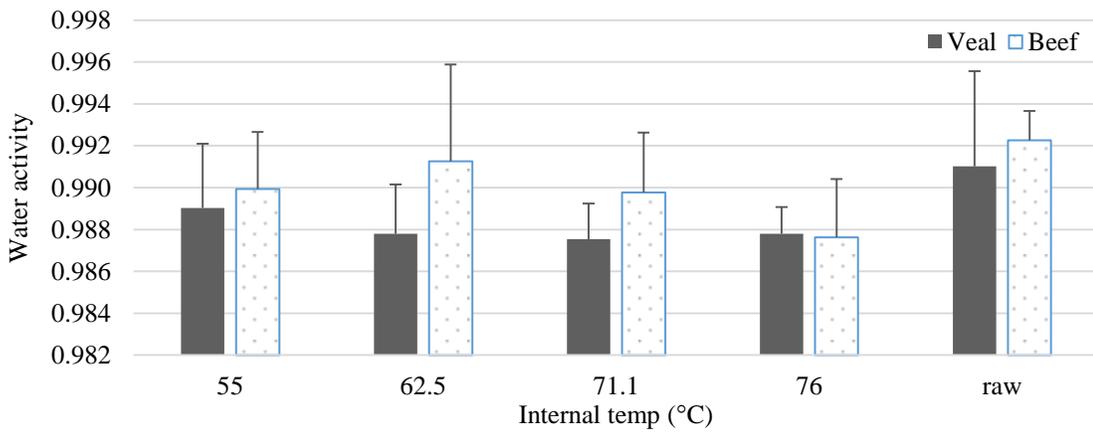


Figure A6: Comparison of water activity for beef and veal patties under different treatments

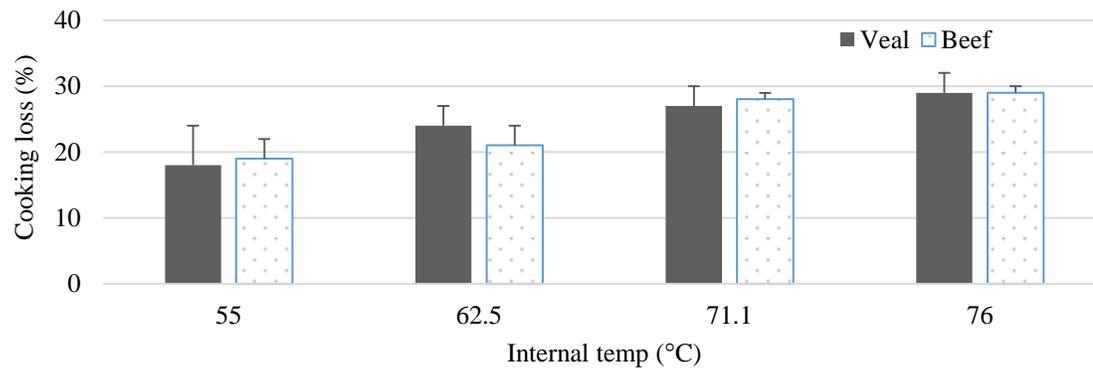


Figure A7: Comparison of cooking loss for beef and veal patties under different treatments

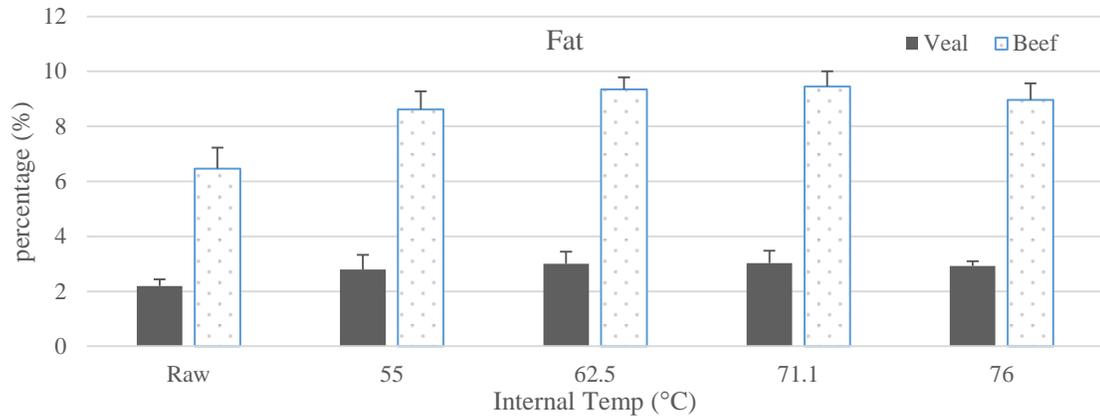


Figure A8: Comparison of change in Fat content.

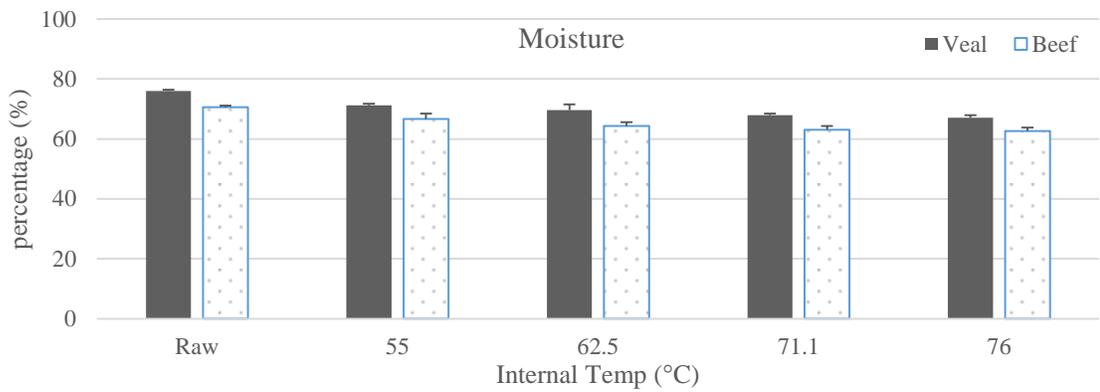


Figure A9: Comparison of change in moisture.

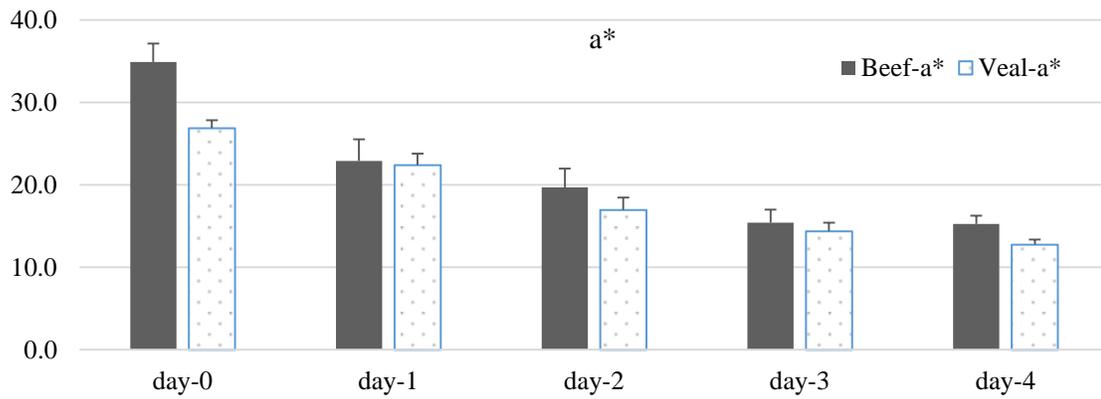


Figure A10: Comparison of change of redness (a^*) throughout storage with different treatments on beef and veal petties.

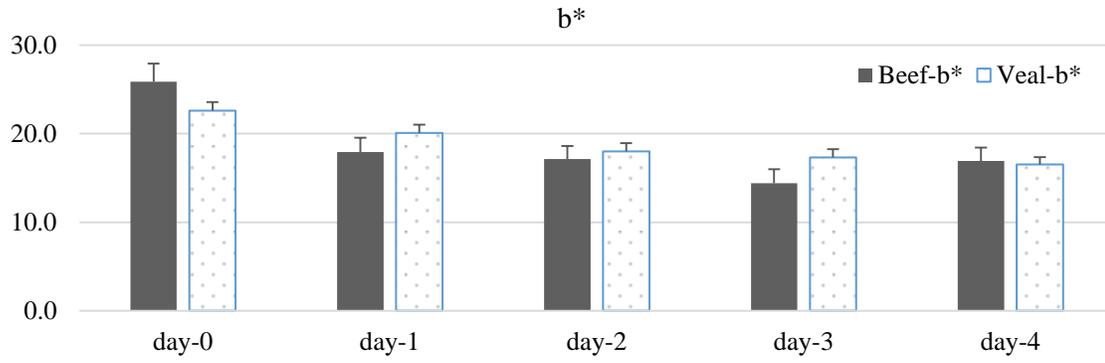


Figure A11: Comparison of change of yellowness (b^*) throughout storage with different treatments on beef and veal patties.

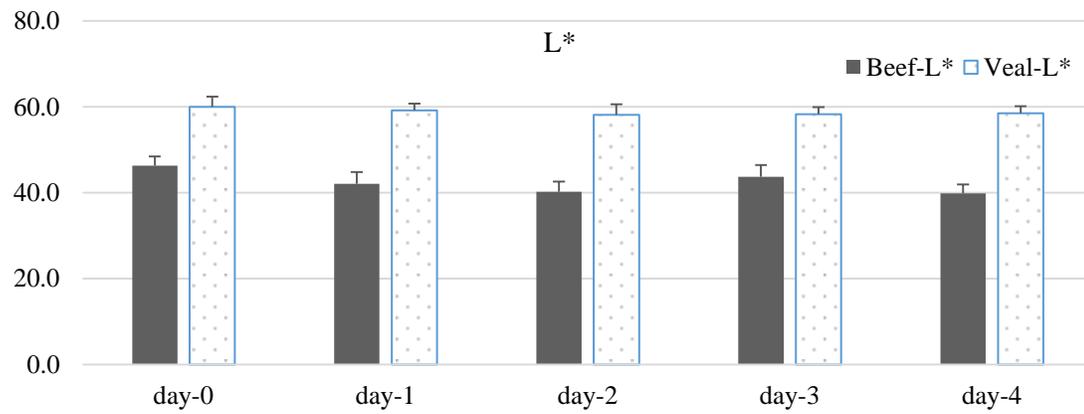


Figure A12: Comparison of change of lightness (L^*) throughout storage with different treatments.

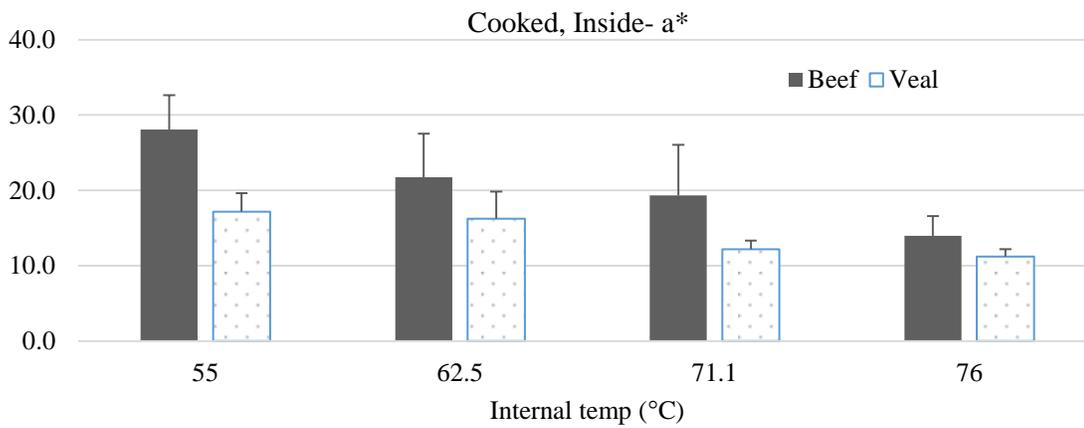


Figure A13: Comparison of the internal redness (a^*) of the patties after different.

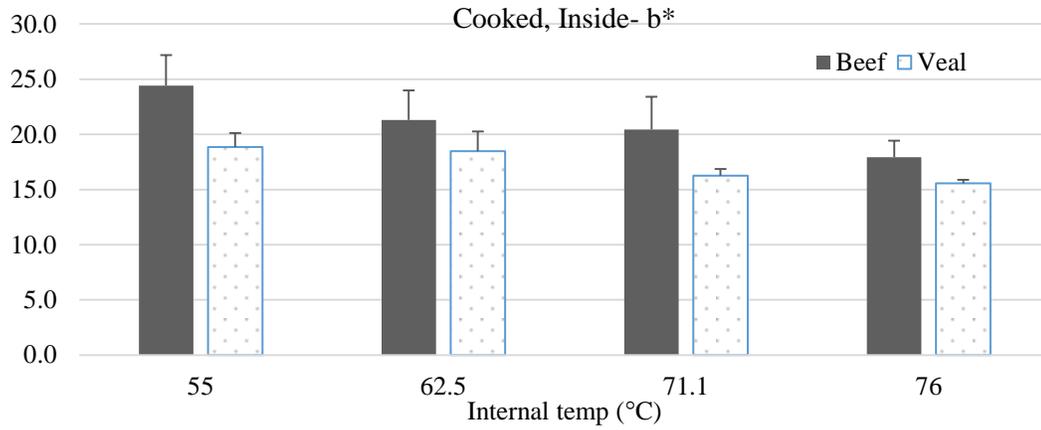


Figure A14: Comparison of the internal yellowness (b^*) of the patties after different treatments.

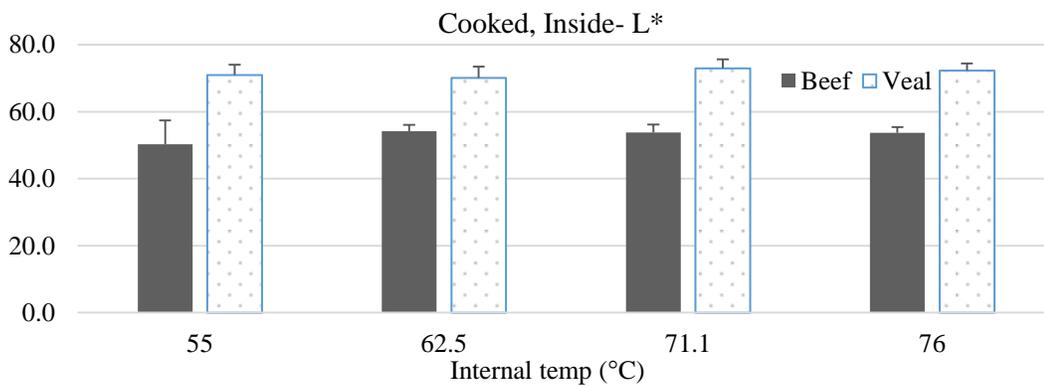


Figure A15: Comparison of the internal lightness (L^*) of the patties after different treatments.

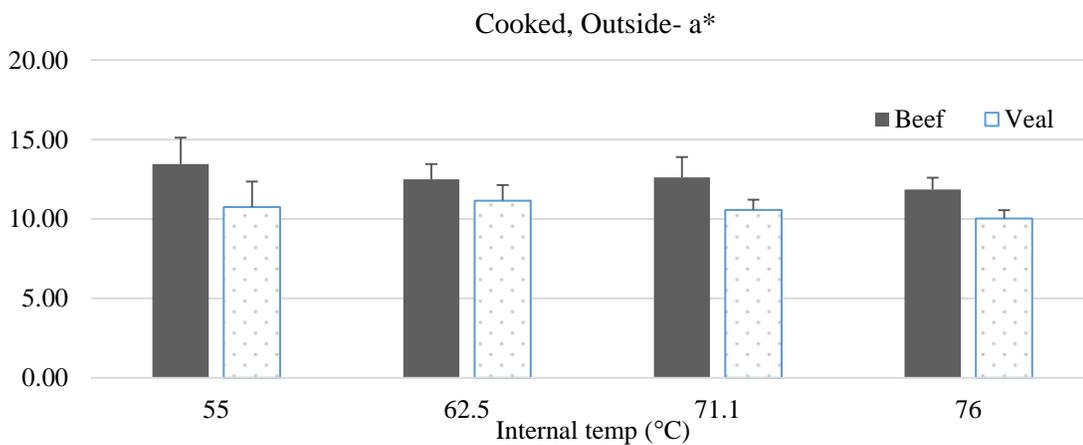


Figure A16: Comparison of the surface redness (a^*) of the patties after different treatments.

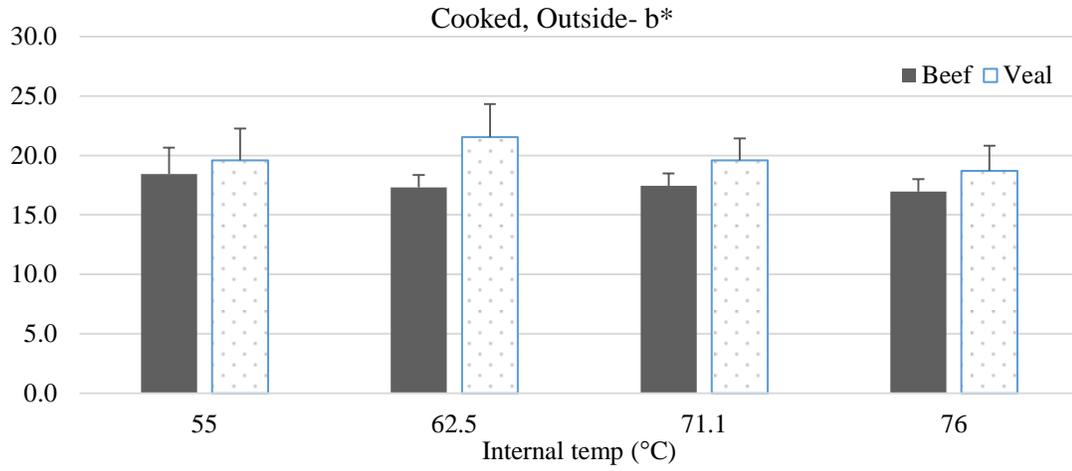


Figure A17: Comparison of the surface yellowness (b^*) of the patties after different treatments.

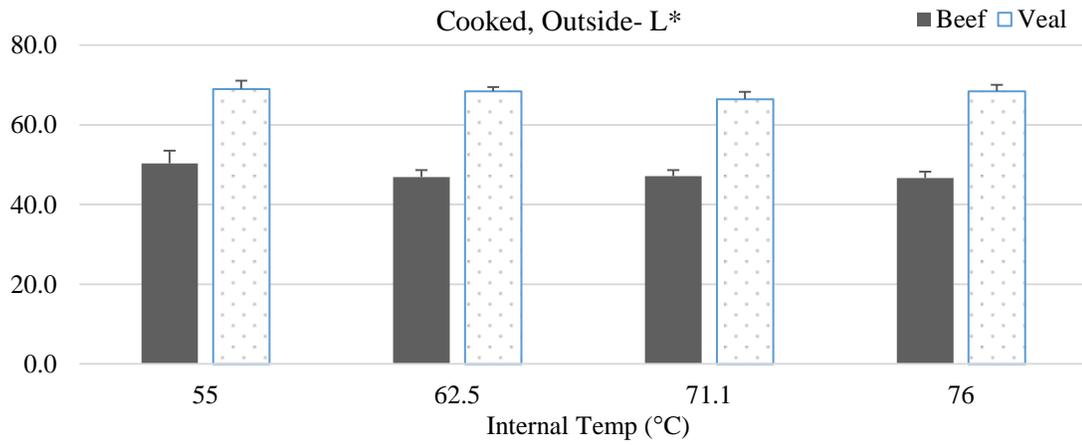


Figure A18: Comparison of the surface lightness (L^*) of the patties after different treatments.

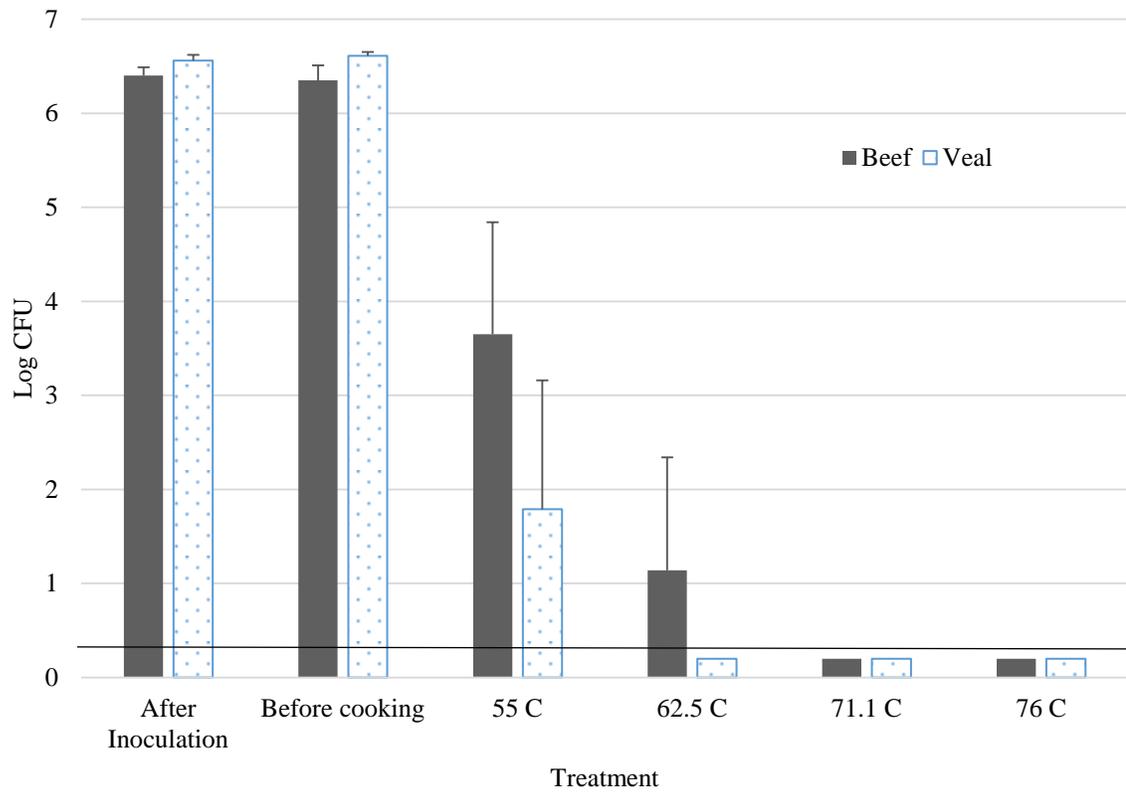


Figure A19: Comparison of inactivation of STEC with different treatments, where the line indicates the detecting limit (0.33 log CFU/mL)