



## Fate of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* During Curing and Drying of Beef Bresaola

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**Abstract:** Manufacturing dry-cured meat products without a thermal lethality step is a growing trend for charcuterie companies in the United States. The United States Department of Agriculture Food Safety and Inspection Service requires that hazards for ready-to-eat meat products be addressed with a scientifically valid Hazard Analysis Critical Control Point system. Because little validation literature exists for these products, an experiment was designed to investigate the safety of beef bresaola. The objective of this study was to determine the reduction of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* during curing and drying of bresaola. Prior to curing, whole beef *semitendinosus* muscle was inoculated with a mixed culture containing 3 strains each of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes*, allowed to air dry (30 min at 23°C), sprayed with a 2.5% Beefside antimicrobial treatment (Birko Corp., Henderson, CO), and allowed to sit overnight in a walk-in cooler (2°C–4°C). Cure (NaNO<sub>3</sub> and NaNO<sub>2</sub>) and salt were applied to the beef surface 24 h after the antimicrobial treatment, and the beef was cured for 28 d (2°C–4°C). Following curing, a proprietary spice mixture was surface coated, and each piece was stuffed into beef casings (115–130 mm). The stuffed bresaola pieces were hung and allowed to dry for 35 d to a target water activity < 0.92 (13.63°C ± 2°C; relative humidity 68% ± 7%). Pathogen populations and water activity were analyzed on days 0, 1, and 2 and then weekly until day 65 of the study. Final reductions of 5.97, 5.98, and 5.44 log<sub>10</sub> colony-forming units (CFU)/cm<sup>2</sup> were achieved on day 65 for *E. coli*, *Salmonella* spp., and *L. monocytogenes*, respectively. During the entire curing and drying process, populations of each species never increased by more than 0.5 log<sub>10</sub> CFU/cm<sup>2</sup>. The critical parameters used to cure and dry this product are sufficient to achieve the minimum 5 log<sub>10</sub> CFU/cm<sup>2</sup> reduction of each pathogen as required by the United States Department of Agriculture Food Safety and Inspection Service to validate process safety.

**Key words:** bresaola, dry-cured, *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*

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## Introduction

Salting, curing, and drying have been utilized as meat preservation methods for thousands of years, and whole-muscle meat products like prosciutto and bresaola were among the first products to be developed by ancient societies throughout Europe (Zeuthen, 2007; Zhou et al., 2010; Ruhlman and Polcyn, 2012). As the charcuterie and salumi industry in the United

States has grown and expanded, artisanal and wholesale producers alike are recreating these ancient products using techniques that have been maintained through the centuries. Traditionally, bresaola was produced with whole beef muscles like *semitendinosus* (eye of round) or *semimembranosus* (inside round), and products were not cooked prior to consumption. Eye of round or similar beef cuts were coated with salt and spices, stuffed into casings, or wrapped in skin and then were hung to be dried. The finished bresaola was sliced and consumed once

enough water had been removed from the product (Zeuthen, 2007; Zhou et al., 2010; Ruhlman and Polcyn, 2012).

Bresaola is generally considered safe to consume without thermal treatment and relies heavily upon hurdle technology and the hurdle effect to achieve bacterial safety (Leistner and Gorris, 1995; Leistner, 2000). For example, bresaola and other whole-muscle charcuterie products rely primarily on the collective use of salt and drying in order to reduce water activity ( $a_w$ ) in the final product, which is detrimental to the survival and growth of any pathogens present (Sperber, 1983; Beales, 2004). These intrinsic and extrinsic stressors for pathogenic cells may be metabolic or structural. Metabolic stress inhibits growth by increasing the osmotic pressure of the meat system, causing bacterial cells to expend a greater amount of energy maintaining internal water and ion homeostasis. Pathogens that are prevented from growing in a low  $a_w$  environment are hindered because energy is used to maintain homeostasis rather than multiplication, and death of the pathogen occurs when the cell has become metabolically exhausted (Sperber, 1983; Beales, 2004). Structural stress of the pathogenic cell can cause morphology changes in shape or even composition changes of the cell membrane, specifically, fatty acids and lipids that play a role in membrane permeability and structural integrity (Harvey and Leach, 1998; Rowan, 1999; Murga et al., 2000; Guerzoni et al., 2001). Several curing formulations, either directly or indirectly, utilize a nitrate or nitrite source. In addition to preventing the germination of *Clostridium botulinum* spores, nitrite, and/or nitrate are bactericidal to other microorganisms that may be present in or on meat products (Majou and Christieans, 2018). Although the mechanism by which nitrate and/or nitrite cause death of pathogens is not completely understood, it is known that once sodium nitrite ( $\text{NaNO}_2$ ) is added to a meat product, some of the  $\text{NaNO}_2$  is converted to nitrous acid, a chemical that negatively impacts bacterial cellular processes (Hospital et al., 2014; Majou and Christieans, 2018).

Despite the effectiveness of hurdle technology for improving the safety of meat products, the presence of pathogens in a raw, ready-to-eat (RTE) product such as bresaola is still of concern. It is well known that beef cattle are a natural reservoir for pathogenic *Escherichia coli*, a microorganism that can be present on beef surfaces contaminated during the harvest and fabrication process (Elder et al., 2000; Barkocy-Gallagher et al., 2005; Duffy et al., 2006; Brichta-Harhay et al., 2008; Castro et al., 2017). According to the United States Department of Agriculture Food Safety and

Inspection Service (USDA-FSIS), *E. coli* O157:H7 (*Ec*) and other Shiga toxin–producing *E. coli* are the pathogens of highest concern in products containing beef. *Ec* may be the most severe among pathogenic cells capable of producing Shiga toxin because of its low infectious dose (10 or fewer viable cells). Infections by this pathogen result in severe disease, especially in immunocompromised individuals (Montville et al., 2012), and the USDA-FSIS considers all Shiga toxin–producing *E. coli* to be an adulterant in beef products (USDA-FSIS, 2014a). Nontyphoidal *Salmonella enterica* ssp. *enterica* (*Sal*) is also a concern in beef products, and although *Sal* does not produce toxin, the bacterium is able to cause severe enterocolitic disease that can result in death (Montville et al., 2012; Fàbrega and Vila, 2013). *Listeria monocytogenes* (*Lm*) is also of great concern for all RTE meat products, especially those that are not subjected to a thermal processing step during production. *Lm* can form and persist in biofilms in the food-processing environment and grow under refrigeration, thus creating the potential for contamination of finished product (Hill et al., 2002; Gandhi and Chikindas, 2007; Swaminathan and Gerner-Smidt, 2007; Ferreira et al., 2014). Because of the growth parameters of *Lm* and high mortality rate caused by listeriosis, the USDA-FSIS employs a zero-tolerance policy for the presence of *Lm* in RTE meat products (USDA-FSIS, 2014b).

The US Code of Federal Regulations states that meat-processing establishments should conduct a hazard analysis of “food safety hazards reasonably likely to occur” and that preventative measures, backed by scientific evidence, be established to control the identified hazards for all products produced (Federal Register, 1996). Additionally, the USDA-FSIS requires that *Ec*, *Lm*, and *Sal* be controlled during the production of beef bresaola ( $\geq 5 \log_{10}$  reduction of pathogens) to ensure product safety (USDA-FSIS, 2017). Experimental trials conducted in a laboratory setting that replicate, as best as possible, the conditions in processing environments are commonly accepted by regulators as a method of validating an individual production process for RTE products (Scott, 2005). Therefore, the objectives of this research were to validate a production process of raw, RTE beef bresaola according to USDA-FSIS regulations and to achieve a  $5 \log_{10}$  reduction in *Ec*, *Sal*, and *Lm* by the end of processing. The results of this experiment could be used as scientifically valid support for the safe production of raw, RTE bresaola for Hazard Analysis Critical Control Point documentation as well as to fill a

significant gap in the literature on the safety of whole-muscle charcuterie products.

## Materials and Methods

### Preparation of pathogens

Beef Round, eye of round subprimals (Institutional Meat Purchasing Specifications 171C) were received frozen directly from a meat processor in the northeast. For each of 4 independent replications, beef subprimals ( $n = 6$ ) were thawed (2°C–4°C) in a walk-in cooler and challenged over the course of curing and drying with a pathogen cocktail of 3 strains each of the following: *Ec*, *Sal* (Typhimurium, Montevideo, and Panama), and *Lm*. Pathogenic cultures were obtained from the American Type Culture Collection (Manassas, VA), the Centers for Disease Control and Prevention (Atlanta, GA), and the Pennsylvania State University Department of Food Science (University Park, PA) (Table 1). Frozen stocks were aseptically transferred to sterile tryptic soy broth (TSB; Hardy Diagnostics, Santa Maria, CA) and aerobically incubated at 37°C for 24 h. The 24-h cultures were then streaked onto selective media and confirmed for pathogen type using agglutination testing (Microgen Bioproducts, Camberley, UK). Single colonies of each pathogen type were transferred to 10 mL of TSB and incubated for 24 h, as previously described. The 10 mL cultures were then transferred to 240 mL of TSB and grown to a cell concentration of  $\sim 8.5 \log_{10}$  colony-forming units (CFU)/mL. Equal volumes (250 mL) of each strain were mixed in a sterile metal bin under a biological safety cabinet to create a total

volume of 2.25 L for the homogenized inoculation immersion bath.

### Inoculation and processing procedures

The experiment was conducted in 4 independent replications in the Penn State Food Safety Pilot Plant, a Centers for Disease Control and Prevention Biosafety Level 2 facility. For each replication, 6 eye of round subprimals ( $n = 6$ ) were submerged in the prepared inoculum ( $\sim 8.5 \log_{10}$  CFU/mL) immersion bath for approximately 30 min under a biological safety cabinet ( $\sim 23^\circ\text{C}$ ) to a target level of 6–7  $\log_{10}$  CFU/cm<sup>2</sup>. The subprimals were transferred to a sterile plastic bin (uncovered) and allowed to dry for approximately 30 min at  $\sim 4^\circ\text{C}$ , rotating approximately every 10 min in the bin. After drying, a 1:40 (2.5% vol/vol) solution of fresh Beefxide antimicrobial (Birko Corp., Henderson, CO) was sprayed onto the meat surface using a hand-pump tank sprayer (H.D. Hudson Mfr. Co., Chicago, IL) to evenly cover all surfaces of the product. Briefly, the spray nozzle was kept approximately 10–12 cm from the product surface. The wand was moved back and forth in a sweeping motion the length of each eye of round from end to end and then repeated in the same pattern backward. Spraying of the product surface was about 15–20 s on each side before the product was rotated 180 degrees and sprayed again to coat all surfaces. The eye of round subprimals then were transferred to  $\sim 4^\circ\text{C}$  walk-in cooler. Twenty-four hours following the antimicrobial spray treatment, the eye of round subprimals were cured by applying half of the total curing mixture, a proprietary blend of salt (3.5% of meat weight) and cure ingredients (NaNO<sub>2</sub> [150 ppm] and sodium nitrate [NaNO<sub>3</sub>; 100 ppm]). After being coated with the dry ingredients, the beef was placed in a food-grade, plastic, sterile meat lug at 4°C, covered, and allowed to cure at 4°C for approximately 7 d before being coated again with the remaining half of the cure mixture. The eye of round subprimals remained in the 4°C walk-in cooler for a total of 4 wk before being hand stuffed into 115- to 130-mm beef bung casings (Globe Casing, Carlstadt, NJ) that had been treated with a 2.5% Beefxide antimicrobial solution prior to use. The bresaola was tied with butcher twine (24 ply; UltraSource USA, Kansas City, MO) and transferred to an AS50 drying cabinet (Impianti Condizionamento Salumifici; Camposanto, Modena, Italy) at 12°C–14°C (average 13.63°C  $\pm$  2°C) and 65%–75% relative humidity (average 68%  $\pm$  7%). Temperature and humidity were monitored using a HOBO UX100-003 data logger (Onset Computer

**Table 1.** Type and source of pathogens

Organism	Source
<i>Escherichia coli</i> O157:H7	ATCC 43865
<i>Escherichia coli</i> O157:H7	ATCC BAA-460
<i>Escherichia coli</i> O157:H7 strain PA-2	Penn State Food Science
<i>Salmonella enterica</i> ssp. <i>enterica</i> serovar Typhimurium	ATCC 14028
<i>Salmonella enterica</i> ssp. <i>enterica</i> serovar Panama	ATCC 7378
<i>Salmonella enterica</i> ssp. <i>enterica</i> serovar Montevideo	CDC 013
<i>Listeria monocytogenes</i> Scott A	Penn State Food Science
<i>Listeria monocytogenes</i> serotype 4b isolate H3396	Penn State Food Science
<i>Listeria monocytogenes</i> serotype 1/2a isolate J1-129	Penn State Food Science

ATCC = American Type Culture Collection; CDC = Centers for Disease Control and Prevention.

Corp., Bourne, MA). Total curing and drying occurred over a period of 65 d to a target surface  $a_w$  of <0.92.

### Sampling for microbial populations and water activity

Samples for microbial populations were taken on day 0 (after inoculation but prior to antimicrobial treatment), day 1 (after antimicrobial treatment but prior to addition of salt and cure ingredients), day 2 (approximately 24 h after addition of salt), and then every 7 d beginning on day 9 until day 65. At each sampling time, 4 eye of round subprimals were randomly selected to evaluate the microbial populations ( $n = 3$ ) and to measure  $a_w$  ( $n = 1$ ). Using a 5 cm × 5 cm stainless steel template, a 25 cm<sup>2</sup> piece was cut from the surface of the meat (avoiding previously sampled areas) using a disposable, sterile scalpel (Bard-Parker; Aspen Surgical, Caledonia, MI) and placed into a 400 mL stomacher bag (BagFilter; Interscience, Woburn, MA). Twenty-five milliliters of 1X phosphate-buffered saline solution (pH 7.2; Hardy Diagnostics, Santa Maria, CA) was added to the sterile stomacher bag, and the sample was homogenized for 3 min at 260 rpm (Stomacher 400 Circulator; Seward Limited, West Sussex, UK). The stomachate from each sample was serially diluted into 9 mL of 1X phosphate-buffered saline, and aliquots of 0.1 mL were spread plated in duplicate onto selective agar to achieve a detection limit of 0.69 log<sub>10</sub> CFU/cm<sup>2</sup>. The stomachate was plated in 0.5 mL aliquots in quadruplicate when microbial populations were below the original detection limit. Enumeration of *Ec*, *Sal*, and *Lm* was performed using Cefixime Tellurite Sorbitol MacConkey agar (Remel, Lenexa, KS), Xylose Lysine Deoxycholate agar (Remel), and modified Oxford agar with antibiotic supplement (BD, Sparks, MD), respectively. Cefixime Tellurite Sorbitol MacConkey agar and Xylose Lysine Deoxycholate agar plates were incubated aerobically at 37°C for 24 h, and modified Oxford agar with antibiotic supplement plates were incubated aerobically for 48 h at 37°C. When no colonies were enumerated, the stomachate sample was enriched (simultaneously during plating) to determine the presence of *Ec*, *Sal*, and *Lm*. Enrichments were performed using the USDA Microbiology Laboratory Guidebook methodology. Gram-negative broth (Difco Laboratories, Franklin Lakes, NJ) was used to enrich for *Ec*, whereas lactose broth (primary; HiMedia, Mumbai, India) and Rappaport-Vassiliadis (secondary; Remel Products, Lenexa, KS) broth was used for determination of *Sal*. University of Vermont Medium (Difco Laboratories)

and Fraser broth (Difco Laboratories) were used as primary and secondary enrichments to determine the presence of *Lm*.  $a_w$  was measured using a calibrated AquaLab 4TE dew point meter (Decagon Devices, Pullman, WA). Briefly, the 25 cm<sup>2</sup> surface piece removed from the surface of the eye of round was trimmed to fit in the round sample cup. The cup was inserted into the chamber and the chamber locked to initiate measurement.

### Statistical analysis

Concentrations of *Ec*, *Sal*, and *Lm* were analyzed independently using a General Linear Model with unique consecutive comparisons ( $\alpha = 0.05$ ) (SAS version 9.4, SAS Institute, Inc., Cary, NC). To maintain statistical power, comparisons were made between the pathogen concentration average from one sampling time and the concentration of the same pathogen on the following sampling time. No comparisons were made between pathogens. Statistical analysis was not used for  $a_w$  data.

## Results

Reductions greater than 5 log<sub>10</sub> CFU/cm<sup>2</sup> were achieved for each pathogen by the end of processing on day 65. *Ec*, *Sal*, and *Lm* achieved final reductions 5.97, 5.98, and 5.44 log<sub>10</sub> CFU/cm<sup>2</sup> ( $P < 0.00001$  for each), respectively (Table 2). Table 2 shows the mean population of each pathogen for each sampling day and the cumulative log<sub>10</sub> reduction during the 65-d process. Steady reductions of each pathogen occurred over the duration of the curing and drying process. The antimicrobial intervention achieved between 0.50 and 0.90 log reductions, depending on pathogen type ( $P < 0.05$  for each). *Lm* was the first pathogen to achieve a 5 log<sub>10</sub> reduction with a reduction of 5.21 log<sub>10</sub> CFU/cm<sup>2</sup> occurring on day 37. *Ec* and *Sal* first achieved 5 log<sub>10</sub> reductions on day 44 with reductions of 5.52 and 5.74 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively ( $P < 0.05$ ). It is important to note that the populations of all 3 pathogens never increased greater than 0.5 log<sub>10</sub> CFU/cm<sup>2</sup> at any time during sampling, indicating that no growth of pathogens occurred during processing. Because the eye of round subprimals were intact whole-muscle roasts and not injected or tenderized, the interior of the bresaola was presumed to be sterile, and only surface measurements of microbial populations are reported. The final surface  $a_w$  of the bresaola was 0.84 (0.88 internal) and was below the target finished  $a_w$  of 0.92. The  $a_w$



**Table 2.** Mean pathogen levels ( $\log_{10}$  CFU/cm<sup>2</sup>  $\pm$  standard error) and cumulative  $\log_{10}$  reduction of *Escherichia coli* O157:H7 (*Ec*), *Salmonella* spp. (*Sal*), and *Listeria monocytogenes* (*Lm*;  $n = 24$ ), and  $a_w$  ( $n = 4$ ) of bresaola at sampling days

Day	<i>Ec</i>	<i>Ec</i> Reduction	<i>Sal</i>	<i>Sal</i> Reduction	<i>Lm</i>	<i>Lm</i> Reduction	$a_w$
0	6.42 $\pm$ 0.03	–	6.38 $\pm$ 0.05	–	6.35 $\pm$ 0.05	–	0.99
1	5.91 $\pm$ 0.08	0.51 ( $P = 0.0004$ )	5.59 $\pm$ 0.05	0.79 ( $P < 0.0001$ )	5.62 $\pm$ 0.05	0.73 ( $P < 0.0001$ )	0.94
2	4.94 $\pm$ 0.07	1.48 ( $P < 0.0001$ )	4.37 $\pm$ 0.10	2.04 ( $P < 0.0001$ )	4.58 $\pm$ 0.10	1.76 ( $P < 0.0001$ )	0.94
9	4.88 $\pm$ 0.10	1.54 ( $P = 0.6978$ )	4.53 $\pm$ 0.12	1.84 ( $P = 0.1616$ )	4.73 $\pm$ 0.12	1.62 ( $P = 0.2382$ )	0.92
16	3.80 $\pm$ 0.05	2.62 ( $P < 0.0001$ )	3.57 $\pm$ 0.07	2.81 ( $P < 0.0001$ )	3.68 $\pm$ 0.07	2.66 ( $P < 0.0001$ )	0.89
23	3.31 $\pm$ 0.18	3.11 ( $P = 0.0006$ )	2.50 $\pm$ 0.20	3.88 ( $P < 0.0001$ )	3.52 $\pm$ 0.17	2.83 ( $P = 0.1784$ )	0.88
30	3.13 $\pm$ 0.07	3.30 ( $P = 0.1954$ )	2.61 $\pm$ 0.07	3.76 ( $P = 0.4049$ )	3.29 $\pm$ 0.10	3.06 ( $P = 0.0557$ )	0.88
37	1.98 $\pm$ 0.12	4.44 ( $P < 0.0001$ )	1.55 $\pm$ 0.04	4.83 ( $P < 0.0001$ )	1.14 $\pm$ 0.10	5.21 ( $P < 0.0001$ )	0.85
44	0.90 $\pm$ 0.12	5.52 ( $P < 0.0001$ )	0.64 $\pm$ 0.09	5.74 ( $P < 0.0001$ )	1.11 $\pm$ 0.04	5.24 ( $P = 0.8556$ )	0.85
51	0.66 $\pm$ 0.18	5.76 ( $P = 0.0957$ )	0.65 $\pm$ 0.17	5.72 ( $P = 0.9261$ )	0.93 $\pm$ 0.09	5.42 ( $P = 0.1303$ )	0.85
58	0.50 $\pm$ 0.10	5.92 ( $P = 0.242$ )	0.41 $\pm$ 0.08	5.97 ( $P = 0.086$ )	0.70 $\pm$ 0.06	5.65 ( $P = 0.0578$ )	0.83
65	0.45 $\pm$ 0.11	5.97 ( $P = 0.7399$ )	0.40 $\pm$ 0.07	5.98 ( $P = 0.9222$ )	0.91 $\pm$ 0.07	5.44 ( $P = 0.0834$ )	0.84

Statistical comparisons of pathogen concentration were made between each sample day consecutively ( $n = 24$ ,  $\alpha = 0.05$ ) within pathogen type.

$a_w$  = water activity; CFU = colony-forming units.

first dropped below 0.92 during curing on day 16 and remained below 0.92 until the end of processing. During the first week of drying (day 30–37), surface  $a_w$  dropped from 0.88 to 0.85.

## Discussion

There has been a recent trend of processing raw, RTE beef products in the US; however, there is little scientific literature supporting their safety. To our knowledge, this bresaola research is the first to demonstrate the viability of a production process for raw, RTE beef challenged with *Ec*, *Sal*, and *Lm* to meet FSIS regulatory standards. Burnham et al. (2008) investigated the lethality of pathogens during the processing of biltong and droëwors, dried beef snacks of South African origins. Of the 2 product types examined, biltong is most similar to bresaola because both are whole muscle pieces. Results from the biltong research showed a 3–4  $\log_{10}$  reduction in *Ec*, *Sal*, and *Lm* from curing and drying alone. The authors concluded that the parameters used to produce biltong were lethal to pathogens, but raw material testing of each production

lot would be needed in combination with the production parameters to validate the process. It is important to note that Burnham et al. (2008) did not incorporate any form of antimicrobial intervention (organic acid spray or dip) that was shown to be effective in the current study; however, the finished  $a_w$  (0.85 and 0.60) was quite lethal to pathogens.  $a_w$  is perhaps one of the most important intrinsic factors deterring growth and limiting survival of microorganisms. Scott (1957) investigated the minimum  $a_w$  for growth of many bacteria, indicating that growth below 0.85 for the pathogens investigated in the current study should not occur. It is difficult to compare in-going salt or curing ingredients between the biltong and bresaola processes because the biltong study measured finished percent water-phase salt and not salt on an in-going basis. The reductions of pathogens during the production of bresaola further demonstrate the utility of hurdle technology in meat processing and relevance to product safety. Although the current experiment did not evaluate the efficacy of utilizing various types of organic acid treatments during the production of bresaola, this research provides support for the use of organic acid (acetic, lactic, citric, peracetic acid, etc.)

treatments of raw meat (and casings) prior to further production steps. Other research in our lab (Gaydos et al., 2016; Rivera-Reyes et al., 2017) indicated that organic acids are effective and important hurdles to add during the production of various comminuted products.

Although no statistical comparisons were made between pathogens on the same day or differing sampling days, it is interesting to note that *Lm* was the first pathogen to achieve a 5 log<sub>10</sub> reduction despite its well-documented resistance to drying and high concentrations of salt (Hill et al., 2002; Gandhi and Chikindas, 2007; Swaminathan and Gerner-Smith, 2007; Ferreira et al., 2014). *Lm* also persisted in higher levels (~1 log<sub>10</sub>) when compared with *Ec* and *Sal* (~0.5 log<sub>10</sub>), which is consistent with Rainaldi et al. (1991), who showed that *Lm* could survive on dried bresaola for 7 d. Attention should be drawn to the numerical increase in the concentration of *Sal* from day 23 to day 30. There was no statistical difference ( $P = 0.4049$ ) between day 23 and day 30 for mean populations of *Sal*, which is further support for the conclusion that this increase is not indicative of true “growth” of the pathogen.

The results of this validation study indicate that the parameters used to cure ( $\geq 3.5\%$  salt, 150 ppm NaNO<sub>2</sub>, and 100 ppm NaNO<sub>3</sub>) and dry (surface  $a_w \leq 0.85$ ) whole-muscle beef products are able to achieve a minimum 5-log reduction of *Ec*, *Sal*, and *Lm*. The combination of the antimicrobial intervention (2.5% [vol/vol] Beefside spray), curing ingredients (salt, NaNO<sub>3</sub>, and NaNO<sub>2</sub>) and refrigerated curing parameters (2°C–4°C for 28 d) as well as the drying schedule (12°C–14°C; 65%–75% relative humidity for 35 d minimum) allow for a consistent decrease in both the pathogen populations and  $a_w$ . The levels of pathogens used in this challenge study indicate a worst-case scenario for pathogens present on beef products that would not likely be present in such high concentrations in a sanitary processing environment using reputable source materials. Although survival of each pathogen type was evident, the combination of hurdles, both intrinsic and extrinsic factors, does not allow for cell recovery and growth on this product.

## Conclusions

Despite lack of scientific literature for the safety of artisanal and dry-cured meat products, consumer demand continues to increase for these types of products. Controlled curing with salt and NaNO<sub>3</sub> and

NaNO<sub>2</sub> is essential for product flavor, color, and safety. Drying, although primarily performed for quality reasons, is paramount to the safety of beef bresaola because a thermal lethality step is not typically utilized. Humidity control during drying will ensure consistent texture and mouthfeel that is expected by the consumer as well as provide critical and consistent reduction in  $a_w$  for both external and internal product surfaces. Meat processors may utilize the results of this research for Hazard Analysis Critical Control Point supporting documentation and scientific validation for the production of raw, RTE bresaola, provided that production procedures follow the previously stated minimum safety parameters for curing and drying as well as incorporate the antimicrobial treatment on raw beef subprimals and casings.

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