Meat and Muscle Biology™

Fate of *Listeria monocytogenes* and Shiga Toxin-Producing *Escherichia coli* on Bresaola Slices During Storage

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Abstract: The viability of multistrain cocktails of genetically marked strains of *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC) were separately monitored on slices of one brand of a commercially produced bresaola (ca. pH 6.7 and a_w 0.899) during extended storage at refrigeration and abusive temperatures. Two slices (ca. 8 g each; ca. 10.2 cm wide, ca. 11 cm long) of bresaola were layered horizontally within a nylon-polyethylene bag. The outer surface of each slice was inoculated (50 µL total; ca. 3.5 log colony-forming units [CFU]/package) with a rifampicin-resistant (100 µg/mL) cocktail of either *L. monocytogenes* (5 strains) or STEC (8 strains). Bags were vacuum-sealed and then stored at 4°C or 10°C for 180 or 90 d, respectively. In each of 5 trials, 3 bags were analyzed for pathogen presence at each sampling interval via the US Department of Agriculture–Agricultural Research Service package rinse method. In general, levels of *L. monocytogenes* and STEC decreased by 3.0 and 2.4 log CFU/package, respectively, after 180 d when bresaola was stored at 4°C. When bresaola was stored at 10°C for 90 d, levels of *L. monocytogenes* and STEC decreased by 2.4 and 3.1 log CFU/package, respectively. Thus, the sliced bresaola evaluated herein did not provide a favorable environment for either persistence or outgrowth of surface-inoculated cells of *L. monocytogenes* or STEC.

Key words: bresaola, dry-cured, Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*
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Introduction

Over the past decade, the art of preparing and assembling artisanal meat products (i.e., charcuterie) has become very popular, both within Europe where it originated and now in the United States as well, particularly among individuals seeking a more social, indulgent, and extraordinary eating experience (Denis, 2018; Nelson, 2018; Gerrard, 2021). Charcuterie typically includes various pork products such as salami, prosciutto, and soppressata, but of late it may also include other species of meat and other types of food like pâté, cheese, crackers, fruits, nuts, and dips to collectively deliver a wide array of distinctive flavors, tastes, and textures (Spiker, 2014; Gerrard, 2021). Bresaola (or brisaola) is one example of a specialty/niche meat that has experienced an increase in popularity in the US (Strange, 2013). This ready-to-eat (RTE), whole-muscle, salted, and aged (2 to 3 months) beef product originates from Northern Italy (Ruhlman and Polcyn, 2013; Volpi, 2022). Bresaola is commonly made with lean beef, typically the eye of round (di Cantoni, 2010). It has a very aromatic, yet delicate, flavor, which is attributable to the mixture of salt, sugar, curing salt, and dry-spice blend, with the latter containing a mixture of spices, such as black pepper, juniper berries, cinnamon, cloves, and bay leaves, applied to the outer surface of the raw meat (Braghieri et al., 2009; di Cantoni, 2010; Ruhlman and Polcyn, 2013;

Picone et al., 2019). The resulting product is then dry-cured under specific conditions of temperature and relative humidity for 60 to 120 d until an average weight loss of ca. 40% is achieved (Paleari et al., 2003; Volpi, 2022). The final product is washed and dried before being vacuum packaged as either whole or sliced for subsequent sale in the refrigerated meats section at retail (Paleari et al., 2003). Bresaola has been produced and consumed for centuries, both across Italy and worldwide, without posing a serious public health risk. Regardless, very little information has been published on the presence or fate of foodborne pathogens on bresaola, and such data are especially lacking for bresaola produced in the US.

Bresaola and related dry-cured red meat products such as cecina de León, Bündnerfleisch, and pastirma, lack a thermal lethality step during manufacture to enhance safety. As for other dry-cured, whole-muscle RTE meat products, the quality and safety attributes of bresaola rely solely on a decrease in water activity (a_w) during its salting/curing and drying/maturation steps (di Cantoni, 2010). The formulation and process for preparing bresaola and the physical-chemical composition of the resulting product (e.g., a neutral to slightly acidic pH [ca. pH 6.0 to pH 6.7] and somewhat high a_w [ca. a_w 0.90 to a_w 0.96]) (Frustoli et al., 2007; Koutsoumanis and Angelidis, 2007) would suggest that cells of bacterial pathogens such as *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC) may find such conditions favorable for their (out)growth or survival (Farber et al., 2007; Porto-Fett et al., 2010; Mataragas et al., 2015; Ducic et al., 2016; Balamurugan et al., 2017; Omer et al., 2018). Moreover, the association of STEC with raw beef, as well as the likelihood for surface contamination with *L. monocytogenes* during slicing, assembling, and/or packaging of bresaola, could also pose a public health concern. The psychrotrophic nature of *L. monocytogenes*, the extended shelf life (e.g., 90 to 180 d at 4°C) of bresaola, and its consumption without further cooking/processing may exacerbate concerns related to product safety. Thus, we monitored the viability of *L. monocytogenes* or STEC that were surface inoculated onto slices of a single brand of a commercial all-beef bresaola during extended storage at 4°C and 10°C to access the safety of this product.

Materials and Methods

Bacterial strains

Pre-sliced commercially produced bresaola was surface inoculated (see below) with either a multistrain cocktail of 8 rifampicin-resistant strains of STEC (100 µg rifampicin/mL; TCI America, Portland, OR) or a multistrain cocktail of 5 rifampicin-resistant strains of *L. monocytogenes* (100 µg rifampicin/mL) (Table 1). The multistrain cocktails of *L. monocytogenes* or STEC were prepared by combining approximately equal volumes of an overnight-grown, stationary-phase cell suspension of each isolate of either *L. monocytogenes* or STEC. Each cocktail was then diluted separately as needed in 0.1% peptone water (Difco, BD, Franklin Lakes, NJ) to achieve a target level of ca. 4.0 log colony-forming units (CFU)/mL. These strains of STEC and *L. monocytogenes* were confirmed, cultured, and/or maintained as described previously (Porto et al., 2002; Luchansky et al., 2008).

Inoculation of bresaola slices

Multiple packages (113 g each, ca. 14 slices per package) from different production lots of a single brand of sliced bresaola (ingredients from label: beef, sea salt, cultured Swiss chard, sugar, natural flavor, pepper, and spices) were purchased from a local supermarket. In addition to the ingredients, the only other information on the label was "To enjoy peak freshness, use within 3 to 4 d once opened." Two slices (ca. 8 g each; ca. 10.2 cm wide, ca. 11 cm long) of bresaola were aseptically transferred from the original package and layered horizontally into a nylon-polyethylene bag (3 mil standard barrier, 12.7 by 17.8 cm, O_2 transmission rate of 0.26 $cm^3/100\ in^2/24$ h at 90% relative humidity at 38°C with a moisture vapor transmission rate of 0.31 g of H_2O per 100 in^2 per 24 h at 22.8°C; PrimeSource, Kansas City, MO). The outer surface of each slice was either inoculated (50 µL total; ca. 3.5 log CFU/package) with the 5-strain cocktail of rifampicin-resistant cells of *L. monocytogenes* or the 8-strain cocktail of rifampicin-resistant cells of STEC. Bags were vacuum-sealed to 950 mBar with a Multivac A300/16 vacuum-packaging unit (Sepp Haggenmüller KG, Wolfertschwenden, Germany) and then stored at 4°C or 10°C for 180 or 90 d, respectively.

Microbiological analyses

The US Department of Agriculture–Agricultural Research Service (USDA-ARS) package rinse method (Luchansky et al., 2002) was used to recover cells of STEC or *L. monocytogenes* that were separately inoculated onto slices of bresaola. In brief, packages stored at 4°C were analyzed on days 0, 7, 14, 21, 28, 35, 42, 60, 90, 120, 150, and 180, whereas packages stored at 10°C

Table 1. Strains of *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC) used in this study.

Bacterial strain	Strain designation	Source	Serotypes
L. monocytogenes	MFS-2	Environmental isolate from a pork processing plant	1/2a
	H7776	Frankfurter isolate	4b
	ScottA	Clinical isolate	4b
	LM-101M	Beef and pork sausage isolate	4b
	F6854	Turkey frankfurter isolate	1/2a
STEC	H30	Isolate from an infant with diarrhea	O26:H11
	CDC 96-3285	Human stool isolate	O45:H2
	CDC 90-3128	Human stool isolate	O103:H2
	ATCC BAA-2326	Human stool isolate	O104:H4
	JB1-95	Clinical isolate	O111:H-
	CDC 97-3068	Human stool isolate	O121:H19
	83-75	Human stool isolate	O145:NM
	USDA-FSIS 011-82	Meat isolate	O157:H7

were analyzed on days 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90. The outer surface of each package was disinfected with a paper towel moistened with 70% ethanol before the package was opened with the aid of alcohol-sterilized scissors. Next, 25 mL of 0.1% sterile peptone water were added to each bag and the contents were manually massaged for ca. 2 min. Portions of the resulting rinsate, with and without prior dilution in 0.1% peptone water, were plated onto sorbitol-MacConkey (SMAC; Difco) or modified Oxford (MOX; Difco) agar plates containing rifampicin (100 µg/mL) to recover surviving cells of STEC or *L. monocytogenes*, respectively. Plates were incubated at 37°C for 24 h (SMAC) or 48 h (MOX) before colonies typical for each pathogen were enumerated and expressed as log CFU/package. When pathogen levels decreased to below the detection limit (≤1.35 log CFU/package) by direct plating, pathogen presence was determined via enrichment as previously described (Hinkens et al., 1996; Cook, 1999).

For enumeration of the aerobic plate count (APC) and lactic acid bacteria (LAB) levels on slices of non-inoculated bresaola, portions of the resulting rinsate, diluted in 0.1% peptone water, were spread plated onto Brain Heart Infusion (BHI; Difco) and de Man, Rogosa, and Sharpe (MRS; Difco) agar plates, respectively. The BHI plates were incubated at 37°C for 24 h, whereas the MRS plates were incubated anaerobically within an anaerobic chamber (10.24% carbon dioxide, 5.11% hydrogen, and balance nitrogen; Whitley DG250; Don Whitley Scientific, West Yorkshire, UK) at 37°C for 48 h. The APC and LAB levels, expressed as log CFU/package, were enumerated on days 0, 90, and 180 or on days 0, 45, and 90 of storage at 4°C or 10°C, respectively.

Chemical analyses

Proximate chemical analyses were conducted on a single ca. 225-g composite representative sample of the single brand of bresaola (prior to inoculation) from each of 4 of the 5 trials/batches that was subsequently inoculated with a target pathogen (Table 2). For comparison purposes, proximate chemical analyses were also conducted on a single ca. 225-g composite representative sample of noninoculated bresaola from one additional commercial brand of bresaola (ingredients from label: beef, sea salt, cultured celery powder, sugar, natural flavor, pepper, spices) (Table 2). Analyses were conducted by a commercial testing laboratory using

Table 2. Proximate composition of two brands of pre-sliced, commercially-produced bresaola.

Analyses	Bresaola[a](Brand A)	Bresaola[b](Brand B)
Ash (%)	6.88 ± 1.01	6.23
Carbohydrates (%)	0.41 ± 0.55	<0.1
Fat (%)	2.16 ± 0.43	1.36
Moisture (%)	53.71 ± 3.29	61.4
Protein (%)	37.61 ± 1.73	32.37
Salt (%)	5.13 ± 0.68	4.64
Nitrite (ppm)	<5.0 ± 0.0	<5.0
Acidity (%; as lactic acid)	0.94 ± 0.53	0.94
pH	6.65 ± 0.48	6.56
Water activity (a_w)	0.899 ± 0.027	0.929

[a]Data are the results from analyses of a single ca. 225-gram composite representative sample of non-inoculated bresaola from four of the five trials/batches purchased at retail (Brand A) (N=4, n=1).

[b]Data are the results from analyses of a single ca. 225-gram composite representative sample of non-inoculated bresaola from one additional commercial brand of bresaola (Brand B) (N=1, n=1).

methods described and approved by the Association of Official Analytical Chemists (AOAC, 2012).

Statistical analyses

In each of 5 trials, 3 samples of sliced bresaola (i.e., 2 slices of bresaola per sample) that were purchased at retail and subsequently inoculated with *L. monocytogenes* or STEC were analyzed for viable cells of each pathogen cocktail at various sampling times during refrigerated storage. For each storage temperature (4°C and 10°C), log CFU values were analyzed using a Pathogen x Storage Days two-way analysis of variance (ANOVA). Heterogeneity of log CFU variability among storage days and nonzero correlation among log CFU values observed at different storage days were incorporated into the ANOVA models by specifying a heterogeneous compound symmetric covariance structure in the SAS PROC MIXED REPEATED statement (SAS v9.4, SAS/STAT v15.2, PROC MIXED, 2021; SAS Institute, Inc., Cary, NC). Pairwise comparisons among storage days means were obtained by specifying SLICE=pathogen in the LSMEANS Pathogen*Days statement, with the option ADJUST=SIDAK to ensure experiment-wise $\alpha = 0.05$ and using the PDMIX800 macro (Saxton, 1998). Comparison of log CFU for *L. monocytogenes* versus STEC at each storage day was accomplished by specifying SLICE=day in the LSMEANS Pathogen*Days statement.

Results and Discussion

In addition to bresaola from Italy, examples of other whole-muscle, dry-cured beef products include cecina de León from Northwest Spain, charqui from South America, carne-de-sol from Brazil, Bündnerfleisch from Switzerland, biltong from South Africa, fenalår from Norway, and pastirma (also known as pasturma or basturma) from Armenia and Turkey (García et al., 1995; Ingham et al., 2006; Ishihara and Madruga, 2013; Jones et al., 2017). Bresaola is frequently consumed as part of antipasto or artisanal meat trays and as a topping on salads and specialty breads. Among the ca. 200 types of cooked, salted, and/or smoked meats available at retail, bresaola is the only delicatessen-type beef product from Italy that carries a Protected Geographical Indication certification and, as such, is highly saleable for various cultures and religions (ItalianFOOD, 2019; European Commission, 2021). Both the popularity and production of bresaola have increased gradually and quantifiably over the past 2 decades across Italy and worldwide: in 2020 almost 13,000 tons were manufactured by the 16 certified Italian producers, with attendant sales of about $450M euros (ItalianFOOD, 2021). In contrast to the high demand for bresaola, there is a general lack of information in the scientific literature about the safety of this product. Whereas the recipe and process for manufacturing bresaola may be sufficient to appreciably lower the risk of illness from foodborne pathogens contributed by the raw materials/ingredients, the likelihood for postprocess (cross) contamination, and especially during slicing or assembly of charcuterie trays, as well as its extended storage period, may allow for pathogen presence and, in turn, persistence or proliferation.

For the purpose of this study, a single brand of beef bresaola purchased at one location of a single food retailer was analyzed for microbial load and chemical composition and then subsequently inoculated with STEC or *L. monocytogenes*. Proximate chemical analyses (Table 2) established that the product tested herein (i.e., Brand A) was somewhat lean (ca. 2.2% fat), with an intermediate a_w (ca. a_w 0.90) and moisture level (ca. 53.2%), a relatively higher salt content (5.1%), and a somewhat neutral pH (ca. pH 6.7). As delineated in Table 2, we evaluated one additional brand of commercial bresaola: the chemical composition of this second brand of bresaola (i.e., Brand B) was similar in composition to the brand herein that was inoculated with the target pathogens. By comparison, Frustoli et al. (2007) reported that 23 samples of commercial bresaola displayed an average pH, a_w, moisture, and salt level of ca. pH 6.0, a_w 0.93, 56.7%, and 3.9%, respectively.

Microbiological analyses of the sliced bresaola purchased for the present study revealed high initial levels (ca. 8.8 log CFU/package) of APC and LAB (data not shown). After storage at 4°C (180 d) or 10°C (90 d), levels of APC increased to ca. 9.8 or 9.2 log CFU/package, respectively, whereas levels of LAB remained relatively unchanged (i.e., ca. 9.0 log CFU/package) (data not shown). Similar results were observed in other studies wherein initial levels of indigenous LAB and APC associated with bresaola ranged from ca. 7.0 to 8.1 log CFU/g, whereas after 90 d of storage at temperatures ranging from 4°C to 21°C, levels of indigenous LAB and APC remained relatively unchanged or increased by only 0.1 to 0.9 log CFU/g (Frustoli et al., 2007; Dalzini et al., 2014). Note: for each of 3 of the 5 trials conducted, 3 samples of bresaola (i.e., 3 slices of bresaola per sample) purchased at retail were analyzed for naturally

occurring cells of *L. monocytogenes* or STEC by direct plating (detection limit of ≤1.35 log CFU/sample) or by enrichment: all samples tested negative (data not shown).

L. monocytogenes and STEC can persist in dried cured meat products at ≥a_w 0.92 (i.e., the water activity level optimum for their growth) during extended storage due to the elaboration of cellular responses allowing for their survival at relatively low a_w levels (Beuchat et al., 2013; Ly et al., 2019). Our results demonstrated that slices of bresaola (a_w 0.899; Table 2) did not provide a favorable environment for outgrowth of surface-inoculated cells of *L. monocytogenes* or STEC during extended storage at refrigeration or slightly abusive storage temperatures (Figures 1 and 2). The longer the time of storage at 4°C or 10°C, the greater ($P < 0.05$) the inactivation of *L. monocytogenes* or STEC on sliced bresaola. In addition, no significant ($P > 0.05$) differences between inactivation of *L. monocytogenes* and STEC were observed after 180 d of storage at 4°C on bresaola, whereas less inactivation ($P < 0.05$) of *L. monocytogenes* cells was observed compared to STEC cells after 90 d of storage at 10°C. More specifically, when bresaola was stored for 180 d at 4°C, *L. monocytogenes* numbers decreased by ≥3.0 log CFU/package (Figure 1), whereas when stored at 10°C for 90 d, pathogen numbers decreased by ca. 2.4 log CFU/package (Figure 2). Likewise, STEC numbers decreased by ca. 2.4 or ≥3.1 log CFU/package when stored at 4°C or 10°C for 180 or 90 d, respectively (Figures 1 and 2). These findings may be attributed to the collective effects of a lower water activity (ca. a_w 0.899) acting in concert with the intrinsic antimicrobial properties of the curing salts and spices used in the formulation and the somewhat elevated levels (and types) of indigenous LAB and APC and associated secondary metabolites.

Various studies estimated the prevalence of STEC in raw beef at 1.1% to 16.8% at levels ranging from <0.52 to 4.03 log CFU/g (Doyle and Schoeni, 1987; Samadpour et al., 2002; Cagney et al., 2004; Carney et al., 2006). Similarly, the prevalence of *L. monocytogenes* in raw beef has been estimated at 0.8% to 28% at levels ranging from <0.3 to 10 to 100 MPN/g (Skovgaard and Morgen, 1988; Inoue et al., 2000; Persavento et al., 2009; Jang et al., 2021). From a food safety perspective, it is significant that research on bresaola and related whole-muscle dried beef products such as cecina de León, pastirma, and charqui have established that drying and curing alone are sufficient to deliver a ca. 2.5 to 4.0-log reduction in levels of STEC, *Salmonella* spp., and *L. monocytogenes* (Ingham et al., 2006; Burnham et al., 2008; Menéndez et al., 2015; Watson et al., 2021). Germane to the focus of the present study, Frustoli et al. (2007) reported that (inoculated) commercial (sliced) beef bresaola (ca. pH 5.6 and a_w 0.94) stored for 90 d at 4°C, 8°C, 15°C, or 21°C did not support outgrowth of *L. monocytogenes*: pathogen numbers were reduced from ca. 1.4 log CFU/g to ca. 0.3 log CFU/g in bresaola stored at 4°C and to below detection (≤0.18 log CFU/g) in bresaola stored at 8°C, 15°C, or 21°C. Likewise, Miraglia et al.

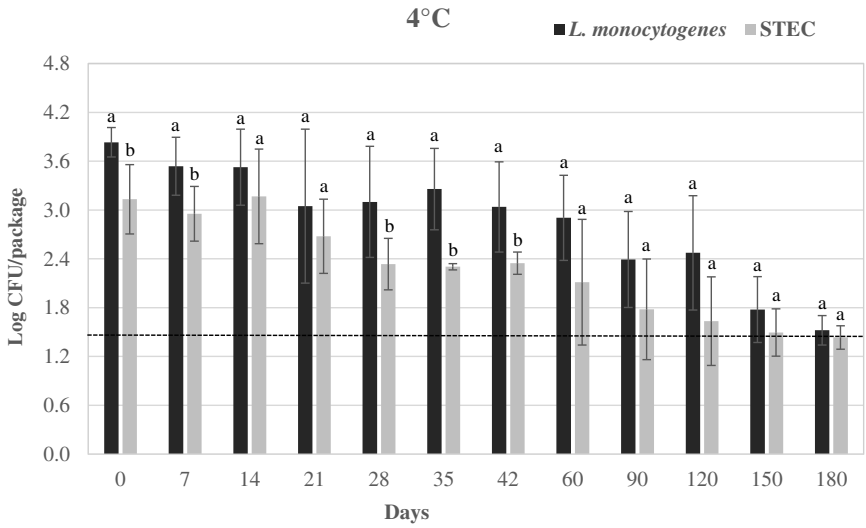

Figure 1. Recovery of Shiga toxin-producing *Escherichia coli* (STEC) and *L. monocytogenes* (log colony-forming units [CFU]/package) inoculated onto slices of bresaola during extended storage at 4°C. For a given storage day, means for surviving levels of *Listeria monocytogenes* and STEC with different lowercase letters are significantly ($P \leq 0.05$) different. Error bars represent the standard deviation of the mean ($N = 5$ trials, $n = 3$ samples per trial). Dotted line denotes the detection limit (≤1.35 log CFU/package) by direct plating.

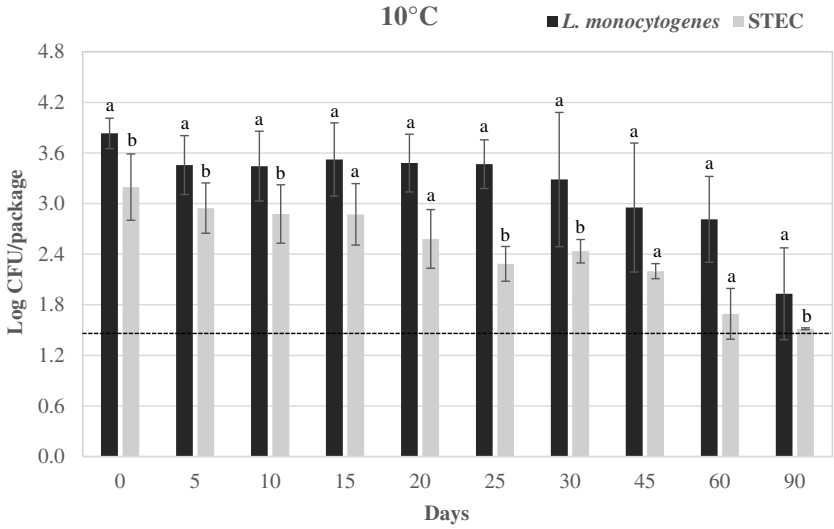

Figure 2. Recovery of Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes* (log colony-forming units [CFU]/package) inoculated onto slices of bresaola during extended storage at 10°C. For a given storage day, means for surviving levels of *L. monocytogenes* and STEC with different lowercase letters are significantly ($P \leq 0.05$) different. Error bars represent the standard deviation of the mean ($N = 5$ trials, $n = 3$ samples per trial). Dotted line denotes the detection limit (≤1.35 log CFU/package) by direct plating.

(2009) reported that pieces or slices of "bresaola della Valtellina IGP" stored at 5°C, 10°C, 15°C, or 20°C for up to 127 d did not support (out)growth of *L. monocytogenes*. Also, Dalzini et al. (2014) reported that slices of bresaola (ca. pH 5.6 and a_w 0.92) prepared from cured turkey breast and stored for 7 d at 5°C and then for 83 d at 8°C resulted in reductions of ca. 0.7 to ≥1.2 log CFU/g in levels of *L. monocytogenes*. As another example, using whole-muscle bresaola produced in the laboratory for research purposes, Watson et al. (2021) monitored the fate of surface-inoculated cells of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* and reported that a >5.0 log CFU/cm^2 reduction in levels of all 3 pathogens was achieved during curing and drying. Lastly, Finazzi et al. (2006) evaluated the behavior of *Salmonella* and *E. coli* O157:H7 on the surface of bresaola during aging for 35 d and reported reductions of ca. 5.0 and 4.0 log CFU/cm^2, respectively. Our results compare favorably with the abovementioned studies, validating that proper storage of dry-cured meats such as bresaola does not support viability or outgrowth of cells of STEC or *L. monocytogenes* that may be present on the surface of such products as a result of postprocess contamination in general or from slicing or improper/extensive handling specifically (Ingham et al., 2004; Finazzi et al., 2006; Frustoli et al., 2007; Miraglia et al., 2009; Dalzini et al., 2014; Watson et al., 2021).

The fact that humans have been preserving meats such as bresaola by drying/curing and salting for hundreds of years, coupled with the absence of recalls and illnesses attributed to bresaola in the US, serves as a testament to its overall wholesomeness. Excluding the present study, few if any data have been published to quantify the fate of pathogens on commercially prepared slices of bresaola during extended storage. The primary focus of the present study was to quantify the fate of *L. monocytogenes* and STEC on the outer surfaces of bresaola slices to simulate what would occur for contaminated slices in direct contact with the packaging material. It would be of keen interest for a future study to determine if viability of the abovementioned target pathogens would differ appreciably within the microenvironment created between slices of bresaola that are stacked within vacuum-sealed packages of retail bresaola compared with otherwise similar slices entirely in direct contact with the packing material. Regardless, it remains possible for process deviations or postprocess contamination of bresaola to occur which, in turn, may allow for harmful bacteria to remain in contact with the finished product. Even if that were to occur, our data establish that bresaola would not support outgrowth or persistence of STEC or *L. monocytogenes*.

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Literature Cited

AOAC. 2012. Official methods of analysis of AOAC International. 19th ed. Rockville, MD.

Balamurugan, S., R. Ahmed, A. Gao, and P. Strange. 2017. Comparison of the fate of the top six non-O157 Shiga-toxin producing *Escherichia coli* (STEC) and *E. coli* O157:H7 during the manufacture of dry fermented sausages. Int. J. Food Microbiol. 259:14–21. https://doi.org/10.1016/j.ijfoodmicro.2017.07.018.

Beuchat, L. R., E. Komitopoulou, H. Beckers, R. P. Betts, F. Bourdichon, S. Fanning, H. M. Joosten, and B. H. Ter Kuile. 2013. Low-water activity foods: Increased concern as vehicles of foodborne pathogens. J. Food Protect. 76:150–172. https://doi.org/10.4315/0362-028X.JFP-12-211.

Braghieri, A., A. Girolami, A. Carlucci, N. Piazzolla, A. M. Riviezzi, and F. Napolitano. 2009. Sensory properties affecting acceptability of "bresaola" from Podolian young bulls. J. Sens. Stud. 24(5):677–697. https://doi.org/10.1111/j.1745-459X.2009.00233.x.

Burnham, G. M., D. J. Hanson, C. M. Koshick, and S. C. Ingham. 2008. Death of *Salmonella* serovars, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes* during the drying of meat: A case studying using biltong and droëwors. J. Food Safety 28(2):198–209. https://doi.org/10.1111/j.1745-4565.2008.00114.x.

Cagney, C., H. Crowley, G. Duffy, J. J. Sheridan, S. O'Brien, E. Carney, W. Anderson, D. A. McDowell, I. S. Blair, and R. H. Bishop. 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. Food Microbiol. 21(2):203–212. https://doi.org/10.1016/S0740-0020(03)00052-2.

Carney, E., S. B. O'Brien, J. J. Sheridan, D. A. McDowell, I. S. Blair, and G. Duffy. 2006. Prevalence and level of *Escherichia coli* O157 on beef trimmings, carcasses and boned head meat at a beef slaughter plant. Food Microbiol. 23(1):52–59. https://doi.org/10.1016/j.fm.2004.12.001.

Cook, L. V. 1999. Isolation and identification of *Listeria monocytogenes* from red meat, poultry, egg, and environmental samples. In: USDA/FSIS Microbiology Laboratory Guidebook. 3rd ed., Revision 2. USDA, Washington, DC.

Dalzini, E., E. Cosciani-Cunico, S. D'Amico, C. Sfameni, B. Bertasi, M. N. Losio, A. Serraino, and P. Daminelli. 2014. Growth potential of *Listeria monocytogenes* in sliced turkey bresaola packed in modified atmosphere. Ital. J. of Food Safety 3:2231. https://doi.org/10.4081/ijfs.2014.2231.

Denis, N. P. 2018. Category spotlight: Contemporary charcuterie: A cut above. https://www.specialtyfood.com/news/article/category-spotlight-contemporary-charcuterie-cut-above/. (Accessed 14 December 2021).

di Cantoni, C. 2010. Le bresaole, origini e caratteristiche. http://www.pubblicitaitalia.com/eurocarni/2010/7/9882.html. (Accessed 3 January 2022).

Doyle, M. P., and J. L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microb. 53(10):2394–2396. https://doi.org/10.1128/aem.53.10.2394-2396.1987.

Ducic, M., N. Klisara, S. Markov, B. Blagojevic, A. Vidkovic, and S. Buncic. 2016. The fate and pasteurization-based inactivation of *Escherichia coli* O157, *Salmonella* Typhimurium and *Listeria monocytogenes* in dry, fermented sausages. Food Control 59:400–406.

European Commission. 2021. Bresaola della Valtellina. https://ec.europa.eu/info/food-farming-fisheries/food-safety-and-quality/certification/quality-labels/geographical-indications-register/details/EUGI00000013402. (Accessed 19 January 2022).

Farber, J. M., F. Pagotto, and C. Scherf. 2007. Incidence and behavior of *Listeria monocytogenes* in meat products. In: E. T. Ryser and E. H. Marth, editors, Listeria, listeriosis, and food safety. 3rd ed. CRC Press, Boca Raton, FL. p. 503–570.

Finazzi, G., P. Daminelli, P. Monastero, A. Aglietta, and P. Boni. 2006. Dynamicof the survival of *Salmonella typhimurium* and *Escherichia coli* O157:H7, in bresaola of Valtellina IGP artificially contaminated. Presented at: 2nd Med-Vet-Net Scientific Meeting on Zoonoses Research in Europe, Malta. May 3–6.

Frustoli, M. A., M. Cigarini, A. Garritani, S. Garulli, N. Bovis, C. Schivazappa, and S. Barbuti. 2007. Andamento di *Listeria monocytogenes* durante la shelf-life di bresaola preaffettata e confezionata in atmosfera protettiva (Fate of *Listeria monocytogenes* during shelf-life of pre-sliced bresaola packaged under modified atmosphere). Ind. Conserve 4:325–331.

García, I., J. M. Zumalacárregui, and V. Díez. 1995. Microbial succession and identification of *Micrococcaceae* in dried beef cecina, an intermediate moisture meat product. Food Microbiol. 12:309–315. https://doi.org/10.1016/S0740-0020(95)80111-1.

Gerrard, M. 2021. What is charcuterie? https://1000oaksbarrel.com/blog/what-is-charcuterie/. (Accessed 26 December 2021).

Hinkens, J. C., N. G. Faith, T. D. Lorang, P. Bailey, D. Buege, C. W. Kaspar, and J. B. Luchansky. 1996. Validation of pepperoni processes for control of *Escherichia coli* O157:H7. J. Food Protect. 59(12):1260–1266. https://doi.org/10.4315/0362-028x-59.12.1260.

Ingham, S. C., D. R. Buege, B. K. Dropp, and J. A. Losinski. 2004. Survival of *Listeria monocytogenes* during storage of ready-to-eat meat products processed by drying, fermentation, and/or smoking. J. Food Protect. 67(12):2698–2702. https://doi.org/10.4315/0362-028x-67.12.2698.

Ingham, S. C., G. Searls, and D. R. Buege. 2006. Inhibition of *Salmonella* serovars, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during dry-curing and drying of meat: A case study with basturma. J. Food Safety 26:160–172.

Inoue, S., A. Nakama, Y. Arai, Y. Kokubo, T. Maruyama, A. Saito, T. Yoshida, M. Terao, S. Yamamoto, and S. Kumagai. 2000.

Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan. Int. J. Food Microbiol. 59(1–2):73–77. https://doi.org/10.1016/s0168-1605(00)00284-1.

Ishihara, Y. M., and M. S. Madruga. 2013. Indicadores de maciez em carnes salgadas e dessecadas: Uma revisão (Tenderness-indicators in salted and dried meat: A review). Ciências Agrárias 34(6):3721–3737. https://doi.org/10.5433/1679-0359.2013v34n6Supl2p3721.

ItalianFOOD. 2019. Speck and bresaola: Plenty of exports. https://news.italianfood.net/2019/01/14/speck-bresaola-plenty-exports/. (Accessed 14 December 2021).

ItalianFOOD. 2021. Strategies Bresaola della Valtellina PGI Consortium are using to counteract the crisis. https://news.italianfood.net/2021/04/20/how-bresaola-della-valtellina-pgi-consortium-is-counteracting-the-crisis/. (Accessed 28 December 2021).

Jang, Y.-S., J.-S. Moon, H. J. Kang, D. Bae, and K.-H. Seo. 2021. Prevalence, characterization, and antimicrobial susceptibility of *Listeria monocytogenes* from raw beef and slaughterhouse environments in Korea. Foodborne Pathog. Dis. 18(6): 419–425. https://doi.org/10.1089/fpd.2020.2903.

Jones, M., E. Arnaud, P. Gouws, and L. C. Hoffman. 2017. Processing of South African biltong – A review. S. Afr. J. Anim. Sci. 47(6):743–757. https://doi.org/10.4314/sajas.v47i6.2.

Koutsoumanis, K., and A. S. Angelidis, 2007. Probabilistic modeling approach for evaluating the compliance of ready-to-eat foods with new European Union safety criteria for *Listeria monocytogenes*. Appl. Environ. Microbiol. 73(15):4496–5004. https://doi.org/10.1128/AEM.00245-07.

Luchansky, J. B., A. C. S. Porto, F. M. Wallace, and J. E. Call, 2002. Recovery of *Listeria monocytogenes* from vacuum-sealed packages of frankfurters: Comparison of the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service product composite enrichment method, the USDA Agricultural Research Service (ARS) product composite rinse method, and the USDA-ARS package rinse method. J. Food Protect. 65(3):567–570. https://doi.org/10.4315/0362-028x-65.3.567.

Luchansky, J. B., R. K. Phebus, H. Thippareddi, and J. E. Call. 2008. Translocation of surface inoculated *Escherichia coli* O157:H7 into beef subprimals following blade tenderization. J. Food Protect. 71(11):2190–2197.

Ly, V., V. R. Parreira, and J. M. Farber. 2019. Current understanding and perspectives on *Listeria monocytogenes* in low-moisture foods. Curr. Opin. Food Sci.. 26:18–24. https://doi.org/10.1016/j.cofs.2019.02.012.

Mataragas, M., A. Bellio, F. Rovetto, S. Astegiano, C. Greci, C. Hertel, L. Decatelli, and L. Cocolin. 2015. Quantification of persistence of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* during manufacture of Italian fermented sausages. Food Control 47:552–559. https://doi.org/10.1016/j.foodcont.2014.07.058.

Menéndez, R. A., E. Rendueles, J. J. Sanz, R. Capita, and C. García-Fernández. 2015. Behavior of *Listeria monocytogenes* in sliced ready-to-eat meat products packaged under vacuum or modified atmosphere conditions. J. Food Protect. 78(10): 1891–1895. https://doi.org/10.4315/0362-028x.jfp-15-103.

Miraglia, V., G. Finazzi, P. Daminelli, E. Bonometti, M. Gregorelli, and P. Boni. 2009. Behaviour of *Listeria monocytogenes* in chunked or sliced seasoned Bresaola della Valtellina IGP. Ind. Aliment.-Italy 48:58–64.

Nelson, A. 2018. Consumers continue to crave craft meats. https://www.foodbusinessnews.net/articles/12821-consumers-continue-to-crave-craft-meats. (Accessed 3 January 2022).

Omer, M. K., A. Á. Ordonez, M. Prieto, E. Skjerve, T. Asehun, and O. A. Alvseike. 2018. A systematic review of bacterial food-borne outbreaks related to red meat and meat products. Foodborne Pathog. Dis. 15(10):598–610. https://doi.org/10.1089/fpd.2017.2393.

Paleari, M. A., V. M. Moretti, G. Beretta, T. Mentasti, and C. Bersani. 2003. Cured products from different animal species. Meat Sci. 63(4):485–489. https://doi.org/10.1016/s0309-1740(02)00108-0.

Persavento, G., B. Ducci, D. Nieri, N. Comodo, and A. Lo Nostro. 2009. Prevalence and antibiotic susceptibility of *Listeria* spp. isolated from raw meat and retail foods. Food Control 21(5):708–713.

Picone, G., I. De Noni, P. Ferranti, M. A. Nicolai, C. Alamprese, A. Trimigno, A. Brodkorb, R. Portmann, A. Pihlanto, S. N. El, and F. Capozzi. 2019. Monitoring molecular composition and digestibility of ripened bresaola through a combined foodomics approach. Food Res. Int. 115:360–368. https://doi.org/10.1016/j.foodres.2018.11.021.

Porto, A. C. S., B. D. G. M. Franco, E. S. Sant'Anna, J. E. Call, A. Piva, and J. B. Luchansky. 2002. Viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, commercially-prepared with and without 2.0 or 3.0% added potassium lactate, during extended storage at 4 and 10° C. J. Food Protect. 65(2):308–315. https://doi.org/10.4315/0362-028x-65.2.308.

Porto-Fett, A. C. S., J. E. Call, B. E. Shoyer, D. E. Hill, C. Pshebniski, G. J. Cocoma, and J. B. Luchansky. 2010. Evaluation of fermentation, drying, and/or high pressure processing on viability of *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella* spp., and *Trichinella spiralis* in raw pork and Genoa salami. Int. J. Food Microbiol. 140:61–75. https://doi.org/10.1016/j.ijfoodmicro.2010.02.008.

Ruhlman, M., and B. Polcyn. 2013. Pâté and terrines: The Cinderella meat loaf. In: Charcuterie: The craft of salting, smoking, and curing. W. W. Norton and Company, Inc., New York. p. 201–251.

Samadpour, M., M. Kubler, F. C. Buck, G. A. Depavia, E. Mazengia, J. Stewart, P. Yang, and D. Alfi. 2002. Prevalence of Shiga toxin-producing *Escherichia coli* in ground beef and cattle feces from King County, Washington. J. Food Protect. 65 (8):1322–1325. https://doi.org/10.4315/0362-028x-65.8.1322.

Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in PROC MIXED. Proc. SAS Users Group Intl., SAS Inst. 23:1243–1246.

Skovgaard, N., and C.-A. Morgen. 1988. Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. Int. J. Food Microbiol. 6(3):229–242. https://doi.org/10.1016/0168-1605(88)90015-3.

Spiker, L. 2014. What the heck is charcuterie? And why you need it at your next party. https://www.theorganickitchen.org/what-

the-heck-is-charcuterie-and-why-you-need-it-at-your-next-party/. (Accessed 16 December 2021).

Strange, C. 2013. Food trend alert: Charcuterie. https://www.wholefoodsmarket.com/blog/food-trend-alert-charcuterie. (Accessed 2 January 2022).

Volpi. 2022. What is bresaola?: The ultimate guide to cured beef. https://www.volpifoods.com/blog/what-is-bresaola-the-ultimate-guide-to-cured-beef/. (Accessed 8 January 2022).

Watson, S. C., N. J. Gaydos, S. R. Egolf, and J. A. Campbell. 2021. Fate of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* during curing and drying of beef bresaola. Meat Muscle Biol. 5:14, 1–8. https://doi.org/10.22175/mmb.11621.