Growth potential of *Listeria monocytogenes* in sliced turkey *bresaola* packed in modified atmosphere

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**Abstract**

According to EC Regulation No 2073/2005, for food business operators that produce ready-to-eat (RTE) product, it is crucial to be able to demonstrate if the product supports the growth of *Listeria monocytogenes*. The objective of the study was therefore to evaluate the behaviour of *L. monocytogenes* in sliced RTE turkey *bresaola* (made by cured turkey breast 4.5% NaCl, 1% sodium lactate, sodium nitrite 150 ppm and flavouring) during the shelf life of the product, simulating a contamination during the slicing operation. Considering a shelf life of 90 days (as defined by manufacturer) the packages of *bresaola* were stored at 5°C for 7 days and at 8°C for the remaining storage time (83 days). *L. monocytogenes* count decreased during storage test from 1.43/1.98 log cfu/g in the three batches tested to 1.03 log cfu/g in one batch and to undetectable levels in the other two batches. The results show that the Investigated product is unable to support the growth of *L. monocytogenes*.

**Materials and Methods**

**Bresaola samples**

Three batches of turkey *bresaola* (turkey breast, 4.5% NaCl, 1% sodium lactate, sodium nitrite 150 ppm and flavouring), sliced and packaged in a modified atmosphere (30% CO2 and 70% N2), were obtained from a local manufacturer. Twenty one packs of each batch for a total of 63 packs (100 g each) of turkey *bresaola*, were used in the study. For each batch 3 packs were used to verify the absence of natural contamination by *L. monocytogenes*; 3 packs were analysed at the beginning of the shelf life (day 0) and 3 packs at the end of the shelf life (90 days after storage at 5°C for 7 days and at 8°C for the remaining 83 days) for the mesophilic lactic acid bacteria (LAB) enumeration and for the measurement of pH; and water activity (a_w). The other 12 packs were used for the surface inoculation test.

**Bacterial cultures and inoculum preparation**

Three strains of *L. monocytogenes*, classified by EcoRI ribotyping, were used in this study: ATCC® 19115™ (DUP 1042) and *L. monocytogenes* field strains Lm171718 and Lm171767 (both DUP 1038) isolated from two different swine sausages. Stock cultures were kept frozen (-80°C) in Brain Heart Infusion (BHI; Oxoid, Basingstoke, UK) broth supplemented with 20% glycerol until use; strains were inoculated into BHI and incubated at 37°C for 24 h and than inoculated in BHI broth and incubated at 8°C to reach the beginning of the stationary phase (Uyttendaele et al., 2004). The three strains cultures, each at the same approximate concentration (ca. 7 log cfu g^-1) were mixed; counts of each culture were confirmed by serial decimal dilution and plating in Agar Listeria Ottaviani Agosti (ALOA; Microbiol Diagnostici, Cagliari, Italy). The multi-strain cocktail was centrifuged for 60 min at 4°C, 4000 g and the pellet re-suspended in sterile physiological solution and appropriately diluted.

**Surface inoculation**

For each batch, 12 packs of *bresaola* were used for experimental inoculation: packs were aseptically opened and the slices were inoculated on the top surface with 1% w/v of the multi-strain cocktail of *L. monocytogenes* to a...
final concentration of about 1.5-2 log cfu/g. The inoculum was distributed over the entire surface with a sterile L-shaped plastic cell spreader (Incofar, Modena, Italy) and then the slices were re-packed into sterile polyethylene bags in modified atmosphere (30% CO₂ and 70% N₂) using S100-Tecnovac equipment [Tecnovac, Grassobbio (BG), Italy]. Inoculated packs were stored at 5°C for 7 days and at 8°C for the remaining 83 days. *Listeria monocytogenes* enumeration was carried out at 0, 40, 60 and 90 days of storage on three replicates samples for each batch.

### Microbiological and physico-chemical analysis

The slices were transferred into plastic one-chamber filter stomacher bags (NEOMED, London, UK) and homogenised 1:3 w:v in sterile peptone water (PW) (CONDA, Madrid, London, UK) and homogenised 1:3 w:v in sterile peptone water (PW) (CONDA, Madrid, London, UK) and homogenised 1:3 w:v in sterile peptone water (PW) (CONDA, Madrid, London, UK) in accordance to ISO/FDIS 1998. The mesophilic lactic acid bacteria (LAB) count was carried out by pour plating 1 mL of appropriate dilution in MRS agar (MRS; Microbiol Diagnostic) incubated at 30°C for 72 h. The pH values were determined using a HI 223 Calibration checkTM Microprocessor pH meter (Hanna Instrument, USA) equipped with a Gel-Glass electrode (Hamilton, Switzerland). Water activity (aw) was measured at 25°C with the aw recorder AquaLab, series 3, Model TE (Decagon Devices, Inc., Pullman, WA, USA) in accordance to ISO/FDIS 21807 (ISO/FDIS, 2004).

### Statistical analysis

Microbiological counting results were expressed as colony forming unit (cfu) per gram. Microbial counts were reported as log cfu/g. The average and standard deviations of microbial counts and physico-chemical values were determined from the average of three samples at each sampling time for each batch. The growth potential (δ) of *Listeria monocytogenes* was calculated as difference between the median concentration at the end of shelf life (day 90) and the median concentration at the beginning of the shelf life (day 0), in three replicates, for three batches. The maximum growth potential (δmax) was calculated as maximal difference between day 90 and day 0 among the 3 batches (EUCRL, 2008).

### Results

Examination of not inoculated samples at the beginning of shelf life revealed the absence of natural *Listeria monocytogenes* contamination in sliced turkey *bresaola*. Average of initial values (N=3 batch; n=3 replicates) of pH and aw were 5.55±0.05 (range of 5.43-5.63) and 0.923±0.010 (range of 0.911-0.939) respectively; at the end of the shelf life, average values of pH and aw were 5.45±0.11 (range of 5.32-5.67), and 0.925±0.008 (range of 0.912-0.939) respectively. The values of pH, mesophilic lactic acid bacteria (LAB) count at days 0 and 90 are reported in Table 1. No significant differences (P>0.05) were observed between LAB log counts at the beginning and at the end of the shelf life; the physico-chemical properties showed moderate but significant changes (P<0.05) during the storage under the defined conditions.

Starting from values of 1.50-1.81 log cfu/g in the three batches at the day of inoculation, *Listeria monocytogenes* count decreased to 1.03±0.07 log cfu/g in batch 1, and below the level of detection (<0.47 log cfu/g) in batches 2 and 3 at the end of the shelf life (90 days). The growth potential (δ) of *Listeria monocytogenes* ranged from -1.32 log cfu/g in batch 2 to -0.58 log cfu/g in batch 1; the maximum growth potential (δmax) of *Listeria monocytogenes* on sliced turkey *bresaola* was -0.58 log cfu/g. The average values of *Listeria monocytogenes* log counts in three contaminated batches are shown in Table 1.

### Discussion and Conclusions

The growth potential of *Listeria monocytogenes* in foods depends from many factors, the most important being the strain(s), injury or stress applied to the strain(s), intrinsic properties of the food (e.g. pH, NaCl content, aw, food composition, associated microflora, antimicrobial constituents) and extrinsic properties (e.g. temperature profile, gas atmosphere) (EUCRL, 2008). In agreement with EC Regulation No. 2073/2005, products with pH 4.4 or aw 0.92, products with pH <5.0 and aw <0.94 and products with a shelf life of less than five days belong to the category of RTE foods that not support the growth of *Listeria monocytogenes*. The regulation also states that other categories of products can also belong at this category, subject to scientific justification. In the present study, the physico-chemical properties (pH and aw) of the sliced turkey *bresaola* showed a

### Table 1. Evolution of *Listeria monocytogenes*, lactic acid bacteria (log cfu/g) and pH on three batches of turkey *bresaola* during the shelf life (average±standard deviation of three samples for each contaminated batch).

|          | Batch 1 |          | Batch 2 |          | Batch 3 |
|----------|---------|----------|---------|----------|---------|
|          | 0       | 40       | 60      | 90       |         |
| *L. monocytogenes* (log cfu/g) | | | | | |
| LAB (log cfu/g) | 1.68±0.28 | 1.50±0.14 | 1.34±0.12 | 1.03±0.07 |         |
| pH | 8.09±0.07 | na | na | 7.90±0.16 | 5.45±0.01 |
| *L. monocytogenes* (log cfu/g) | | | | | |
| LAB (log cfu/g) | 1.81±0.17 | nd | nd | na | 5.35±0.04 |
| pH | 8.16±0.03 | na | na | 5.29±0.19 | 5.53±0.04 |
| *L. monocytogenes* (log cfu/g) | | | | | |
| LAB (log cfu/g) | 1.50±0.13 | 1.13±0.32 | 0.99±0.07 | nd |         |
| pH | 8.05±0.15 | na | na | 7.81±0.22 | 5.58±0.11 |

LAB, lactic acid bacteria; na, not analysed; nd, not detected.
variability between the different batches that do not allow to clearly define the product category with regard of supporting or not *L. monocytogenes* growth. The results of the present study showed that the investigated sliced turkey *bresaola* do not support the growth of *L. monocytogenes* even when turkey *bresaola* were stored in condition of moderate thermal abuse.

The observed decrease of pH during the 90 days of storage (from 5.51/5.60 to 5.35/5.58) in the three batches may have influenced the survival of *L. monocytogenes* but cannot explain alone the decrease in *L. monocytogenes* count given that the growth/no growth interfaces of *Listeria* reported by Le Marc et al. (2002) are 5.50 and 4.6-4.7 respectively at 5 and 10°C; similarly the minimal *a_0* levels for growth and survival of *L. monocytogenes* were reported to be 0.90-0.92 with lower survival rates in NaCl adjusted media.

The results underscore that *L. monocytogenes* count reduction could be explained by the sum of different factors, such as values of pH (Buchanan et al., 1993) and *a_0* (Sabatakou et al., 2001), the addition of nitrite (Kouakou et al., 2009) and packaging in modified atmosphere (MAP) (Sørheim et al., 2008) reportd that a minimum 30 ppm nitrite will enhance the antilisterial activity of bacteria in typical dry fermented product. Meat Sci 62:323-9.

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