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 sliced *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*.

Introduction

Listeria monocytogenes is widely distributed in nature and the association of this pathogen with meat and slaughter environment is well established (Autio *et al*., 2000). *L. monocytogenes* may contaminate meat products through raw materials, but several studies revealed that environmental contamination is the most frequent source of *L. monocytogenes* in finished products (Barbuti and Parolari, 2002; Colak *et al*., 2007). In deli ready-to-eat (RTE) meats, slicing equipment was demonstrated to be a vehicle for *L. monocytogenes* contamination (Lin *et al*., 2006). The scientific report of EFSA (2010) reported that *L. monocytogenes* was detected in 1.5% of the 3636 units of poultry meat products tested, ranging from 0 to 7.5% positive units in broiler meat and from 0 to 11.8% in turkey meat; in RTE poultry meats the proportion of *L. monocytogenes* positive units ranged between 0.1 to 1.6%. The Community legislation (Regulation No. 2073/2005/EC) lays down food safety criteria for *L. monocytogenes* in RTE: *L. monocytogenes* must not be present in levels exceeding 100 colony forming units (cfu)/g during the shelf life of a product; in addition, in RTE foods that are able to support the growth of the bacterium, *L. monocytogenes* must not be present in 25 g at the time of leaving the production plant unless the producer can demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout its shelf life. Little is known about the fate of *L. monocytogenes* on different types of deli meats in particular on sliced deli meats; the objective of the study was therefore to evaluate the behaviour of *L. monocytogenes* in sliced RTE turkey *bresaola* made by cured turkey breast 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 sliced *bresaola* were stored at 5°C for 7 days to simulate optimal storage temperature at the producer plant, and at 8°C for the remaining storage time (83 days) to simulate home storage considering that more than 50% of the European household fridges hold temperature beyond 6-8°C (AFFSA, 2008).

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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% CO_2 and 70% N_2), 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 *Eco*RI 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° 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 % v:w of the multi-strain cocktail of *L. monocytogenes* to a

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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% CO_2 and 70% N_2) 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. *L. 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, Spain) for 3 min in a Stomacher 400 blender (Seward Medical, London, UK). Decimal dilutions in sterile PW were prepared from each bag. To verify the absence of natural contamination on control samples, at time zero the qualitative analysis for *L. monocytogenes* was performed according to ISO 11290-1 (ISO, 1996). Quantitative analysis for *L. monocytogenes* enumeration was performed on contaminated samples according to ISO 11290-2 (ISO, 1998). The mesophilic lactic acid bacteria (LAB) count was carried out by pour plating 1 mL of appropriate dilution in MRS agar (MRS; Microbiol Diagnostici) 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 (a_w) 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. Analysis of variance (ANOVA) was carried out to evaluate the difference of pH, a_w and microbial counts at different storage time. The significance were statistically analysed by Student *t*-test at a 95% confidence interval ($P<0.05$) using R statistical software version 2.7.0 (R Development Core Team, 2008).

The growth potential (δ) of *L. 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 (δ_{max}) 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 *L. monocytogenes* contamination in sliced turkey *bresaola*. Average of initial values (N=3 batch; n=3 replicates) of pH and a_w 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 a_w 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, *L. 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 *L. monocytogenes* ranged from -1.32 log cfu/g in batch 2 to -0.58 log cfu/g in batch 1; the maximum growth potential (δ_{max}) of *L. monocytogenes* on sliced turkey *bresaola* was -0.58 log cfu/g. The average values of *L. monocytogenes* log counts in three contaminated batches are shown in Table 1.

Discussion and Conclusions

The growth potential of *L. 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, a_w, 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 a_w <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 *L. 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 a_w) of the sliced turkey *bresaola* showed a

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Table 1. Evolution of *L. 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).

	Day			
	0	40	60	90
Batch 1				
L. monocytogenes (log cfu/g)	1.68±0.28	1.50±0.14	1.34±0.12	1.03±0.07
LAB (log cfu/g)	8.09±0.07	na	na	7.90±0.16
pH	5.60±0.03	na	na	5.45±0.01
Batch 2				
L. monocytogenes (log cfu/g)	1.81±0.17	nd	nd	nd
LAB (log cfu/g)	8.16±0.03	na	na	8.29±0.19
pH	5.51±0.07	na	na	5.35±0.04
Batch 3				
L. monocytogenes (log cfu/g)	1.50±0.13	1.13±0.32	0.99±0.07	nd
LAB (log cfu/g)	8.05±0.15	na	na	7.81±0.22
pH	5.55±0.04	na	na	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_w 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_w (Sabatakou *et al*., 2001), the addition of nitrite (Kouakou *et al*., 2009) and packaging in modified atmosphere (MAP) (Sørheim *et al*., 2004); also, even the indigenous microbiota of RTE meat products could affect the behavior of *L. monocytogenes* (Lecompte *et al*., 2008; Ross *et al*., 2009; Mellefont and Ross, 2007) by competition for carbohydrates, acids and bacteriocins production (Serraino *et al.*, 2013). In particular Glass *et al*. (2008) reportd that a minimum 30 ppm nitrite will enhance the antilisterial activity of lactate in RTE poultry meat and that 150 ppm of sodium nitrite doubles the lag-phase of *L. monocytogenes*. The combination of 2% sodium lactate and 0.5% of acetate prevent pathogen growth in sliced pork saveloy with a little as 60 ppm of sodium nitrite (Junker *et al*., 2000). The presented data are currently needed, given the lack of similar information in the literature, and may be a useful scientific tool for food manufacturers that produce RTE meat products with similar characteristics, to demonstrate whether their product supports the growth of *L. monocytogenes* or not, in accordance with EC Regulation No. 2073/2005 (European Commission, 2005).

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