Decrease of Salmonella and Escherichia coli O157:H7 counts during dry-aging of beef but potential growth of Listeria monocytogenes under certain dry-aging conditions

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Abstract

Dry-aging of beef comprises the storage of carcasses and (sub)primal cuts at a low temperature and relative humidity for a prolonged period, aiming to increase the sensory quality of meat. Limited data are available on the survival and potential growth of pathogens on the surface of beef during dry-aging. Therefore, this study evaluates the changes in Salmonella, Shiga toxin-producing Escherichia coli O157:H7 and Listeria monocytogenes counts during dry-aging. A mixture of pathogenic strains was inoculated on the surface of beef loins, which were stored under four different process conditions (2°C and 6°C × relative humidity 75 and 85% during 42 days). Salmonella and E. coli O157:H7 counts significantly decreased during dry-aging. The daily reductions varied from -0.07 to -0.14 log_{10} CFU and from -0.09 to -0.14 log_{10} CFU, respectively, depending on the loin, matrix and condition. The reduction of L. monocytogenes was slower, with a maximum of -0.07 log_{10} CFU/day. L. monocytogenes counts increased with 1.0 log_{10} CFU on the lean meat of one loin with pH >6.0 at the end of dry-aging, indicating that this pathogen can potentially grow under certain dry-aging conditions.

Keywords: dry aging, meat, food safety, process hygiene, Salmonella, STEC, Listeria
1. Introduction

Dry-aging of meat is a process whereby mainly beef carcasses and (sub)primal cuts are stored at a low temperature and relative humidity (RH) for a certain period. With the development of vacuum packaging, carcass aging was largely replaced by wet-aging because the latter resulted in a better economic value. However, during the last decade consumers in different parts of the world showed an increasing interest in dry-aged beef due to the improved tenderness and the unique flavour of such meat (Dashdorj et al., 2016). Several factors influence the flavor and tenderness of dry aged beef. Despite the contradicting results of experimental studies in scientific literature, Terjung and colleagues (2021) concluded that dry-aging should be done at about 2°C, 85% RH and 0.5 m/s air flow during about 40 days. A reduced RH and a good air velocity during the dry-aging process leads initially to a quick drying and concomitant reduction of water activity at the surface of the meat. Different studies showed that drying of the meat surface combined with a low temperature considerably reduced the growth of the microflora present on the meat during the dry-aging process (Ahnström et al., 2006; DeGeer et al., 2009; Li et al., 2014). Nevertheless, microbiological counts on dry-aged beef loins and steaks have been shown to vary greatly under field conditions in Belgium (Gowda et al., 2022), reflecting also the vast differences in processes that are used in practice. In this cross-sectional survey, comprising 15 food business operators, temperatures during dry-aging were set between -1°C and 3°C (measured between 0°C and 5.9°C), RH between 40% and 70% (measured between 50% and 90%), and aging times between 3 and 10 weeks.

Cattle can carry foodborne pathogens such as Salmonella, Shiga toxin-producing Escherichia coli, and Listeria monocytogenes in the intestines and especially on the hide at the moment of slaughter (Dewell et al., 2008; Nastasijevic et al., 2007; Reid et al., 2002; Rhoades et al., 2009). During slaughter, pathogens may be transferred to carcasses during dehiding and evisceration (Beach et al., 2002; Bricha-Harhay et al., 2008; Rhoades et al., 2009; Thomas et
As such, foodborne pathogens may be introduced into the beef production chain, which can potentially be a risk for human health. However, data on the survival and potential growth of these pathogens on the surface of beef during dry-aging are very limited (Knudsen et al., 2011; Tittor et al., 2011). Since the minimal growth temperature of Salmonella and E. coli is 6 °C and 7 °C, respectively (ICMSF 1996), these pathogens are not expected to grow when the temperature remains below the legally required 7°C (Regulation (EC) No 853/2004, 2004). In contrast, the minimal growth temperature of L. monocytogenes (from 0 °C up to 2 °C) (ICMSF 1996) may result in the growth of this pathogen during the dry-aging process, even if the temperature is below 7°C. Therefore, the aim of the present study was to explore the changes in Salmonella, Shiga toxin-producing E. coli O157:H7 and L. monocytogenes counts on beef loins during dry-aging under different combinations of temperature (2°C and 6°C) and relative humidity (75% and 85%).

2. Material and methods

2.1. Study design

Beef loins were inoculated with a mixture of pathogens, and were dry-aged under different conditions, using a 2 × 2 factorial design for temperature (2°C and 6°C) and relative humidity (75% and 85%), with two replications. The evolution in microbial counts was determined for lean tissue and adipose tissue separately (split-plot design, with loin as whole-plot). Microbiological counts were assessed at different time points, up to 6 weeks after inoculation (longitudinal data).

2.2. Bacterial strains and culture conditions

In the present study, Salmonella, Shiga toxin-producing Escherichia coli O157:H7 and L. monocytogenes strains belonging to the culture collection from the Laboratory of Food
Microbiology and Food Preservation, Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, were used for inoculation of the loins: one S. Thompson (S688; isolated from cilantro), two S. Typhimurium (S689 and S883; environmental isolates), three E. coli O157:H7 (E844, E846 and E990; bovine, beef and human isolate, respectively), two L. monocytogenes serotype 4b (L392 and L394; isolated from liver paste and cheese, respectively) and one L. monocytogenes serotype 1/2b (L491; isolated from tuna spread). All strains, stored at -80 °C, were revived in Tryptic Soy Broth (TSB; Oxoid, Basingstoke, UK) during 18-24 h at 37 °C. Each culture was subsequently streaked on the appropriate selective agar medium: Xylose Lysine Deoxycholate agar (XLD; Oxoid) for Salmonella, Sorbitol MacConky agar supplemented with cefixime and potassium tellurite (CT-SMAC; Oxoid) for E. coli O157:H7 and Listeria Ottaviani and Agosti (ALOA; Biolife, Milan, Italy) for L. monocytogenes. After incubation for 18-24 h at 37 °C, a typical colony was inoculated into TSB and again incubated for 18-24 h at 37 °C. Next, Tryptic Soy Agar (TSA; Oxoid) slants were inoculated with a loop from the broth and incubated for 18-24 h at 37 °C. A full loop of each slant culture was transferred into 10 ml TSB and incubated for 24 h at 37 °C, after which 0.1 ml was transferred to another 10 ml TSB. After incubation for 24 h at 37 °C, 1 ml of each of the 9 incubated broths was transferred into a sterile tube and mixed using a vortex. This initial culture mixture was first 4-fold and then once 10-fold diluted in 0.85% NaCl in order to obtain an inoculum solution containing 10^7 cfu/ml of each bacterial species. The number of each bacterial species in the solution was determined by plating out on the appropriate selective agar media.

**2.3. Dry-aging conditions**

For the dry-aging, a drying cabinet Matton, type RKM/DKM (Buysse Food Machinery, Wetteren, Belgium) was used, which has a variation of 0.5 °C and 5% RH under normal
operating conditions. Temperature and relative humidity within the equipment was controlled, and were monitored daily.

The beef samples were subjected to the following four dry-aging conditions: (A) 6°C and 75% RH, (B) 2°C and 75% RH, (C) 6°C and 85% RH, and (D) 2°C and 85% RH. For each condition, two replicates were dry-aged simultaneously in the same cabinet. The experiments for the different conditions were performed serially, with thorough cleaning and disinfection of the drying cabinet between each condition.

2.4. Preparation of beef samples and inoculation procedure

For each condition, two beef loins (*M. longissimus thoracis et lumborum*) were purchased from a local beef cutting plant. The loins were packed in a plastic foil (not vacuum) and stored for ten days post mortem ≤ 4°C. Each beef loin was between 80-90 cm long (loin containing seven ribs) and originated from a different culled Holstein cow. Upon arrival at the laboratory, the loins were cut in two equal parts (Supplemental file, Figure S1). From one part of each loin, the adipose and the superficial connective tissue were removed in order to obtain a large surface of lean meat. This was done under hygienic conditions (in a room at 10°C, disinfected table and knives and wearing disposable gloves, but no other specific measures were taken to prevent bacterial contamination). The other part was left intact. Then, seven surfaces of 25 cm² were delineated using a sterile stainless-steel template (5 × 5 cm) and surgical knife on the surface of both parts of each loin. Each delineated surface was inoculated by spreading 100 µl of the inoculum solution over the surface using a sterile Drigalski spatula. After drying of the inoculum for 15 min, the four parts were placed in the drying cabinet adjusted to the targeted temperature and relative humidity, for a total of six weeks.

2.5. Quantification of pathogens
On day 1, 4, 8, 14, 21, 28, 35 and 42, one inoculated surface was collected from each loin part using a sterile surgical knife and forcep, and placed in a stomacher bag. To all samples (25 cm² each), 25 ml of 0.1% peptone water was added. After homogenization using a stomacher for 1 minute, appropriated agar media were used to enumerate the different types of microorganisms. Homogenates were plated on XLD, CT-SMAC and ALOA to enumerate *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes*, respectively. In order to obtain a detection limit of 1 CFU/cm², 1 ml of each homogenate was spread over three plates of the same agar medium. To recover and to enumerate sublethally injured cells of *Salmonella* and *E. coli* O157:H7, the thin agar layer method was applied, as described by Wu, Fung, Kang, & Thompson (2001). Therefore, on the day of use, 25 ml of both XLD and CT-SMAC agar medium was poured into petri plates. After solidification of the selective agar medium, 7 ml of TSA was spread over all agar media, and plates were used to enumerate *Salmonella* and *E. coli* O157:H7 as described above.

**2.6. Background flora**

On day 0 and day 42, 25 cm² of the superficial adipose tissue and lean meat was collected from the respective parts of all loins, using a sterile stainless-steel template, similar as described above. To enumerate the background flora, the following agar plates and incubation conditions were used: 1) Plate Count Agar (Oxoid) incubated at 22 °C for 5 days for Total Aerobic Count, 2) Violet Red Bile Glucose Agar (Bio-Rad, Temse, Belgium) incubated at 37 °C for 1 day for Enterobacteriaceae, 3) Pseudomonas C.F.C. agar (Oxoid) incubated at 25 °C for 2 days for *Pseudomonas* spp.; 4) De Man, Rogosa and Sharpe agar (Oxoid) incubated at 22 °C for 5 days for Lactic Acid Bacteria, and 5) Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Oxoid) incubated at 25 °C for 3 days for yeasts and molds. The pour plate method was used for Enterobacteriaceae with a detection limit of 1 CFU/cm² and the spread plate method for all other microbial flora with a detection limit of 10 cfu/cm². On DRBC agar plates, yeast and molds were enumerated separately.
2.7. pH measurements

The pH of the loins was measured internally in duplicate on day 0 and 42, using a pH apparatus Knick portamess 654 (Knick Elektronische Messgeräte GmbH, Berlin, Germany) with an electrode LoT406-M6-DXK-S7/25 (Mettler-Toledo, Urdorf, Switzerland).

2.8. Statistical analysis

All analyses and visualisations were performed in RStudio using R version 4.0.2. The mean difference in pH at day 0 versus day 42 was tested using a linear mixed model, including a random intercept for loin to account for the dependence of samples within loins (Bates et al., 2015). We didn’t test for differences in pH between each of the conditions since our study was not designed to detect such differences.

Bacterial counts were log$_{10}$ transformed for analysis. Background flora were summarized descriptively (median, minimum and maximum values). The reduction of pathogens (in log$_{10}$ CFU/cm$^2$) was calculated by subtracting the initial inoculation level (i.e. day 0). For negative samples, a value of zero was used to calculate the reduction. Linear regression models without intercept were fitted per loin/matrix, using the reduction (in log$_{10}$ CFU/cm$^2$) as outcome and time (in days) as a continuous variable. Decimal reduction times (D-values) were then calculated as the negative reciprocal of the slopes (De Jesus and Whiting, 2003). Due to the explorative nature of this study, only two replicates were performed per condition, and the level of reduction between conditions was therefore not compared using a formal analysis.

Sublethality of Salmonella and E. coli O157:H7 cells was estimated by subtracting the counts using the selective agar medium from the counts using the overlay method (both expressed as log$_{10}$ CFU/cm$^2$). Only samples with countable numbers (i.e. above the detection limit) on both agar media were taken into account.

3. Results
3.1. Loin characteristics

The pH at the start of the dry-aging process (day 0) ranged from 5.34 to 5.68 (mean 5.50) for 7 out of 8 loins (Supplemental file, Table S1). After dry-aging (day 42), the pH values varied between 5.60 and 5.99 (mean 5.77, n=7). For one loin (loin 1 within condition D), the pH had a value of 6.21 at day 0 and increased to 6.59 on day 42. The pH of the eight loins significantly increased with a mean value of 0.28 (95% CI: 0.21-0.35; p < 0.001) during the 42 days of dry-aging.

Enumeration results of background flora before and after dry-aging are listed in Table 1. The total aerobic counts on day 0 varied between <1 and 4.0 log\(_{10}\) CFU/cm\(^2\) on lean meat and between 2.1 and 4.2 log\(_{10}\) CFU/cm\(^2\) on adipose tissue. Enterobacteriaceae and moulds were not detected in more than half of the cases (median below the limit of detection; Table 1). At the start of the experiments, lactic acid bacteria, Pseudomonas spp. and yeasts were detected in numbers up to 3.4, 4.2, and 6.3 log\(_{10}\) CFU/cm\(^2\), respectively. At the end of dry-aging, microbial counts were highly variable between different loins, with occasionally high counts of total aerobic counts, lactic acid bacteria, Pseudomonas spp. and yeasts (Table 1).

3.2. Evolution of pathogens over time

The inoculum solution (containing a mixture of three strains of each of the three pathogens) was prepared aiming to achieve 4 log\(_{10}\) CFU/cm\(^2\) per pathogen. This inoculation level was achieved for most conditions, though at 2°C and 75% RH, the level was approximately 1 log higher (Supplemental file, Table S2).

The number of Salmonella, E. coli O157:H7 and L. monocytogenes was determined at different time points and the reductions were calculated, relative to the initial inoculation level. Individual observations per time point are shown in Figure 1, Figure 2, and Figure 3, respectively. The reduction of Salmonella, E. coli O157:H7 and L. monocytogenes during the
dry-aging process was predicted for each of the loins (and for lean and adipose tissue separately) using linear models. The values for the resulting slopes are provided in Supplemental file, Table S3, and are visualized in Figure S2. The corresponding D-values per condition, matrix, and replicate can be found in Table 2.

A significant linear decrease over time was predicted for *Salmonella* for both tissue types on all loins (Figure 1 and Supplemental file, Figure S2). The daily reductions varied between -0.07 and -0.14 log$_{10}$ CFU/day on adipose tissue and between -0.07 and -0.12 log$_{10}$ CFU/day on lean meat (Supplemental file, Table S3). The corresponding D-values varied between 7 and 14 days, depending on the loin, matrix, and condition (Table 2). With the exception of lean meat in condition A (6°C, 75% RH), both replicates yielded very similar results for *Salmonella*. For all conditions, a reduction of at least 3 log was predicted after 42 days of dry-aging. On adipose tissue, a reduction over 4 log was observed after 42 days for the loins under condition A and B (6°C and 2°C, 75% RH). For loins in condition D (2°C, 85% RH), a similar level of reduction (approx. 4 log$_{10}$ CFU) was observed for both tissue types.

Similar to *Salmonella*, dry-aging caused a statistically significant reduction of *E. coli* O157:H7 on all loins, for both tissue types (Figure 2 and Supplemental file, Figure S2). The estimated reduction on each of the loins varied between -0.14 and -0.11 log$_{10}$ CFU/day on adipose tissue and between -0.13 and -0.09 log$_{10}$ CFU/day on lean meat (Supplemental file, Table S3). The predicted D-values varied between 7 and 11 days (Table 2), corresponding to a reduction of more than 3.8 log$_{10}$ CFU after 42 days of dry-aging. For most of the loins, *E. coli* O157:H7 numbers were estimated to decrease faster on adipose tissue than on lean meat, as demonstrated by the lower D-values for adipose tissue compared to lean meat of the same loin (Table 2).

The reduction of *L. monocytogenes* was not statistically significant for all loins (Figure 3 and Supplemental file, Figure S2). For loins in which a significant reduction was observed, the maximum estimated reduction was -0.06 log$_{10}$ CFU/day on adipose tissue and -0.07 log$_{10}$
CFU/day on lean meat (Supplemental file, Table S3). The estimated D-values varied between 14 and 42 days (Table 2). Although on certain loins a reduction of almost $3 \log_{10}$ CFU was predicted after 42 days of dry-aging (D-value around 14 days), for other loins only about 1 log reduction was predicted by the end of dry-aging (D-value around 42 days). *L. monocytogenes* significantly increased for one replicate of lean meat at condition D ($2^\circ$C, 85% RH), resulting in a 1 log higher number at day 42 than the initial inoculated number of *L. monocytogenes* (Supplemental file, Figure S2).

The sublethality for *Salmonella* and *E. coli* O157:H7 is presented in Supplemental file, Figure S3. For both tissues, the median of the differences between the counts on thin overlay plates and the selective plates was mostly around 0 log$_{10}$ cfu/cm$^2$ in each of the four conditions.

**4. Discussion**

In the present study, the evolution of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* during the dry-aging of beef was evaluated, using different temperatures (2 °C and 6 °C) and relative humidities (75% and 85%). On all loins, *Salmonella* and *E. coli* O157:H7 significantly decreased during dry-aging for 42 days.

For *Salmonella* and especially for *E. coli* O157:H7, dry-aging for 6 weeks resulted in a reduction of at least $3 \log_{10}$ CFU/cm$^2$. Tittor et al. (2011) observed a mean reduction of 4.65 log$_{10}$ CFU/cm$^2$ for *Salmonella* and 4.76 log$_{10}$ CFU/cm$^2$ for *E. coli* O157:H7 on lean meat and adipose tissue collected from beef carcasses during 28 days at 3°C and a RH of 80%. Moreover, Knudsen et al. (2011) demonstrated that the *Salmonella* reduction during dry-aging was serotype and even strain dependent, with daily reductions varying between -0.113 and -0.216 log$_{10}$ CFU/cm$^2$ over a period of 14 days (at 1 °C in a conventional refrigerator with a RH varying between 70 and 100%). In the present study, we found (slightly) slower daily reductions of *Salmonella*, varying between -0.07 and -0.14 log$_{10}$ CFU. Since we used the initial inoculation level as the maximum reduction (i.e., for samples below the limit of detection), the estimates...
for daily reductions and the corresponding D-values may be biased when a considerable number of observations were negative. As such, for *Salmonella* and *E. coli* O157:H7, several samples of condition A and D were negative near the end of aging period due to a steep decrease during the first days. Therefore, the actual reduction during these conditions may be underestimated in our study, and the reported estimates can thus be considered conservative. Similar to Knudsen and colleagues (2011), we also assumed a log-linear reduction over time, which seemed very plausible in most cases, though a non-linear shape for the survivor curve (Geeraerd et al., 2005) may be more appropriate in other cases. Indeed, the reduction of both pathogens seemed more pronounced during the initial days of dry-aging, on adipose tissue in particular. To represent contamination of the meat originating from an animal source in the slaughterhouse (rather than from the environment), the bacteria in the present study were grown at 37°C prior inoculation, which was also applied by Knudsen and colleagues (2011), whereas Tittor and colleagues (2011) used a cocktail of frozen cells. The use of cold adapted cells may result in a different survival ability during the dry-aging of beef and should still be assessed. Additionally, only stationary phase cells were used in the present study, so the potential effect of starting physiology of the cells on the potential growth/survival during dry-aging is yet to be elucidated.

Comparison of the counts for *Salmonella* and *E. coli* O157:H7 obtained by the thin layer agar and direct enumeration method on the selective agar revealed that the differences varied around zero. This indicates that the presence of sublethally injured cells of both pathogens was in most cases very limited and that the observed reductions are mainly due to dying of the cells. With around 1 to 3 log_{10} CFU reductions after 42 days in most cases, the decline of *L. monocytogenes* was less pronounced than those of *Salmonella* and *E. coli* O157:H7. Da Silva and colleagues (2019) used *L. innocua* as a surrogate for *L. monocytogenes* and observed a 2.4 and 3.4 log CFU/g reduction after 42 days at 75% RH, an air velocity of 2 m/s, at 2°C and 8°C, respectively. In our study, a large difference in *L. monocytogenes* counts was observed between
both replicates of condition D (2°C, 85% RH), particularly for lean meat. A reduction was observed on one loin (with a pH < 6), whereas *L. monocytogenes* counts increased on the other loin (with a pH > 6). This may indicate that the pH of the meat could have an important effect on the survival and potential growth of *L. monocytogenes* during dry-aging of beef.

Although there doesn’t seem to be a large difference between the temperature and RH combinations that were evaluated during this study, and some differences were observed between replicates, several other factors may have contributed to the observed differences as well. Also general microbiological counts varied substantially between loins in this study, and a similarly large variability was also observed under routine practices (Gowda et al., 2022). In the present study, a mixture of three strains was used of each pathogen to account for possible variation among strains, yet the growth/survival using single strains deserves to be performed to account for strain variability. In addition, three different pathogens were evaluated simultaneously to increase efficiency of our study, though the effect of potential interactions between pathogens was not assessed. The causal effect of distinctive factors contributing to the microbiological load and safety as well as the (sensory) quality of dry-aged beef is still not fully elucidated (Terjung et al., 2021). The development of guidelines and criteria for the safe production of dry-aged beef requires a better knowledge on the (combination of) factors for growth/no-growth of psychrothrophic pathogens, such as *L. monocytogenes*. Therefore, future studies should assess the causal relations of different factors, including pH, temperature, relative humidity and background flora. Only a limited number of conditions were tested during the present study (2°C and 6°C, combined with 75% and 85% RH), which don’t fully represent the true variation of practices that are used in routine production (Gowda et al., 2022). Nevertheless, we observed that in most cases *L. monocytogenes* counts significantly decline during dry-aging, and when they are able to multiply, growth seems to be slower compared to growth on vacuum packed beef (Grau and Vanderlinde, 1990).
Despite the difference in pH of the lean meat of the two loins in condition D (2°C and 85% RH), the level of reduction for *Salmonella* and *E. coli* was similar for both loins. Ribeiro and colleagues (2021) found similar bacterial plate counts on dry-aged beef from higher pH carcasses (average pH 6.69) compared to normal pH (average pH 5.47), whereas for wet-aged beef significantly higher numbers were found on beef with a higher pH. Although our study indicates that meat with a high pH might present a higher risk for *L. monocytogenes*, this doesn’t seem apparent for *Salmonella* nor *E. coli* O157:H7.

5. Conclusions

Our study confirms that pathogens such as *Salmonella* and *E. coli* O157:H7 decrease during dry-aging, as at least a three log reduction was observed for both pathogens under all conditions after 42 days. To the best of our knowledge, this is the first study indicating the potential growth of *L. monocytogenes* during dry-aging of beef. Contrarily to *Salmonella* and *E. coli* O157:H7, the reduction over time was small for *L. monocytogenes*, and we found that this pathogen can potentially grow, even if the temperature is within the legal requirements of dry-aging. In order to obtain more insight in the effect of the processing conditions (temperature and relative humidity) and other factors (such as pH and background flora) on the evolution of *L. monocytogenes* during dry-aging, more extensive studies are necessary.

6. Author contributions

I. Van Damme: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – Original Draft, Writing – Review & Editing; S. Varalakshmi: Investigation, Writing – Original Draft, Writing – Review & Editing; L. De Zutter: Conceptualization, Methodology, Supervision, Writing – Original Draft, Writing – Review & Editing, Resources, Funding acquisition; E. Vossen: Conceptualization, Methodology, Investigation, Project administration, Writing – Review & Editing, Resources; S. De Smet:
7. Competing interests

The authors declare that they have no competing interests.

8. Funding

This research was done in the framework of the Cornet project OPTIDRYBEEF financed by the Flemish government (Agentschap Innoveren en Ondernemen - AIO) and Flanders’ Food. S. Varalakshmi received an Netaji-Subhas ICAR International fellowship of the Indian Council of Agricultural Research (ICAR). The funders had no role in the study design, data collection, analysis, interpretation of data, nor in writing of the report.

9. Data statement

The data underlying the conclusions of this manuscript are available on Zenodo ([dataset] Van Damme et al., 2021).

10. Acknowledgements

Buysse Food Machinery is acknowledged for kindly supplying the ripening cabinet. S. Coolsaet and N. Lyen are acknowledged for their skilful technical support.
References


De Jesus, A.J., Whiting, R.C., 2003. Thermal inactivation, growth, and survival studies of *Listeria monocytogenes* strains belonging to three distinct genotypic lineages. J. Food...


traditional dry ageing or vacuum package ageing. Meat Sci. 97, 433–42.  
https://doi.org/10.1016/j.meatsci.2014.03.014


Effects of simulated dry and wet chilling and aging of beef fat and lean tissues on the reduction of *Escherichia coli* O157:H7 and *Salmonella*. J. Food Prot. 74, 289–293.

https://doi.org/10.4315/0362-028X.JFP-10-295

Figures

Figure 1. Reduction of *Salmonella* spp. numbers (in log_{10} CFU/cm²) on lean meat and adipose tissue during dry-aging of beef loins at different conditions. Observations on lean meat are presented as triangles, and adipose tissue as circles. The different replicates (two per condition) are indicated with a solid (replicate 1) or dashed (replicate 2) line. Reductions are calculated relative to the initial inoculation level. The dashed horizontal line indicates the maximum reduction that could be detected, and is the negative value of the initial inoculation level. Negative samples are plotted on the latter line.
Figure 2. Reduction of *Escherichia coli* O157:H7 numbers (in log_{10} CFU/cm²) on lean meat and adipose tissue during dry-aging of beef loins at different conditions.

Observations on lean meat are presented as triangles, and adipose tissue as circles. The different replicates (two per condition) are indicated with a solid (replicate 1) or dashed (replicate 2) line. Reductions are calculated relative to the initial inoculation level. The dashed horizontal line indicates the maximum reduction that could be detected, and is the negative value of the initial inoculation level. Negative samples are plotted on the latter line.
**Figure 3.** Evolution of *Listeria monocytogenes* numbers (in $\log_{10}$ CFU/cm$^2$) on lean meat and adipose tissue during dry-aging of beef loins at different conditions. Observations on lean meat are presented as triangles, and adipose tissue as circles. The different replicates (two per condition) are indicated with a solid (replicate 1) or dashed (replicate 2) line. Reductions are calculated relative to the initial inoculation level.
Table 1. Background flora on lean and adipose tissue of loins before and after dry-aging (n = 8).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Start (day 0)</th>
<th>End (day 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adipose tissue</td>
<td>Lean tissue</td>
</tr>
<tr>
<td>Total aerobic count</td>
<td>2.9 [2.1, 4.2]</td>
<td>2.3 [&lt;1, 4.0]</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>&lt;0 [&lt;0, 2.8]</td>
<td>&lt;0 [&lt;0, 3.1]</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>1.2 [&lt;1, 2.2]</td>
<td>&lt;1 [&lt;1, 3.4]</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>2.1 [&lt;1, 4.2]</td>
<td>&lt;1 [&lt;1, 4.2]</td>
</tr>
<tr>
<td>Moulds</td>
<td>&lt;1 [&lt;1, 1.0]</td>
<td>&lt;1 [&lt;1, 4.2]</td>
</tr>
<tr>
<td>Yeasts</td>
<td>&lt;1 [&lt;1, 1.9]</td>
<td>&lt;1 [&lt;1, 6.3]</td>
</tr>
</tbody>
</table>

Median [minimum, maximum] values in log$_{10}$ CFU/cm$^2$. To determine the median, the limit of detection was used for negative samples (i.e. 0 log$_{10}$ CFU/cm$^2$ for Enterobacteriaceae and 1 log$_{10}$ CFU/cm$^2$ for the other microorganisms). Counts at the end of dry-aging are a combination of the four dry-aging conditions. Disaggregated data can be found on Zenodo ([dataset] Van Damme et al., 2021).

Table 2. Decimal reduction times of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on lean meat and adipose tissue of dry-aged beef loins subjected to different dry-aging conditions, assuming a linear reduction over time. The numbers represent the estimated D-values (in days) for each of the two replicates (R1 and R2) per condition.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Condition</th>
<th><em>Salmonella</em> spp.</th>
<th><em>E. coli</em> O157:H7</th>
<th><em>L. monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R1</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>6°C, 75% RH</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2°C, 75% RH</td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>6°C, 85% RH</td>
<td>13</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2°C, 85% RH</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Lean meat</td>
<td>6°C, 75% RH</td>
<td>12</td>
<td>9</td>
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</tr>
<tr>
<td></td>
<td>2°C, 75% RH</td>
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<tr>
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<td>6°C, 85% RH</td>
<td>14</td>
<td>14</td>
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<tr>
<td></td>
<td>2°C, 85% RH</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

*NA*: not applicable: a negative D-value was obtained, indicating growth instead of reduction.