1   **Decrease of *Salmonella* and *Escherichia coli* O157:H7 counts during dry-aging of**

2   **beef but potential growth of *Listeria monocytogenes* under certain dry-aging**

3   **conditions**

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1

19   **Abstract**

20   Dry-aging of beef comprises the storage of carcasses and (sub)primal cuts at a low temperature

21   and relative humidity for a prolonged period, aiming to increase the sensory quality of meat.

22   Limited data are available on the survival and potential growth of pathogens on the surface of

23   beef during dry-aging. Therefore, this study evaluates the changes in *Salmonella*, Shiga toxin-

24   producing *Escherichia coli* O157:H7 and *Listeria monocytogenes* counts during dry-aging. A

25   mixture of pathogenic strains was inoculated on the surface of beef loins, which were stored

26   under four different process conditions (2°C and 6 °C × relative humidity 75 and 85% during

27   42 days). *Salmonella* and *E. coli* O157:H7 counts significantly decreased during dry-aging. The

28   daily reductions varied from -0.07 to -0.14 $\log_{10}$ CFU and from -0.09 to -0.14 $\log_{10}$ CFU,

29   respectively, depending on the loin, matrix and condition. The reduction of *L. monocytogenes*

30   was slower, with a maximum of -0.07 $\log_{10}$ CFU/day. *L. monocytogenes* counts increased with

31   1.0 $\log_{10}$ CFU on the lean meat of one loin with pH >6.0 at the end of dry-aging, indicating that

32   this pathogen can potentially grow under certain dry-aging conditions.

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34   **Keywords:** dry aging, meat, food safety, process hygiene, *Salmonella*, STEC, *Listeria*

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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; Brichta-Harhay et al., 2008; Rhoades et al., 2009; Thomas et

61 al., 2012). As such, foodborne pathogens may be introduced into the beef production chain,

62 which can potentially be a risk for human health. However, data on the survival and potential

63 growth of these pathogens on the surface of beef during dry-aging are very limited (Knudsen et

64 al., 2011; Tittor et al., 2011). Since the minimal growth temperature of *Salmonella* and *E. coli*

65 is 6 °C and 7 °C, respectively (ICMSF 1996), these pathogens are not expected to grow when

66 the temperature remains below the legally required 7°C (Regulation (EC) No 853/2004, 2004).

67 In contrast, the minimal growth temperature of *L. monocytogenes* (from 0 °C up to 2 °C)

68 (ICMSF 1996) may result in the growth of this pathogen during the dry-aging process, even if

69 the temperature is below 7°C. Therefore, the aim of the present study was to explore the changes

70 in *Salmonella*, Shiga toxin-producing *E. coli* O157:H7 and *L. monocytogenes* counts on beef

71 loins during dry-aging under different combinations of temperature (2°C and 6°C) and relative

72 humidity (75% and 85%).

73 **2. Material and methods**

74 **2.1. Study design**

75 Beef loins were inoculated with a mixture of pathogens, and were dry-aged under different

76 conditions, using a 2 × 2 factorial design for temperature (2°C and 6°C) and relative humidity

77 (75% and 85%), with two replications. The evolution in microbial counts was determined for

78 lean tissue and adipose tissue separately (split-plot design, with loin as whole-plot).

79 Microbiological counts were assessed at different time points, up to 6 weeks after inoculation

80 (longitudinal data).

81 **2.2. Bacterial strains and culture conditions**

82 In the present study, *Salmonella*, Shiga toxin-producing *Escherichia coli* O157:H7 and

83 *L. monocytogenes* strains belonging to the culture collection from the Laboratory of Food

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84   Microbiology and Food Preservation, Department of Food Safety and Food Quality, Faculty of

85   Bioscience Engineering, Ghent University, were used for inoculation of the loins: one *S.*

86   Thompson (S688; isolated from cilantro), two *S.* Typhimurium (S689 and S883; environmental

87   isolates), three *E. coli* O157:H7 (E844, E846 and E990; bovine, beef and human isolate,

88   respectively), two *L. monocytogenes* serotype 4b (L392 and L394; isolated from liver paste and

89   cheese, respectively) and one *L. monocytogenes* serotype 1/2b (L491; isolated from tuna

90   spread). All strains, stored at -80 °C, were revived in Tryptic Soy Broth (TSB; Oxoid,

91   Basingstoke, UK) during 18-24 h at 37 °C. Each culture was subsequently streaked on the

92   appropriate selective agar medium: Xylose Lysine Deoxycholate agar (XLD; Oxoid) for

93   *Salmonella*, Sorbitol MacConky agar supplemented with cefixime and potassium tellurite (CT-

94   SMAC; Oxoid) for *E. coli* O157:H7 and Listeria Ottaviani and Agosti (ALOA; Biolife, Milan,

95   Italy) for *L. monocytogenes*. After incubation for 18-24 h at 37 °C, a typical colony was

96   inoculated into TSB and again incubated for 18-24 h at 37 °C. Next, Tryptic Soy Agar (TSA;

97   Oxoid) slants were inoculated with a loop from the broth and incubated for 18-24 h at 37 °C. A

98   full loop of each slant culture was transferred into 10 ml TSB and incubated for 24 h at 37 °C,

99   after which 0.1 ml was transferred to another 10 ml TSB. After incubation for 24 h at 37 °C, 1

100   ml of each of the 9 incubated broths was transferred into a sterile tube and mixed using a vortex.

101   This initial culture mixture was first 4-fold and then once 10-fold diluted in 0.85% NaCl in

102   order to obtain an inoculum solution containing 10^7 cfu/ml of each bacterial species. The

103   number of each bacterial species in the solution was determined by plating out on the appropiate

104   selective agar media.

2.3. Dry-aging conditions

106   For the dry-aging, a drying cabinet Matton, type RKM/DKM (Buysse Food Machinery,

107   Wetteren, Belgium) was used, which has a variation of 0.5 °C and 5% RH under normal

108 operating conditions. Temperature and relative humidity within the equipment was controlled,

109 and were monitored daily.

110 The beef samples were subjected to the following four dry-aging conditions: (A) $6°C$ and 75%

111 RH, (B) $2°C$ and 75% RH, (C) $6°C$ and 85% RH, and (D) $2°C$ and 85% RH. For each condition,

112 two replicates were dry-aged simultaneously in the same cabinet. The experiments for the

113 different conditons were performed serially, with thorough cleaning and disinfection of the

114 drying cabinet between each condition.

115 **2.4. Preparation of beef samples and inoculation procedure**

116 For each condition, two beef loins (*M. longissimus thoracis et lumborum*) were purchased from

117 a local beef cutting plant. The loins were packed in a plastic foil (not vacuum) and stored for

118 ten days post mortem $\leq 4°C$. Each beef loin was between 80-90 cm long (loin containing seven

119 ribs) and originated from a different culled Holstein cow. Upon arrival at the laboratory, the

120 loins were cut in two equal parts (Supplemental file, Figure S1). From one part of each loin, the

121 adipose and the superficial connective tissue were removed in order to obtain a large surface of

122 lean meat. This was done under hygienic conditions (in a room at $10°C$, disinfected table and

123 knives and wearing disposable gloves, but no other specific measures were taken to prevent

124 bacterial contamination). The other part was left intact. Then, seven surfaces of 25 cm^2 were

125 delineated using a sterile stainless-steel template (5×5 cm) and surgical knife on the surface

126 of both parts of each loin. Each delineated surface was inoculated by spreading 100 μl of the

127 inoculum solution over the surface using a sterile Drigalski spatula. After drying of the

128 inoculum for 15 min, the four parts were placed in the drying cabinet adjusted to the targeted

129 temperature and relative humidity, for a total of six weeks.

130 **2.5. Quantification of pathogens**

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131 On day 1, 4, 8, 14, 21, 28, 35 and 42, one inoculated surface was collected from each loin part

132 using a sterile surgical knife and forcep, and placed in a stomacher bag. To all samples (25 cm^2

133 each), 25 ml of 0.1% peptone water was added. After homogenization using a stomacher for 1

134 minute, appropriated agar media were used to enumerate the different types of microorganisms.

135 Homogenates were plated on XLD, CT-SMAC and ALOA to enumerate *Salmonella*, *E. coli*

136 O157:H7 and *L. monocytogenes*, respectively. In order to obtain a detection limit of 1 CFU/cm^2,

137 1 ml of each homogenate was spread over three plates of the same agar medium. To recover

138 and to enumerate sublethally injured cells of *Salmonella* and *E. coli* O157:H7, the thin agar

139 layer method was applied, as described by Wu, Fung, Kang, & Thompson (2001). Therefore,

140 on the day of use, 25 ml of both XLD and CT-SMAC agar medium was poured into petri plates.

141 After solidification of the selective agar medium, 7 ml of TSA was spread over all agar media,

142 and plates were used to enumerate *Salmonella* and *E. coli* O157:H7 as described above.

143 **2.6. Background flora**

144 On day 0 and day 42, 25 cm^2 of the superficial adipose tissue and lean meat was collected from

145 the respective parts of all loins, using a sterile stainless-steel template, similar as described

146 above. To enumerate the background flora, the following agar plates and incubation conditions

147 were used: 1) Plate Count Agar (Oxoid) incubated at 22 °C for 5 days for Total Aerobic Count,

148 2) Violet Red Bile Glucose Agar (Bio-Rad, Temse, Belgium) incubated at 37 °C for 1 day for

149 *Enterobacteriaceae*, 3) Pseudomonas C.F.C. agar (Oxoid) incubated at 25 °C for 2 days for

150 *Pseudomonas* spp.; 4) De Man, Rogosa and Sharpe agar (Oxoid) incubated at 22 °C for 5 days

151 for Lactic Acid Bacteria, and 5) Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Oxoid)

152 incubated at 25 °C for 3 days for yeasts and molds. The pour plate method was used for

153 *Enterobacteriaceae* with a detection limit of 1 CFU/cm^2 and the spread plate method for all

154 other microbial flora with a detection limit of 10 cfu/cm^2. On DRBC agar plates, yeast and

155 molds were enumerated separately.

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2.7. pH measurements

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157 The pH of the loins was measured internally in duplicate on day 0 and 42, using a pH apparatus

158 Knick portamess 654 (Knick Elektronische Messgeräte GmbH, Berlin, Germany) with an

159 electrode LoT406-M6-DXK-S7/25 (Mettler-Toledo, Urdorf, Switserland).

2.8. Statistical analysis

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161 All analyses and visualisations were performed in RStudio using R version 4.0.2. The mean

162 difference in pH at day 0 versus day 42 was tested using a linear mixed model, including a

163 random intercept for loin to account for the dependence of samples within loins (Bates et al.,

164 2015). We didn't test for differences in pH between each of the conditions since our study was

165 not designed to detect such differences.

166 Bacterial counts were log_{10} transformed for analysis. Background flora were summarized

167 descriptively (median, minimum and maximum values). The reduction of pathogens (in log_{10}

168 CFU/cm^2) was calculated by subtracting the initial inoculation level (i.e. day 0). For negative

169 samples, a value of zero was used to calculate the reduction. Linear regression models without

170 intercept were fitted per loin/matrix, using the reduction (in log_{10} CFU/cm^2) as outcome and

171 time (in days) as a continuous variable. Decimal reduction times (D-values) were then

172 calculated as the negative reciprocal of the slopes (De Jesus and Whiting, 2003). Due to the

173 explorative nature of this study, only two replicates were performed per condition, and the level

174 of reduction between conditions was therefore not compared using a formal analysis.

175 Sublethality of *Salmonella* and *E. coli* O157:H7 cells was estimated by substracting the counts

176 using the selective agar medium from the counts using the overlay method (both expressed as

177 log_{10} CFU/cm^2). Only samples with countable numbers (i.e. above the detection limit) on both

178 agar media were taken into account.

3. Results

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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

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204   dry-aging processs was predicted for each of the loins (and for lean and adipose tissue

205   separately) using linear models. The values for the resulting slopes are provided in

206   Supplemental file, Table S3, and are visualized in Figure S2. The corresponding D-values per

207   condition, matrix, and replicate can be found in Table 2.

208   A significant linear decrease over time was predicted for *Salmonella* for both tissue types on

209   all loins (Figure 1 and Supplemental file, Figure S2). The daily reductions varied between -0.07

210   and -0.14 $\log_{10}$ CFU/day on adipose tissue and between -0.07 and -0.12 $\log_{10}$ CFU/day on lean

211   meat (Supplemental file, Table S3). The corresponding D-values varied between 7 and 14 days,

212   depending on the loin, matrix, and condition (Table 2). With the exception of lean meat in

213   condition A (6°C, 75% RH), both replicates yielded very similar results for *Salmonella*. For all

214   conditions, a reduction of at least 3 log was predicted after 42 days of dry-aging. On adipose

215   tissue, a reduction over 4 log was observed after 42 days for the loins under condition A and B

216   (6°C and 2°C, 75% RH). For loins in condition D (2°C, 85% RH), a similar level of reduction

217   (approx. 4 $\log_{10}$ CFU) was observed for both tissue types.

218   Similar to *Salmonella*, dry-aging caused a statistically significant reduction of *E. coli* O157:H7

219   on all loins, for both tissue types (Figure 2 and Supplemental file, Figure S2). The estimated

220   reduction on each of the loins varied between -0.14 and -0.11 $\log_{10}$ CFU/day on adipose tissue

221   and between -0.13 and -0.09 $\log_{10}$ CFU/day on lean meat (Supplemental file, Table S3). The

222   predicted D-values varied between 7 and 11 days (Table 2), corresponding to a reduction of

223   more than 3.8 $\log_{10}$ CFU after 42 days of dry-aging. For most of the loins, *E. coli* O157:H7

224   numbers were estimated to decrease faster on adipose tissue than on lean meat, as demonstrated

225   by the lower D-values for adipose tissue compared to lean meat of the same loin (Table 2).

226   The reduction of *L. monocytogenes* was not statistically significant for all loins (Figure 3 and

227   Supplemental file, Figure S2). For loins in which a significant reduction was observed, the

228   maximum estimated reduction was -0.06 $\log_{10}$ CFU/day on adipose tissue and -0.07 $\log_{10}$

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229 CFU/day on lean meat (Supplemental file, Table S3). The estimated D-values varied between

230 14 and 42 days (Table 2). Although on certain loins a reduction of almost 3 $\log_{10}$ CFU was

231 predicted after 42 days of dry-aging (D-value around 14 days), for other loins only about 1 log

232 reduction was predicted by the end of dry-aging (D-value around 42 days). *L. monocytogenes*

233 significantly increased for one replicate of lean meat at condition D (2°C, 85% RH), resulting

234 in a 1 log higher number at day 42 than the initial inoculated number of *L. monocytogenes*

235 (Supplemental file, Figure S2).

236 The sublethality for *Salmonella* and *E. coli* O157:H7 is presented in Supplemental file, Figure

237 S3. For both tissues, the median of the differences between the counts on thin overlay plates

238 and the selective plates was mostly around 0 $\log_{10}$ cfu/cm^2 in each of the four conditions.

239 **4. Discussion**

240 In the present study, the evolution of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* during

241 the dry-aging of beef was evaluated, using different temperatures (2 °C and 6 °C) and relative

242 humidities (75% and 85%). On all loins, *Salmonella* and *E. coli* O157:H7 significantly

243 decreased during dry-aging for 42 days.

244 For *Salmonella* and especially for *E. coli* O157:H7, dry-aging for 6 weeks resulted in a

245 reduction of at least 3 $\log_{10}$ CFU/cm^2. Tittor et al. (2011) observed a mean reduction of 4.65

246 $\log_{10}$ CFU/cm^2 for *Salmonella* and 4.76 $\log_{10}$ CFU/cm^2 for *E. coli* O157:H7 on lean meat and

247 adipose tissue collected from beef carcasses during 28 days at 3°C and a RH of 80%. Moreover,

248 Knudsen et al. (2011) demonstrated that the *Salmonella* reduction during dry-aging was

249 serotype and even strain dependent, with daily reductions varying between -0.113 and -0.216

250 $\log_{10}$ CFU/cm^2 over a period of 14 days (at 1 °C in a conventional refrigerator with a RH varying

251 between 70 and 100%). In the present study, we found (slightly) slower daily reductions of

252 *Salmonella*, varying between -0.07 and -0.14 $\log_{10}$ CFU. Since we used the initial inoculation

253 level as the maximum reduction (i.e., for samples below the limit of detection), the estimates

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254 for daily reductions and the corresponding D-values may be biased when a considerable number

255 of observations were negative. As such, for *Salmonella* and *E. coli* O157:H7, several samples

256 of condition A and D were negative near the end of aging period due to a steep decrease during

257 the first days. Therefore, the actual reduction during these conditions may be underestimated in

258 our study, and the reported estimates can thus be considered conservative. Similar to Knudsen

259 and colleagues (2011), we also assumed a log-linear reduction over time, which seemed very

260 plausible in most cases, though a non-linear shape for the survivor curve (Geeraerd et al., 2005)

261 may be more appropriate in other cases. Indeed, the reduction of both pathogens seemed more

262 pronounced during the initial days of dry-aging, on adipose tissue in particular. To represent

263 contamination of the meat originating from an animal source in the slaughterhouse (rather than

264 from the environment), the bacteria in the present study were grown at 37°C prior inoculation,

265 which was also applied by Knudsen and colleagues (2011), whereas Tittor and colleagues

266 (2011) used a cocktail of frozen cells. The use of cold adapted cells may result in a different

267 survival ability during the dry-aging of beef and should still be assessed. Additionally, only

268 stationary phase cells were used in the present study, so the potential effect of starting

269 physiology of the cells on the potential growth/survival during dry-aging is yet to be elucidated.

270 Comparison of the counts for *Salmonella* and *E. coli* O157:H7 obtained by the thin layer agar

271 and direct enumeration method on the selective agar revealed that the differences varied around

272 zero. This indicates that the presence of sublethally injured cells of both pathogens was in most

273 cases very limited and that the observed reductions are mainly due to dying of the cells.

274 With around 1 to 3 $\log_{10}$ CFU reductions after 42 days in most cases, the decline of

275 *L. monocytogenes* was less pronounced than those of *Salmonella* and *E. coli* O157:H7. Da Silva

276 and colleagues (2019) used *L. innocua* as a surrogate for *L. monocytogenes* and observed a 2.4

277 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,

278 respectively. In our study, a large difference in *L. monocytogenes* counts was observed between

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279   both replicates of condition D (2°C, 85% RH), particularly for lean meat. A reduction was

280   observed on one loin (with a pH < 6), whereas *L. monocytogenes* counts increased on the other

281   loin (with a pH > 6). This may indicate that the pH of the meat could have an important effect

282   on the survival and potential growth of *L. monocytogenes* during dry-aging of beef.

283   Although there doesn't seem to be a large difference between the temperature and RH

284   combinations that were evaluated during this study, and some differences were observed

285   between replicates, several other factors may have contributed to the observed differences as

286   well. Also general microbiological counts varied substantially between loins in this study, and

287   a similarly large variability was also observed under routine practices (Gowda et al., 2022). In

288   the present study, a mixture of three strains was used of each pathogen to account for possible

289   variation among strains, yet the growth/survival using single strains deserves to be performed

290   to account for strain variability. In addition, three different pathogens were evaluated

291   simultaneously to increase efficiency of our study, though the effect of potential interactions

292   between pathogens was not assessed. The causal effect of distinctive factors contributing to the

293   microbiological load and safety as well as the (sensory) quality of dry-aged beef is still not fully

294   elucidated (Terjung et al., 2021). The development of guidelines and criteria for the safe

295   production of dry-aged beef requires a better knowledge on the (combination of) factors for

296   growth/no-growth of psychrothrophic pathogens, such as *L. monocytogenes*. Therefore, future

297   studies should assess the causal relations of different factors, including pH, temperature,

298   relative humidity and background flora. Only a limited number of conditions were tested during

299   the present study (2°C and 6°C, combined with 75% and 85% RH), which don't fully represent

300   the true variation of practices that are used in routine production (Gowda et al., 2022).

301   Nevertheless, we observed that in most cases *L. monocytogenes* counts significantly decline

302   during dry-aging, and when they are able to multiply, growth seems to be slower compared to

303   growth on vacuum packed beef (Grau and Vanderlinde, 1990).

304 Despite the difference in pH of the lean meat of the two loins in condition D (2°C and 85%

305 RH), the level of reduction for *Salmonella* and *E. coli* was similar for both loins. Ribeiro and

306 colleagues (2021) found similar bacterial plate counts on dry-aged beef from higher pH

307 carcasses (average pH 6.69) compared to normal pH (average pH 5.47), whereas for wet-aged

308 beef significantly higher numbers were found on beef with a higher pH. Although our study

309 indicates that meat with a high pH might present a higher risk for *L. monocytogenes*, this doesn't

310 seem apparent for *Salmonella* nor *E. coli* O157:H7.

5. Conclusions

312 Our study confirms that pathogens such as *Salmonella* and *E. coli* O157:H7 decrease during

313 dry-aging, as at least a three log reduction was observed for both pathogens under all conditions

314 after 42 days. To the best of our knowledge, this is the first study indicating the potential growth

315 of *L. monocytogenes* during dry-aging of beef. Contrarily to *Salmonella* and *E. coli* O157:H7,

316 the reduction over time was small for *L. monocytogenes*, and we found that this pathogen can

317 potentially grow, even if the temperature is within the legal requirements of dry-aging. In order

318 to obtain more insight in the effect of the processing conditions (temperature and relative

319 humidity) and other factors (such as pH and background flora) on the evolution of

320 *L. monocytogenes* during dry-aging, more extensive studies are necessary.

6. Author contributions

322 **I. Van Damme**: Conceptualization, Methodology, Formal analysis, Data curation,

323 Visualization, Writing – Original Draft, Writing – Review & Editing; **S. Varalakshmi**:

324 Investigation, Writing – Original Draft, Writing – Review & Editing; **L. De Zutter**:

325 Conceptualization, Methodology, Supervision, Writing – Original Draft, Writing – Review &

326 Editing, Resources, Funding acquisition; **E. Vossen**: Conceptualization, Methodology,

327 Investigation, Project administration, Writing – Review & Editing, Resources; **S. De Smet**:

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Conceptualization, Methodology, Resources, Supervision, Funding acquisition, Writing – Review & Editing.

7. Competing interests

8. Funding

9. Data statement

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

10. Acknowledgements

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Figures

Figure 1. Reduction of *Salmonella* spp. 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. The dashed horizontal line

indicates the maximum reduction that could be detected, and is the negative value of the initial

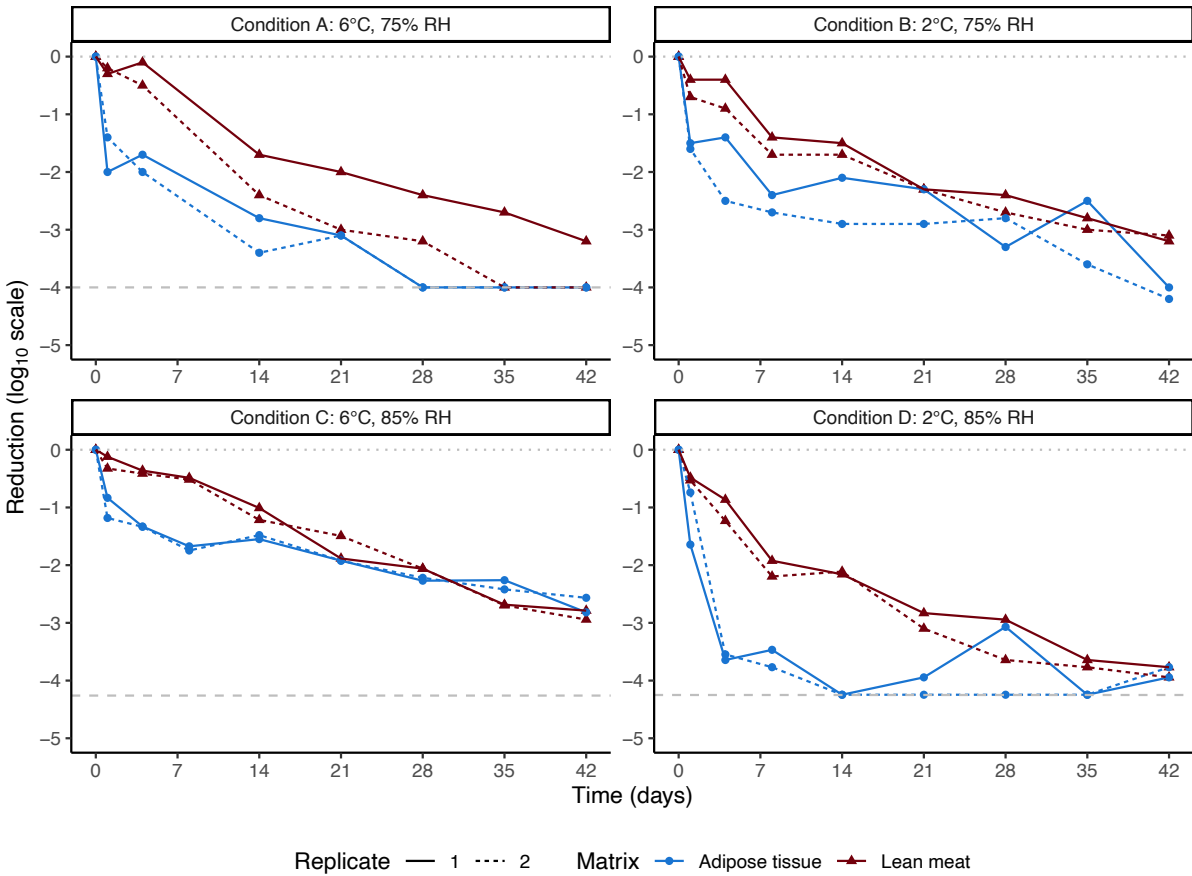inoculation level. Negative samples are plotted on the latter line.

435 **Figure 2.** Reduction of *Escherichia coli* O157:H7 numbers (in $\log_{10}$ CFU/cm^2) on lean meat

436 and adipose tissue during dry-aging of beef loins at different conditions.

437 Observations on lean meat are presented as triangles, and adipose tissue as circles. The different

438 replicates (two per condition) are indicated with a solid (replicate 1) or dashed (replicate 2) line.

439 Reductions are calculated relative to the initial inoculation level. The dashed horizontal line

440 indicates the maximum reduction that could be detected, and is the negative value of the initial

441 inoculation level. Negative samples are plotted on the latter line.
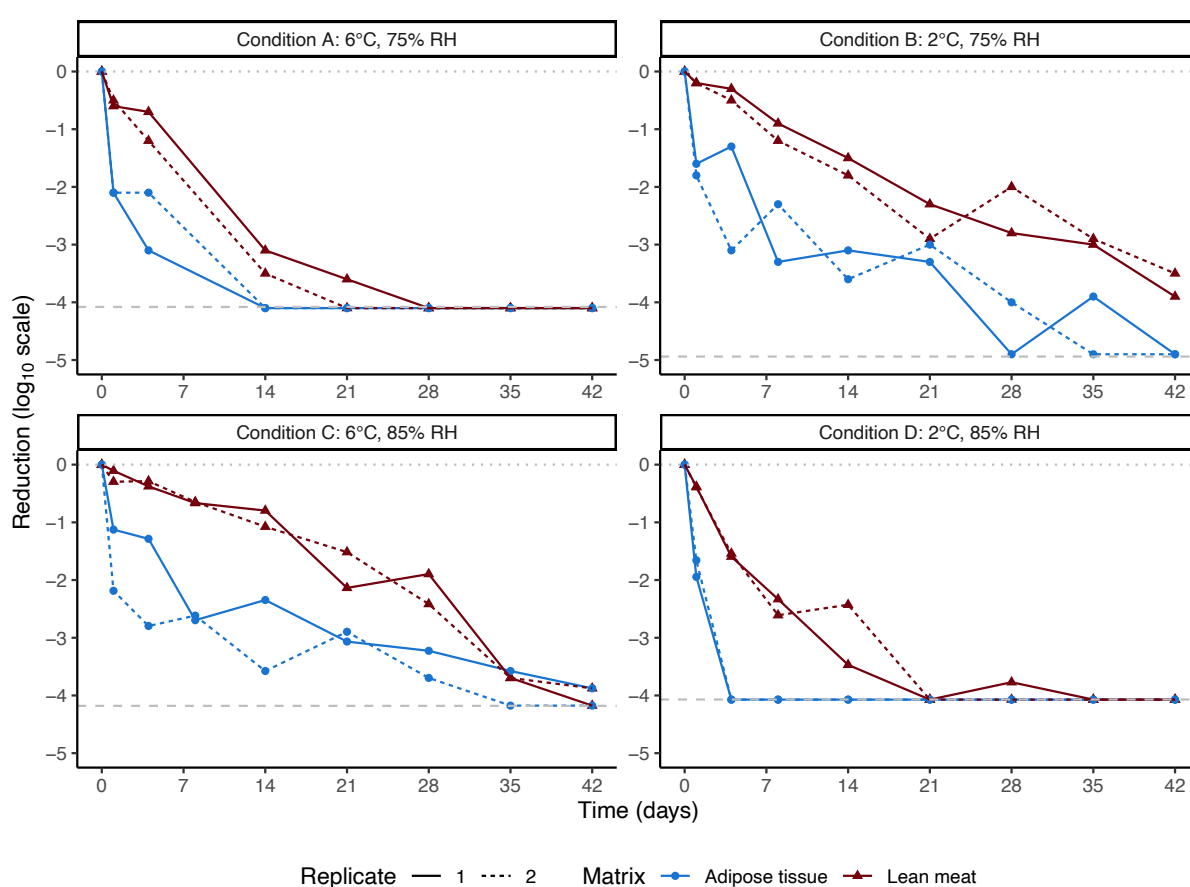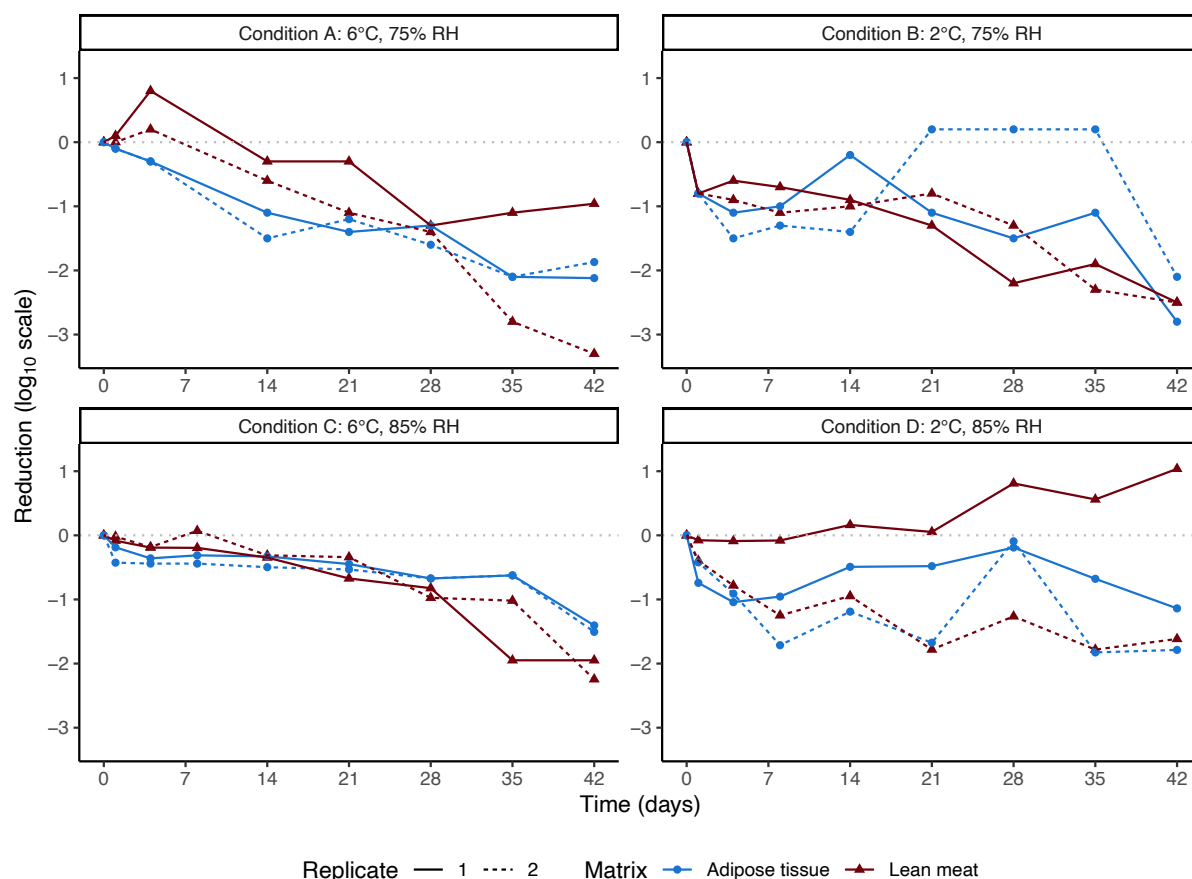

442

443   **Figure 3.** Evolution of *Listeria monocytogenes* numbers (in $\log_{10}$ CFU/cm^2) on lean meat and

444   adipose tissue during dry-aging of beef loins at different conditions.

445   Observations on lean meat are presented as triangles, and adipose tissue as circles. The different

446   replicates (two per condition) are indicated with a solid (replicate 1) or dashed (replicate 2) line.

447   Reductions are calculated relative to the initial inoculation level.

448

449

Tables

Table 1. Background flora on lean and adipose tissue of loins before and after dry-aging (n = 8).

Microorganism	Start (day 0)		End (day 42)	
	Adipose tissue	Lean tissue	Adipose tissue	Lean tissue
Total aerobic count	2.9 [2.1, 4.2]	2.3 [<1, 4.0]	5.1 [2.4, 7.8]	2.5 [1.3, 4.2]
Enterobacteriaceae	<0 [<0, 2.8]	<0 [<0, 3.1]	1.6 [<0, 2.5]	0.4 [<0, 2.6]
Lactic acid bacteria	1.2 [<1, 2.2]	<1 [<1, 3.4]	1.5 [<1, 7.0]	1.9 [<1, 4.0]
Pseudomonas spp.	2.1 [<1, 4.2]	<1 [<1, 4.2]	4.0 [<1, 7.4]	<1 [<1, 2.5]
Moulds	<1 [<1, 1.0]	<1 [<1, 4.2]	<1 [<1, 1.9]	<1 [<1, 1.6]
Yeasts	<1 [<1, 1.9]	1.1 [<1, 6.3]	3.5 [<1, 7.0]	1.5 [<1, 4.2]

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.

Matrix	Condition	*Salmonella* spp.		*E. coli* O157:H7		*L. monocytogenes*	
		R1	R2	R1	R2	R1	R2
Adipose tissue	6°C, 75% RH	8	8	8	8	18	18
	2°C, 75% RH	10	9	7	7	19	42
	6°C, 85% RH	13	13	9	8	38	35
	2°C, 85% RH	8	7	7	7	42	21
Lean meat	6°C, 75% RH	12	9	8	8	36	14
	2°C, 75% RH	12	11	11	11	16	17
	6°C, 85% RH	14	14	11	11	23	27
	2°C, 85% RH	9	9	8	8	NA*	20

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