Italian Journal of Food Safety

https://www.pagepressjournals.org/index.php/ijfs/index

eISSN 2239-7132

Ital J Food Saf 2026 [Online ahead of print]

Please cite this article as:

Savini F, Indio V, Prandini L, et al. **Outside in: assessment of microbial composition of the crust of dry-aged beef and its relevance in relation to food business operator practices**. *Ital J Food Saf* doi:10.4081/ijfs.2026.14620

Submitted: 03-11-2025
Accepted: 21-01-2026

Outside in: assessment of microbial composition of the crust of dry-aged beef and its relevance in relation to food business operator practices

Federica Savini,[1] Valentina Indio,[1] Laura Prandini,[1] Federico Tomasello,[1,2] Alessandra De Cesare,[1] Chiara Oliveri,[1] Alessandro Seguino,[1] Elena Zanato,[1] Andrea Serraino[1]

[1]Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Italy; [2]IRTA-Food Quality and Technology, Monells (Girona), Spain

Correspondence: Federica Savini, Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Italy. E-mail: federica.savini3@unibo.it

Key words: meat sterility, GHP, GMP, shotgun metagenomics, microbial ecology, culture independent technique.

Contributions: Federica Savini: conception and design, analysis and interpretation of data, writing – original draft preparation, project administration. Valentina Indio, Elena Zanato: analysis and interpretation of data, writing – original draft preparation. Laura Prandini: analysis and interpretation of data. Federico Tomasello: analysis and interpretation of data. Alessandra De Cesare, Chiara Oliveri, Alessandro Seguino: writing – review and editing. Andrea Serraino: conception and design, resources, project administration, writing – review and editing.

Conflict of interest: the authors declare no conflicts of interest.

Ethics approval and consent to participate: not applicable.

Availability of data and materials: the sequencing data generated during this study can be found at the SRA-NCBI bioportal under the projec id PRJNA1344894

Funding: this research was funded by the project “Contratto di filiera, Alleviamo Sostenibile, DM n. 0673777 del 22/12/2021, V Avviso n. 0182458 del 22/04/2022 e s.m." CUP - CFDA5243/07/J79I21000140001”

Abstract
Dry aging of beef has recently been defined in Delegated Regulation 1141/2023, amending Regulation 853/2005. The delegated regulation lists specific measures to be applied when processing such a product. Specifically, a point is dedicated to the crust trimming that should be carried out in a hygienic manner, since the interventions performed at the end of the process might determine contamination of the edible parts.
Nevertheless, despite the punctual application of good hygiene practices (GHP) and good manufacturing practices (GMP), a certain degree of contamination with pathogenic and spoilage microorganisms of the cut portions cannot be avoided, as demonstrated by some authors reporting contamination of the inner parts of dry-aged meat.
In order to investigate the level of contamination occurring in field conditions during trimming and portioning, we performed two different trials: the sterility trial with the aim of evaluating the sterility of the inner parts of beef during aging and the contamination trial to assess the transfer of microbial populations from the outer to the inner part of the dry-aged beef. All tests were performed by means of cultural and non-cultural methods.
Results of the sterility trial show that a very limited percentage of non-host DNA is present in the inner parts of the meat starting from the beginning of the test and that the detectable DNA increases slightly during the time of aging. Besides, the contamination trial results showed that the contamination of the trimmed meat is qualitatively and quantitatively related to the contamination of the crust.
As a consequence, adherence to GHP and GMP during trimming and handling of dry-aged meat according to scientific literature is crucial to avoid/minimize cross-contamination since our data clearly demonstrate that processing practices are fully reflected in the final product quality.

Introduction
A large diversity of meat products exists on the market; among them, dry-aged beef has gained significant attention in recent years for its enhanced flavor, tenderness, and overall quality. Dry-aging of meat is a specialized process in which meat is matured in controlled conditions for an extended period, ranging from a couple of weeks to several months up to one year. During this process, enzymatic reactions and moisture evaporation occur, leading to the development of a unique texture in the meat.
Following the increasing popularity that the technique is gaining among food business operators and private consumers, as a result of a request from the European Commission, a Scientific Opinion entitled "Microbiological safety of aged meat" has recently been released from EFSA (EFSA Panel on Biological Hazards *et al*., 2023). In the document, the BIOHAZ panel concluded that such a product "…does not create a higher public health risk than fresh meat if certain requirements are complied with".
Based on the aforementioned EFSA Opinion Regulation 1141/2023 (European Commission, 2024) has been issued, amending Annex III of Regulation 853/2004 that includes now the definition of dry aging as follows: "the storage of fresh meat in aerobic conditions of hanging carcasses or cuts either unpacked or packed in bags permeable to water vapour in a refrigerated room or cabinet and left to age for several weeks at controlled environmental conditions of temperature, relative humidity and airflow". Even though the Regulation is very specific in terms of environmental conditions and duration of the process, it opens to the possibility for food business operator (FBO) to apply other combinations of process parameters and to process meat of other species if they demonstrate to the satisfaction of the Competent Authority that equivalent guarantees are provided on the safety of the meat.
Literature has to date reported that throughout the process, the total bacteria count (TBC) increases on the crust, with psychotropic bacteria and Pseudomonas accounting for the majority, but different studies report varying situations (Gowda *et al*., 2022; Savini *et al*., 2024), probably due to the application of customized process parameters within the cabinets.

Based on mathematical models, among pathogenic microorganisms, *Listeria monocytogenes* is regarded as the only one capable of growing in aging conditions on beef (EFSA Panel on Biological Hazards *et al*., 2023), while studies *in vivo* have demonstrated that not only is no growth evidenced, but also a decrease might take place throughout the process (Muniz da Silva *et al*., 2019; Gowda *et al*., 2022; Savini *et al*., 2025). Given the scarcity and the discrepancy among the available data, good hygiene practices (GHPs) specific to the production and storage of dry-aged meat are defined within Regulation 2024/1141, reporting that "when the crust is trimmed, such trimming shall be carried out in a hygienic manner". Additionally, it is stated that "the dry crust should not be used for the preparation of other products as it contains most of the microbial contamination unless subsequently treatments such as heat or high pressure are applied to eliminate any pathogens present".

Nevertheless, despite the punctual application of GHP and good manufacturing practices (GMP), a certain degree of contamination with pathogenic and spoilage microorganisms of the cut portions cannot be avoided, as demonstrated by some authors reporting contamination of the inner parts of dry aged meat (Mikami *et al*., 2021; Gowda *et al*., 2022).

This study was conducted mimicking FBOs' operational conditions applied when aging beef up to 60 days within dedicated cabinets, with the objective to assess the effect on the bacterial contamination load of the portioning phases.

Internal portions of meat intended for human consumption are generally assumed to be sterile, but since about 80% of the microbes inhabiting the human body cannot be found via conventional culture-based techniques (Chen *et al*., 2023), we have analyzed samples coupling shotgun metagenomics and culture methods. Additionally, we have conducted a sterility assessment to determine the bacterial load present on the internal portions of aseptically collected samples throughout the aging period.

Through our analysis, we aim to contribute to the understanding of the relationship between hygienic practices and the quality of dry-aged meat, providing insights into the necessary precautions and control measures for implementing the shelf life of safe and high-quality production.

Materials and Methods

Sampling plan

Two different trials were performed, the first with the aim to assess the sterility of the inner parts of beef during ageing (Sterility Trial - ST) and the second to assess the transfer of microbial populations from the outer to the inner part of the dry-aged beef during trimming and portioning under field conditions (Contamination Trial - CT). In both trials, the ageing was performed within a Stagionello® Meat Curing Device cabinet, equipment designed and patented (European Patent No. EP 2769276B1; Canadian Patent No. CA2852650) for meat dry aging. The parameters set during the 60 days of aging were 1 ± 2 °C of temperature, RH 78±7% and ventilation 2.0 m/s. This combination of parameters has already been validated (Savini *et al*., 2024) in accordance with EU Regulation 2024/1141.

For the ST, a total of 4 loins (*longissimus dorsi*) from the same slaughtering batch, weighing between 3.5 kg and 5.5 kg, were purchased by a local supplier, sourced from Holstein male beef cattle of average age of 23 months and 360 Kg. After slaughter, the half carcasses were chilled (0±3ºC) for 4 days, after which the loins were removed and vacuum-packed for 8 days till the delivery to the laboratory. Each loin was considered a sampling unit, that were randomly dedicated to a collection timepoint, namely 0, 30, 45 and 60 days (identified as T0, T30, T45, and T60) of dry-aging.

For the CT, 3 Black Angus (mean age 26 months) loins of the same slaughtering batch were purchased vacuum-packed by the same local supplier. Each loin, of approximately 5,5 kg, was considered a sample unit. After slaughter, the half carcasses were cold stored (0±3ºC) for 4 days, and subsequently rib steak was removed, and loins were vacuum packaged for till the arrival at the laboratory within 4 days**.** Samples were collected after 0, 30, 45 and 60 days (identified as T0, T30, T45 and T60).

All the entailed cuts were of normal bloomed beef color and did not present quality defects.

Sample collection

For the ST, at each sampling point, a heated branding iron (5x2 cm) was used to sterilize the surface of the lean meat of the loin for 60 seconds, allowing the cauterization to reach a depth of 3 mm. Then, the cauterized external part was removed using a sterile scalpel (Figure 1A) and a sample of at least 10 grams was excised from the more internal muscle using sterile instruments (Figure 1B). Half of the sample was immediately stored at -80°C for subsequent metagenomic analyses, while the remaining part was directly plated on blood agar dishes and cultured in fluid thyoglicollate medium (Oxoid Ltd., Basingstoke, UK). Samples for the CT were performed both on the surface and the inner parts of Black Angus loins; the first ones consisted of 10 g of the exposed muscular part of the loins, measuring 3±2 mm thickness. For the inner samples, a 3 cm thick steak was obtained by cutting with cleaned and disinfected knives and a bonesaw after removal of all the crust (about 1-2 cm). The described collection procedure was performed in order to mimic the real scenario applied by an FBO within its facility. A portion of the samples was stored at -80°C for subsequent metagenomic analyses, and another portion was used for total bacterial count and Enterobacteriaceae count according to ISO 48332:2013 and 215282:2017, respectively.

DNA extraction and shotgun metagenomic sequencing

The 12 samples of ST and 8 of CT for metagenomic investigation were thawed at 4°C for 4 hours and diluted 1:1 in 25 mL of sterile molecular biology-grade water. Homogenization was performed using a stomacher (MAYO HG 400V, Italy) at standard speed for 1 minute, followed by centrifugation at 9980× g for 20 minutes at 4°C. Total DNA was extracted from the resulting pellet and from one aliquot of nuclease-free water for molecular biology (Sigma, Milan, Italy) (*i.e.*, negative control) using a bead-beating protocol in combination with the PowerFood® Microbial DNA Isolation Kit (MO BIO-Qiagen) following the manufacturer's instructions. The quality and quantity of the extracted DNA were assessed using a BioSpectrometer® (Eppendorf, Milan, Italy). DNA libraries were then prepared by fragmentation and adapter ligation using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). The libraries concentrations and lengths were quantified using the 5200 Fragment Analyzer System (Agilent, Milan, Italy). Shotgun metagenomic sequencing was performed on the NovaSeq 6000 (Illumina) in paired-end mode with a read length of 2 × 150 bp. Sequencing data are publicly available at the SRA-NCBI bioportal under the project ID PRJNA1344894.

Sequences were processed in order to remove adapters and low-quality tails with AdapterRemoval (Schubert *et al*., 2016). The finished data were thus aligned against the *Bos taurus* genome (bosTau9) with bowtie2 in order to identify and remove the host (bovine) genome. Subsequently, metagenome and microbial composition analysis was conducted by adopting the kaiju algorithm (Menzel *et al*., 2016) and using the nr_euk dataset including bacteria, viruses, fungi and parasites as reference. Taxonomic classification was carried out, and the abundances of each taxon were calculated as percent relative abundance.

Statistical analyses

Statistical analysis of sequencing results was conducted in the R environment using the packages: t_test (rstatix) for comparative analysis of relative abundance among the groups of interest, ggplot2 to construct histograms, Heatmap (ComplexHeatmap) to construct heatmaps, and perform clustering (R Core Team 2022).

The metagenomics data of the Angus samples were analyzed with reference to the fungal component, the presence of spoilage microorganisms and *pathogenic bacteria* considered from the EFSA Opinion on microbiological safety of aged meat (2023).

Correlation analyses of genera abundance (expressed as Log_{10} RPM) between inner and outer surfaces at each timepoint was performed adopting a linear regression model.

The difference in sequencing abundance of non-host genomes reported in Holstein internal samples at different time points was evaluated by t-tests.

Results

The culture examination performed during the ST showed no growth on the blood agar plates and fluid thyoglicollate broth at any timepoint.

Metagenomic analysis of the ST showed that the non-host genome identified throughout time in the inner parts of the meat slightly shifts from a minimum of 0.59% at T0 (B3) to a maximum of 0.79% at T60 (B2) (Table 1). Going into more detail, only a small part of the non-host genome can be mapped on microbial genome, with the highest percentage of reads that belonged to the kingdom of bacteria (min 0.0147 at T0 B1; max 0.1475 at T60 B3), and to a negligible extent to eukariota (min 0.008 T0 B1; max 0.067 T60 B3) mainly represented by fungi and SARs (*i.e.*, the supergroup including *Stramenopiles*, *Alveolata* and *Rhizaria*). The overall percentage of non-host reads didn't change significantly during time ($p>0.05$), both for bacteria and eukaryota reads.

Statistical analyses performed by T test showed a significant difference for bacterial reads between T0 and T30 and for eukaryotic reads between T0 and T45

Reads of bacteria were quantified in the tested samples at each sampling time and belonged to four phyla (Figure 2): Bacillota and Pseudomonadota (*Supplementary Table 1*). While the Bacillota followed a fluctuating trend, in contrast, Pseudomonadota grew throughout the process. Specifically, a peak is described for both Pseudomonadota and Bacillota at T60, but evaluating the trend of the Log RPM, only Pseudomonadota appeared to have a constant increase during time (Figure 2).

Within the phylum Bacillota, the identified genera were *Staphylococcus*, *Streptococcus*, *Brochotrix* and *Enterococcus,* while within the phylum Pseudomonadota *Pseudomonas*, *Photobacterium*, *Klebsiella* and *Shewanella* were the most represented.

Regarding the CT of Black Angus loins in all samples, a low overall biomass of non-host DNA was detected in the inner meat samples compared with the outer ones.

Specifically, foreign DNA to host DNA on the external surface started from 20% at T0, followed by a decline at T30, with an increase at subsequent sampling times until it reached 91% of extracted DNA at T60. On the contrary, the inner parts showed a more static and much less abundant composition with values ranging from 4.98% at T0, followed by a decline in line with the external composition at T30, reaching 2.56%, a value that is stable until T60 (Figure 3).

Within the non-host genome, the microbial component that could be mapped belongs mostly to the kingdom of bacteria, ranging from 0.0796% at T to 1.4637% at T0 in the inner samples, and from 1.0308% at T15 to 41.6737% at T60 in the external samples (Table 2). On the other hand, the microbial Eukaryotic component is low and equally represented in all the analysed samples, and it is not higher than 0.0107%. Regarding the specific microorganisms, in both the inner and outer samples in almost all timepoints, the most represented phyla were Pseudomonadota, Bacillota, Bacteroidota and Actinomycetota (Figure 4). The most represented phyla remained stable over time during sampling, whereas the quantification of different phyla showed a progressive increase in bacterial load on the external surface. This phenomenon is not reflected in the IN samples, which consistently exhibit the same amount of non-host genome.

The linear regression analysis calculated between samples collected from the inner and the outer surface indicates a statistically significant positive correlation ($p\leq0.001$) during the first three time points (Figure 5), with a progressively decreasing correlation coefficient (T0 $R^2 = 0.51$; T30 $R^2 = 0.42$; T45 $R^2 = 0.11$), that is no longer maintained at the end of the process (T60 $R^2 = 0.00$). The only differences emerging from the inner *vs.* outer comparison pertain to poorly represented taxa (or those with p-value values above the significance threshold), indicating that contaminations in the inner part of the meat reflect those on the external surface. A higher correlation coefficient indicates a stronger relationship between the inner

and the external contamination. This suggests that the microbial communities detected in the inner portion of the meat largely mirror those present on the external surface during the first phases of dry-aging and not in the finishing, supporting the idea that potential contaminations originate externally and subsequently penetrate inward, rather than arising from distinct endogenous sources.
Analyses regarding the pathogenic bacteria (Figure 6) that can contaminate carcasses demonstrate that a very low number of reads is present on the surface, and generally lower amounts are reported on the inner samples if compared to the outer surface. This is evident for *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp. and *Yersinia* spp, while for *Staphylococcus* spp. and *Campylobacter* spp. similar values are reported in the outer and inner samples.
In relation to the fungal component, the very poor contamination of the outer samples did not show changes percentage-wise of *Debaryomyces*, *Aspergillus*, and *Penicillium* over the 60-days maturation period, similarly a very low number of reds was evidenced in the inner samples.
Among spoilage bacteria only for *Pseudomonas* the relative abundance increased over time, while for the other analysed bacteria a general decrease at the end of the process with a fluctuating trend over time can be evidenced. Exceptions are played by *Carnobacterium* and *Brochotrix* that have a fluctuating trend. Inner samples generally present a very low number of reads for all listed microorganism but *Enterobacteriaceae*, *Streptococcus* and *Enterococcus* that tend to reflect the load of the outer samples.
Total bacterial count in the outer and inner samples showed a trend roughly correspondent to the non host genome trend increasing in outer samples from 6.42±0.92 log CFU/g at T0 to 7.48±0.40 log CFU/g at T60 in the outer samples and from 3.51±1.15 log CFU/g at T0 to 3.02±1.04 log CFU/g at T60 in the inner samples. *Enterobacteriaceae* count resulted often under the quantification limit (10 UFC/g), more frequently in outer samples at the end of the ageing and in the inner samples in all the sampling points. Countable samples never exceeded 4.17 log CFU/g in the outer samples and 3.08 log CFU/g in the inner samples.

Discussion

In this study, we evaluated the sterility of the internal portions of meat and how trimming and portioning activities carried out by food industry operators handling dry-aged meat during 60 days of aging can influence product contamination of the inner portions at the end of the process in operational conditions. This experiment was organized to further investigate the observations of previous studies reporting that contamination can be evidenced in the internal parts of dry-aged meat (Capouya *et al*., 2020). In parallel, we investigated the microbiome of the inner and outer parts of the muscle.
Internal portion of meat is generally considered sterile at slaughtering, with contamination that should be limited to the meat surface, unless the animal is affected by bacteriaemia or in case contamination occurs during slaughtering. However, these two cases are uncommon in commercial conditions, and the results of this study confirmed that the inner part of the meat is sterile. On the other hand, some Authors (Gowda *et al*., 2022) occasionally reported high numbers of aerobic and anaerobic psychrotropic bacteria (>3 $\log_{10}$ CFU/g) or the presence of high total bacterial, coagulase-negative and lactic acid bacteria count (Mikami *et al*., 2021), highlighting the need for further studies. It is currently unclear whether the presence of bacteria in the inner part of the meat is due to the migration of bacteria from the surface to the inner part of the loin during the drying process, to the growth of bacteria that were already present inside the meat at the start of the process, or simply to possible contaminations during the trimming process.
In our study the reads detected by shotgun sequencing in the internal samples collected during the ST, which resulted sterile by cultural methods belonged to genera (*Staphylococcus*, *Streptococcus*, *Brochotrix*, *Enterococcus*, *Pseudomonas*, *Photobacterium*, *Klebsiella* and *Shewanella*) usually detected on the surface of dry aged meat (Campbell *et al*., 2001; Di Paolo *et al*., 2023; Savini *et al*., 2024). These results might be explained by the presence of viable bacteria unable to replicate or to the presence of

non-viable DNA introduced by means of the sampling instruments (*i.e.*, scalpel, forceps and tubes), airborne contaminants or a partial dragging from the outer surface despite the use of sterile tools and a rigorous sterilization of the surface of the meat.
The reads obtained in the deep samples in the CT were three times the order of magnitude of the ones collected with sterile tools, leading to the assignment of the results in the complex, to a DNA transfer from the crust rather than to a specific contamination inside the meat. The presence of contaminating DNA is indeed a particular challenge for samples containing a microbial mass where the starting material may be effectively swamped by the contaminating DNA (Salter *et al*., 2014).
In this regard, the sampling technique for the ST encompassed a first step where all microorganisms were thermally inactivated by means of direct burning and DNA was severely degraded, and subsequently, to minimize contamination, a progressively smaller surface was sampled from the outer to the inner layer, coupled with changing both scalpel and forceps, avoiding the collection of beef that had been in contact with the contaminated instruments. Eventual soil-airborne contamination cannot be excluded since sampling within the area of a Bunsen burner allows for providing an aseptic field in terms of live microorganisms but not of the absence of nucleic acids. Last but not least, the collection instruments themselves can be considered sterile but not DNA-free. Indeed, sterile swabs can contain numbers of microbial DNAs comparable to skin and oral ones (Kang *et al*., 2021). Since the sampling was performed in aseptic conditions in a non-DNA-free environment and using sterile but not DNA-free tools, the number of reads detected in this study might be roughly considered a threshold for sterile meat. This hypothesis is further corroborated by the absence of viable microorganisms on the cultures starting from the same sampling specimen.
The microbiome data regarding the contamination occurring during steak portioning demonstrate that the microbiological composition of trimmed meat generally reflects the external one in terms of both quality and quantity of microorganisms, in agreement with previous findings (Da Silva Bernardo *et al.*, 2021; Gowda *et al*., 2022). The results of the total bacterial count confirm the trend observed by metagenomic analysis.
In general, experimental studies indicate that, an increase of the surface TBC happens during aging up to 5-6 Log CFU/cm^2 in the first phases of the ageing and in some cases higher microbial counts are reported; similar values were also detected in steaks and in samples collected in commercial facilities or in samples at retail (Savini *et al*., 2024) and in the present study. Being the crust contamination directly implicated in that of the edible part, a pivotal role is also played by its quality. In this respect, a marginal role is played by the post-slaughter microbial diversity since it is demonstrated (Ryu *et al*., 2018; Savini *et al*., 2024), and further confirmed by our data, that regardless of the initial composition microbial population tends to become homogeneous throughout time. Specifically, *Pseudomonas* spp. tends to replace the other bacteria during ageing without signs of evident spoilage (Lee *et al*., 2019). Pseudomonads are psychrotrophic microorganisms capable of growing rapidly due to short generation time and usually dominate the spoilage consortium of bacteria under aerobic conditions (Stanbridge and Davis, 1998; Koutsoumanis *et al*., 2006). Interestingly, recent findings have evidenced that *Pseudomonas* spp. can also grow under strictly anaerobic conditions for at least 14 days (Hilgarth *et al*., 2019). Within our study, the two other most represented genera of spoilage bacteria are *Carnobacterium* (belonging to the lactic acid bacteria group) and *Brochotrix* spp., both listed with *Pseudomonas* as the main meat spoilage bacteria at refrigeration temperatures (EFSA, 2024) when aerobically stored, but also under anaerobic conditions (Ercolini *et al*., 2006; Húngaro *et al.*, 2016). In addition, the same microorganisms are listed as the most represented in dry-aged beef by other authors (Gowda *et al*., 2022), and that must be considered in future studies on the assessment of the shelf life of trimmed dry-aged meat.
Within the EFSA Opinion on microbiological safety of aged meat, information among FBOs has been collected by means of a questionnaire, revealing that the shelf-life for unpacked dry-aged beef steaks was

typically 4 days but ranged from 2 to 10 days, while in modified atmosphere it was 5 days, and if vacuum packed it increased to 18 days, ranging from 5 to 30 days.
To our knowledge only one study was conducted, on water buffalo meat, to evaluate the microbial behaviour during the shelf life of trimmed dry aged meat; in that study TBC, *Brochothrix termosphacta* count and lactic acid bacteria count were evaluated with the latter appearing to be of significant importance in the spoilage of trimmed dry aged meat and that should be included in the studies on the shelf life of trimmed dry aged meat.

Conclusions

The high sensitivity of the non-culture-dependent analysis helps identify different/more species than allowed by cultural methods, as culturable microorganisms may only represent a small fraction of the total microbiota (Duthoo *et al*., 2022). In our study, the penetration of microorganisms from the crust to the deeper parts of the meat cannot be demonstrated since the inner parts of the meat remained sterile by cultural examination during all the aging process. Results show that a very limited percentage of not host DNA is present in the inner parts of the meat since the beginning of the test and that the DNA detectable increase slightly during the time of ageing alongside with the increase of the external part of the meat: the overall results suggest that the DNA found in the deep samples can be due to transfer that occurred during sampling despite strict sterility procedures and more analysis should consider basal levels of contamination of the surgical tools as well as evaluate meta transcriptomics analyses for meat.
The CT results showed that the contamination of the trimmed meat is qualitatively and quantitatively related to the contamination of the crust. Adherence to GHP and GMP during trimming and handling of dry-aged meat according to scientific literature is crucial in order to avoid/minimize cross-contamination, since our data clearly demonstrate that processing practices are fully reflected in the final product quality. Shelf life studies on the trimmed dry-aged meat are lacking: this work contributes to the scientific knowledge useful to design future studies.

References

Campbell RE, Hunt MC, Levis P, Chambers E, 2001. Dry-aging effects on palatability of beef longissimus muscle. J Food Sci 66:196-9.

Capouya R, Mitchell T, Clark DI, Clark DL, Bass P, 2020. A survey of microbial communities on dry-aged beef in commercial meat processing facilities. Meat Muscle Biol 4:10373.

Chen J, Li T, Ye C, Zhong J, Huang JD, Ke Y, Sun H, 2023. The lung microbiome: a new frontier for lung and brain disease. Int J Mol Sci 24:2170.

Da Silva Bernardo AP, Muniz Da Silva AC, Saraiva Ferreira FM, Da Silva Do Nascimento M, Bertelli Pflanzer S, 2021. The effects of time and relative humidity on dry-aged beef: Traditional versus special bag. Food Sci Technol Int 27:626-34.

Di Paolo M, Ambrosio RL, Lambiase C, Vuoso V, Salzano A, Bifulco G, Barone CMA, Marrone R, 2023. Effects of the aging period and method on the physicochemical, microbiological and rheological characteristics of two cuts of charolais beef. Foods 12:531.

Duthoo E, De Reu K, Leroy F, Weckx S, Heyndrickx M, Rasschaert G, 2022. To culture or not to culture: Careful assessment of metabarcoding data is necessary when evaluating the microbiota of a modified-atmosphere-packaged vegetarian meat alternative throughout its shelf-life period. BMC Microbiol 22:34.

EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bover-Cid S, Chemaly M, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Blagojevic B, Van Damme I, Hempen M, Messens W, Bolton D, 2023. Microbiological safety of aged meat. EFSA J 21:e07745.

Ercolini D, Russo F, Torrieri E, Masi P, Villani F, 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl Environ Microbiol 72:4663-71.

European Commission, 2024. Commission Delegated Regulation (EU) No. 2024/1141 of 14 December 2023 amending annexes II and III to Regulation (EC) No. 853/2004 of the European Parliament and of the Council as regards specific hygiene requirements for certain meat, fishery products, dairy products and eggs. In: Official Journal, EN L series, 19/04/2024.

Gowda TKGM, De Zutter L, Van Royen G, Van Damme I, 2022. Exploring the microbiological quality and safety of dry-aged beef: a cross-sectional study of loin surfaces during ripening and dry-aged beef steaks from commercial meat companies in Belgium. Food Microbiol 102:103919.

Hilgarth M, Lehner EM, Behr J, Vogel RF, 2019. Diversity and anaerobic growth of Pseudomonas spp. Isolated from modified atmosphere packaged minced beef. J Appl Microbiol 127:159-74.

Húngaro HM, Caturla MYR, Horita CN, Furtado MM, Sant'Ana AS, 2016. Blown pack spoilage in vacuum-packaged meat: a review on clostridia as causative agents, sources, detection methods, contributing factors and mitigation strategies. Trends Food Sci Technol 52:123-38.

ISO, 2013. Microbiology of the food chain. Horizontal method for the enumeration of microorganisms. Colony count at 30°C by the surface plating technique. ISO Norm 4833-2:2013. International Organization for Standardization ed., Geneva, Switzerland.

ISO, 2017. Microbiology of the food chain. Horizontal method for the detection and enumeration of Enterobacteriaceae. Colony-count technique. ISO Norm 21528-2:2017. International Organization for Standardization ed., Geneva, Switzerland.

Kang Y, Ji X, Guo L, Xia H, Yang X, Xie Z, Shi X, Wu R, Feng D, Wang C, Chen M, Zhang W, Wei H, Guan Y, Ye K, Zhao G, 2021. Cerebrospinal fluid from healthy pregnant women does not harbor a detectable microbial community. Microbiol Spectr 9:e00769-21.

Koutsoumanis K, Stamatiou A, Skandamis P, Nychas GJE, 2006. Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat and validation of the model under dynamic temperature conditions. Appl Environ Microbiol 72:124-34.

Lee HJ, Yoon JW, Kim M, Oh H, Yoon Y, Jo C, 2019. Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. Meat Sci 153:152-8.

Menzel P, Ng KL, Krogh A, 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nat Commun 7:11257.

Mikami N, Toyotome T, Yamashiro Y, Sugo K, Yoshitomi K, Takaya M, Han KH, Fukushima M, Shimada K, 2021. Dry-aged beef manufactured in Japan: Microbiota identification and their effects on product characteristics. Food Res Int 140:110020.

Muniz da Silva ACM, de Oliveira Pena P, Pflanzer SB, da Silva do Nascimento M, 2019. Effect of different dry aging temperatures on Listeria innocua as surrogate for Listeria monocytogenes. Meat Sci 157:107884.

Ryu S, Park MR, Maburutse BE, Lee WJ, Park DJ, Cho S, Hwang I, Oh S, Kim Y, 2018. Diversity and characteristics of the meat microbiological community on dry aged beef. J Microbiol Biotechnol 28:105-8.

Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW, 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol 12:87.

Savini F, Indio V, Panseri S, Chiesa L, Negri A, Grassi Scalvini F, De Cesare A, Mazzoni M, Clavenzani P, Prandini L, Tomasello F, Terefe Mekonnen Y, Giacometti F, Serraino A, 2024. Impact of dry aging on quality parameters and microbiological safety of beef. LWT, 203:116390.

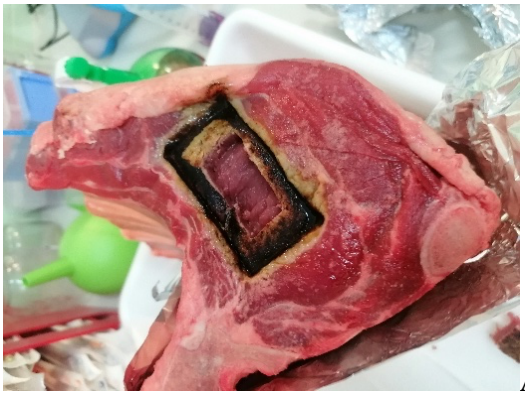
Savini F, Prandini L, Indio V, Tomasello F, Seguino A, De Cesare A, Panseri S, Giacometti F, Delibato E, Bardasi L, Taddei R, Serraino A, 2025. Behaviour of Listeria monocytogenes and Yersinia enterocolitica during beef dry-aging up to 60 days. Int J Food Microbiol 429:110999.

Schubert , Lindgreen S, Orlando L, 2016. AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Res Notes 9:88.

Stanbridge LH, Davis AR, 1998. The microbiology of chill-stored meat. In: Board RG, Davies AR, eds. The microbiology of meat and poultry. Blackie Academic and Professional, London, UK, pp 174-219.

Online supplementary material

Supplementary Table 1. List of the most represented phyla in the inner and crust samples.


A

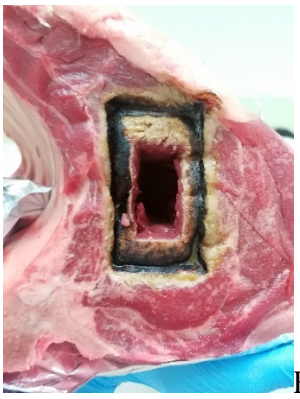

B

Figure 1. For the Sterility Trial, after cauterization of a 5×2 cm area, the superficial beef (approx. 3 mm depth) was excised (A), and a sample weighing min 10 grams was collected from the internal areas (B). The beef obtained from B has been used as a sample.

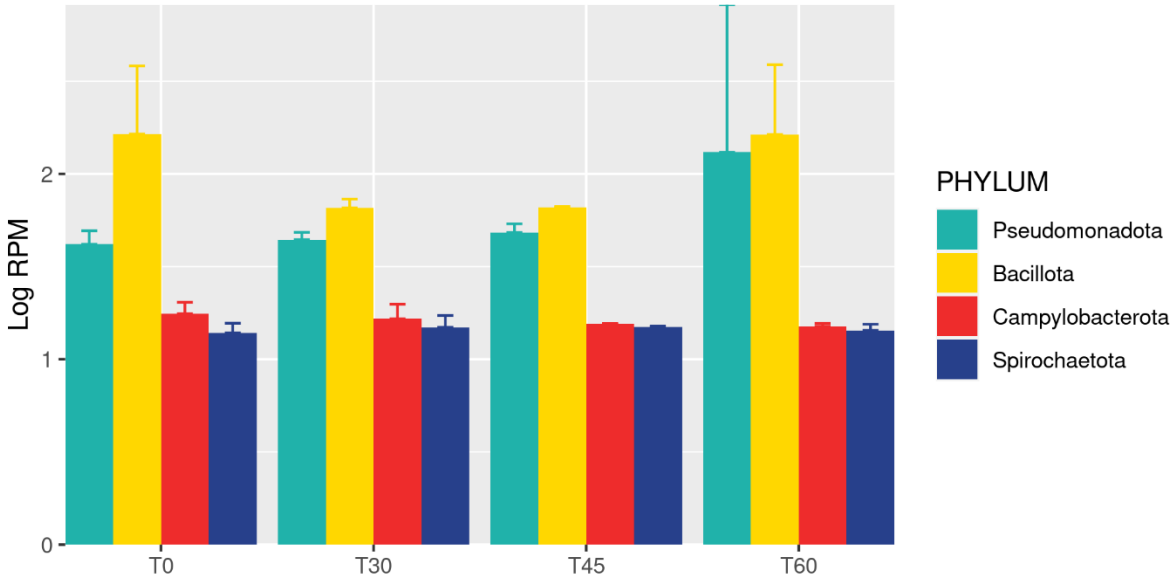


Figure 2. Representation of the inner composition of the bacterial genome retrieved within the sterility test.

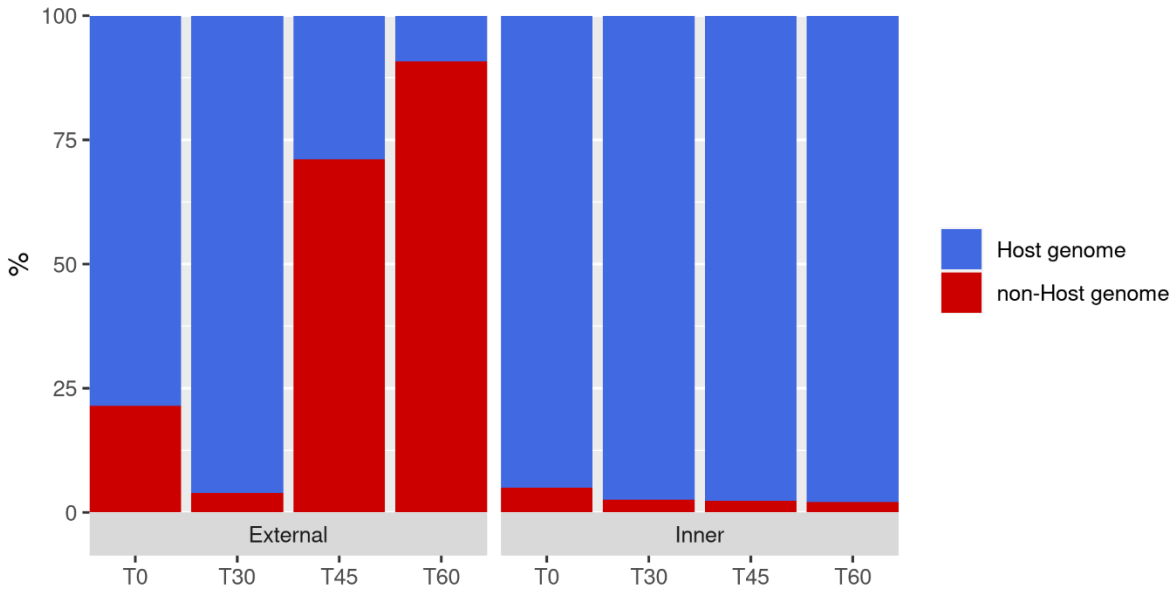


Figure 3. Graphical representation of the composition of the host vs non host genome on external and internal samples of Angus loins (CT).

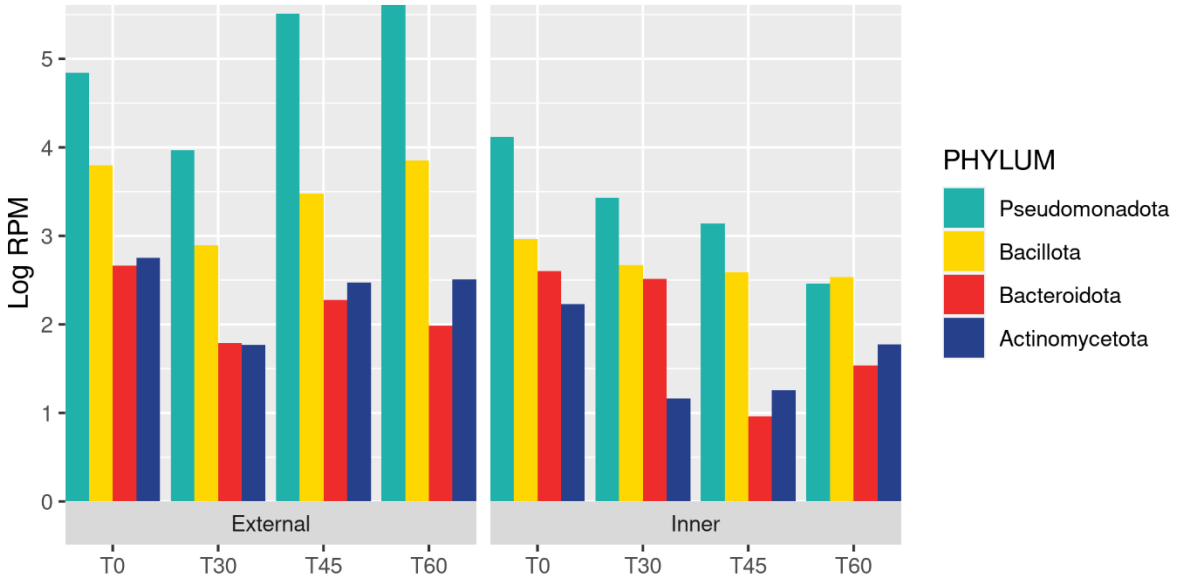


Figure 4. Graphical representation of the composition of the external and internal composition of the Angus samples (CT) at the phylum level.

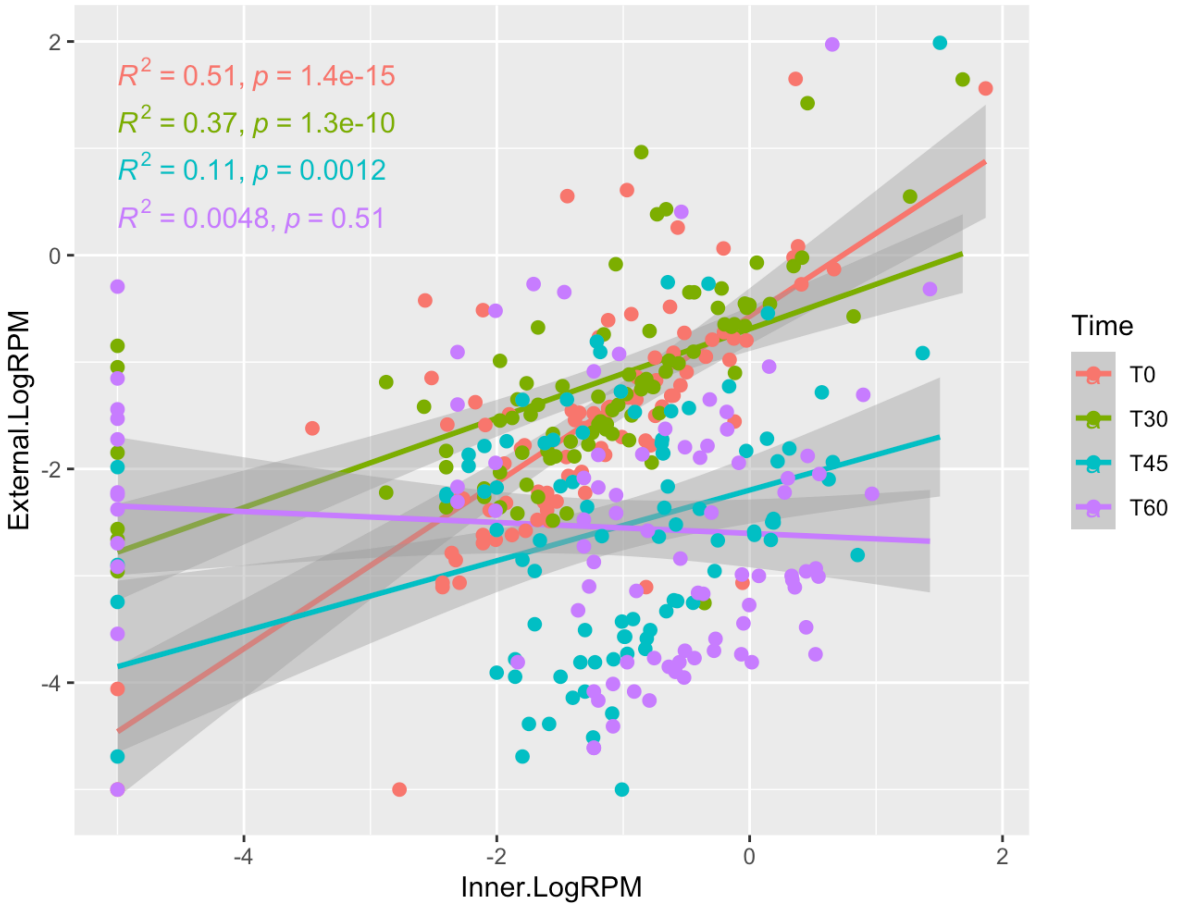


Figure 5. Linear regression showing the correlation between inner and external surface of meat at T0 (red), T30 (green), T45 (light blue), T60 (purple).

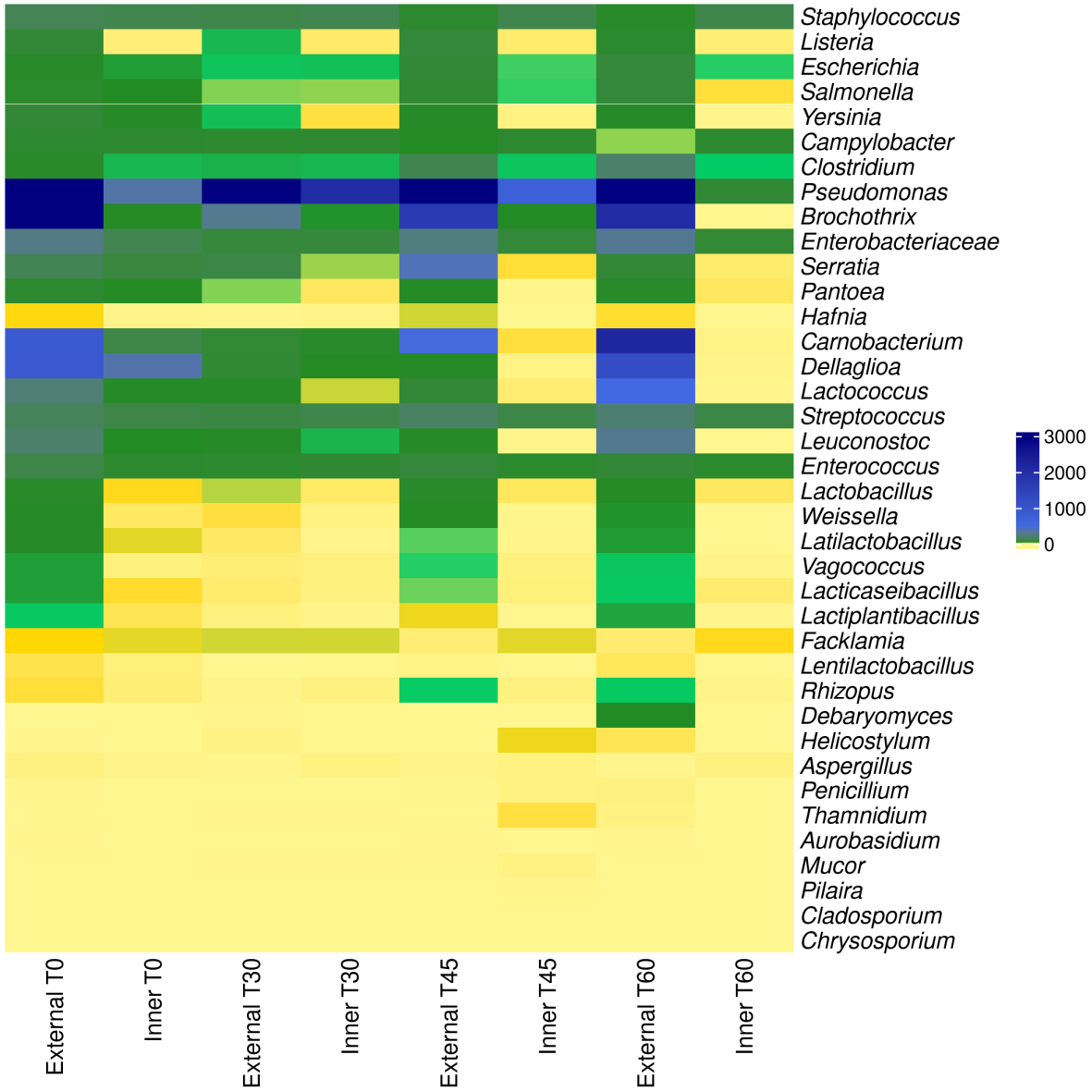


Figure 6. Heatmap representing the distribution of the pathogenic and spoilage bacteria, moulds and yeasts reported in the EFSA opinion "Microbiological safety of aged meat".

Table 1. Abundance of non-host reads reported in Holstein internal samples (mean of 3 batches) in Sterility Trial test over time; different letters in the same column show significant difference by T test (p<0.01).

Time point	Bacteria reads	% bacteria reads	Microbial eukaryota reads	% eukaryota reads
T0	2999 [a]	0.03	460 [a]	0.00
SD	1325	0.01	326	0.00
T30	6513 [b]	0.01	2594 [a]	0.01
SD	111	0.00	2035	0.00
T45	6668 [b]	0.01	2365 [b]	0.01
SD	858	0.00	75	0.00
T60	23980 [b]	0,06	3631 [b]	0.03