



eISSN 2239-7132

Italian Journal of Food Safety

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

Publisher's Disclaimer. E-publishing ahead of print is increasingly important for the rapid dissemination of science. The Early Access service lets users access peer-reviewed articles well before print/regular issue publication, significantly reducing the time it takes for critical findings to reach the research community.

These articles are searchable and citable by their DOI (Digital Object Identifier).

The **Italian Journal of Food Safety** is, therefore, E-publishing PDF files of an early version of manuscripts that have undergone a regular peer review and have been accepted for publication, but have not been through the copyediting, typesetting, pagination, and proofreading processes, which may lead to differences between this version and the final one.

The final version of the manuscript will then appear in a regular issue of the journal.

The E-publishing of this PDF file has been approved by the authors.

Please cite this article as:

Savini F, Indio V, Giacometti F, et al. **Microbiological safety of dry-aged meat: a critical review of data gaps and research needs to define process hygiene and safety criteria.** *Ital J Food Saf* doi:10.4081/ijfs.2024.12438

Submitted: 28-02-2024

Accepted: 03-04-2024

 © the Author(s), 2024
Licensee PAGEPress, Italy

Note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries should be directed to the corresponding author for the article.

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

**Microbiological safety of dry-aged meat:
a critical review of data gaps and research needs to define process hygiene and safety criteria**

Federica Savini,¹ Valentina Indio,¹ Federica Giacometti,² Yitagele Terefe Mekkonen,¹
Alessandra De Cesare,¹ Laura Prandini,¹ Raffaele Marrone,³ Alessandro Seguino,¹
Marika Di Paolo,³ Valeria Vuoso,³ Federico Tomasello,¹ Andrea Serraino¹

¹Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Ozzano dell'Emilia; ²Department of Animal Medicine, Production and Health, University of Padova, Legnaro; ³Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Italy

Correspondence: Valentina Indio, Department of Veterinary Medical Science, Alma Mater Studiorum University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy. E-mail: valentina.indio2@unibo.it

Key words: dry-aged meat, spoilage microorganism, pathogenic microorganism, microbiological criteria, molds.

Contributions: FS, investigation, data curation, writing - original draft, review and editing; VI, FG, ADC, AS, writing - review and editing; YTM, investigation; LP, data curation; RM, writing - original draft; MDP, VV, FT, investigation, data curation; AS, conceptualization investigation; writing - review and editing. All authors have commented and approved the final version as submitted.

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

Ethics approval and consent to participate: not applicable.

Funding: this research was funded under 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 funded by the Ministry of Agriculture, Food Sovereignty and Forests.

Availability of data and materials: data available from the corresponding author upon request.

Conference presentation: this paper was presented at the XXXII National Conference of the Italian Association of Veterinary Food Hygienists (AIVI), September 13-15, 2023, Maierato, Italy.

Abstract

Dry-aged meat is gaining popularity among food business operators and private consumers. The process is carried out in aerobic conditions by hanging beef carcasses or placing subprimal or primal cuts in a dedicated cabinet for several weeks or even months while controlling the environment through the management of process parameters such as temperature, relative humidity, and airflow. In this review, we present a critical evaluation of the literature to evaluate tools to manage the process to guarantee food safety and identify critical control points, as well as good hygienic and manufacturing practices.

In controlled aging conditions, only *Listeria monocytogenes* and *Yersinia enterocolitica* can multiply, while a reduction in the number of *Salmonella* spp. and *Escherichia coli* O157:H7 is generally reported. *Enterobacteriaceae* usually decrease on the surface of the meat during maturation; thus, for the purpose of the hygienic evaluation of the production process, a count no higher than that of unaged meat is expected. Besides, various studies report that the total bacterial count and the spoilage microorganisms significantly increase on the surface of the meat, up to 5-6 Log CFU/g in the absence of visible spoilage. Bacteria of the *Pseudomonas* genus tend to progressively replace other microorganisms during maturation; thus, the total mesophilic or psychrophilic bacterial load is not a good indicator of process hygiene for aged meat.

Critical parameters for the control of the process are temperature, relative humidity, and ventilation, which should be monitored during the process. For this reason, equipment designed and certified for dry aging must be used, and the manufacturer must validate the process. Food business operators must apply general good manufacturing practices (GMP) and good hygiene practices (GHP) for meat processing and some GMP and GHP specific for dry aging.

Several research needs were identified, among them the evolution of the populations of *L. monocytogenes* and *Y. enterocolitica* and the microbiology of the inner parts of the dry-aged meat.

Introduction

Meat consumers are demanding products of high and consistent quality with a distinctive flavor and aroma able to provide a particular sensorial experience when consumed (Álvarez *et al.*, 2021). Dry-aging is a process used by high-end food service restaurants and upscale meat markets to enhance the palatability of meat, encompassing a combination of biochemical/biophysical processes that are naturally caused by a complex group of endogenous proteases and lipases altering the muscle structural integrity, meat color surface, and palatability (Khazzar *et al.*, 2023). Indeed, proteolysis causes muscle tissue to mature, producing a typical taste with a unique flavor and improving the tenderness and juiciness of meat.

For centuries, cold storing was a common way for butchers to preserve and tenderize beef, but the advent of vacuum packaging, along with increased efficiencies in beef processing and transportation, determined the abandonment of dry aging in favor of the wet aging process (Dashdorj *et al.*, 2016), where beef is put in a vacuum-sealed package and stored in a controlled environment for a specific period of time. In recent years, the dry aging technique has attracted the attention of retailers, the food industry, and restaurants in the United States, Australia (Meat and Livestock Australia, 2016), Asia (Dashdorj *et al.*, 2016), and also Europe (EFSA BIOHAZ Panel *et al.*, 2023). The process is carried out in aerobic conditions by hanging beef carcasses or subprimal or placing primal cuts in a refrigerated room and aging for several weeks or even months while controlling the environment through the management of process parameters, namely temperature, relative humidity (RH), and air flow.

Dry aging can be performed in meat processing plants, in which the primal or subprimal cuts are processed for about 7-28 days, and then, after cutting and packing, they are ready for sale. Dry-aged steaks are offered mostly in fine restaurants, upscale grocery stores, gourmet steak companies, and specialized meat shops (Dashdorj *et al.*, 2016). On the other hand, always more frequently, small producers sell their products directly to consumers at meat boutiques or restaurants. This business

approach is made possible by the commercial availability of small maturation chambers used by food business operators (FBO) for aging conducted for up to 180-240 days (Dashdorj *et al.*, 2016). Rezende de Souza *et al.*, (2021), Gowda *et al.*, (2022), Lancaster *et al.*, (2022) and EFSA BIOHAZ Panel *et al.* (2023), have highlighted that most of the producers neither control, nor monitor the relevant process parameters, and in the case these are controlled, a high variability is evident among different FBO.

This is of particular importance since the recent EFSA opinion on the microbiological safety of aged meat (EFSA BIOHAZ Panel *et al.*, 2023) stated that ageing can achieve similar or lower loads of microbiological hazards and spoilage bacteria than the \log_{10} increases predicted for standard fresh meat preparation, but only if defined and controlled conditions are applied. In the document, the experts have in addition pointed out the absence of a shared definition among European Union Member States of the term “dry aged meat”.

The fate of microorganisms during dry aging is determined by their initial contamination and by the environment in the maturation chamber that can significantly influence the increase or decline of specific populations. A multitude of bacteria, yeasts, and molds can grow on dry aged meat given its high nutrient content and the favorable substrate, pH, and water activity (a_w) values. Contamination can occur during slaughtering, dressing and preparation phases, or at the retail level from operators, environment and equipment. During dry aging, the contamination can also be advantaged by the continuous loading of loins into cabinets, adopted by most of the companies (Gowda *et al.*, 2022) rather than the application of an all in-all out production.

The good hygienic practices normally used for meat processing, together with hazard analysis critical control point (HACCP) principles can be applied at each stage along the meat chain. On the other hand, specific HACCP plan and good manufacturing practices (GMP)/good hygiene practices (GHP) for dry ageing should be applied to control the aging process. Guidelines for the safe production of aged meat have been drafted from the Meat Livestock Australia (Meat and Livestock Australia, 2016) and U.S. Meat Export Federation (USMEF, 2014) to help FBO produce safe and standardized dry-aged meat. In addition, information available in the literature was recently revised by EFSA BIOHAZ Panel *et al.* (2023). According to the Commission Delegated Regulation (EU) 2024/1141 (European Commission, 2024) meat subject to dry-ageing must be stored at a surface temperature of -0.5 - 3.0°C , with a RH of a maximum of 85 % and an airflow ranging from 0,2 to 0,5 m/s in a dedicated room or cabinet for a maximum of 35 days; alternatively FBO may apply other combinations of surface temperature, RH, airflow and time, if they demonstrate that equivalent guarantees are provided on the safety of the meat. Nevertheless, data on the airflow effect on surface drying of the meat, the role of competitive microflora on the pathogenic microorganisms eventually present on meat, and indications of microbiological criteria to assess the hygienic status of the process, are lacking, together with indications of requirements for equipment and for the process.

Based on the necessity to guarantee food safety, and identify the critical control points and recommendations for the production of dry-aged meat, we analyzed the literature concerning dry aging and evaluated microbiological safety and microbiological hygienic criteria and good manufacturing practices during dry aging processing. Special attention was paid to process equipment and parameters applied to small maturation chambers used in restaurants and meat boutiques where meat is processed and sold directly to consumers. Lastly, we got into some consideration about regulatory aspects and the environmental and operational conditions inside the cabinets that may result in a significant impact on the evolution of microbial population and their impact on the design of facilities for dry aging.

Regulatory aspects, processing facilities and process parameters

Both terms aging or maturation are used to describe the meat process. From the regulatory point of view, Commission Delegated Regulation (EU) 2024/1141 defines “dry-ageing” as the storage of fresh meat in aerobic conditions of hanging carcasses or cuts either unpacked or packed in bags

permeable to water vapor in a refrigerated room or cabinet and left to age for several weeks at controlled environmental conditions of temperature, RH and airflow (European Commission, 2024). Similarly, USDA (2024) defines dry-aging as the process carried out in aerobic conditions of hanging beef carcasses or subprimal or placing primal cuts either unpacked or packed in bags permeable to water vapor in a refrigerated room and left to age for several weeks or even months at controlled environmental conditions of temperature, RH and airflow.

During dry aging, the process parameters influence the evolution of the proteolytic cascade directly affecting the microbiological safety of the dry-aged meat. Temperature and its stability have a critical importance for the achievement of desired meat characteristics being its increase directly proportional to the speed of enzymatic process maturation. Higher temperatures also promote the multiplication of spoilage and pathogenic microorganisms that may give rise to off odors or unsafe products (Dashorj *et al.*, 2016; Rezende de Souza *et al.*, 2021).

Similarly, controlled RH of the air plays a crucial role in the dry-aging process because too high humidity corresponds to higher spoilage bacterial growth, with consequent production of off-flavors, while too low humidity values will result in a lower yield of the process and less juiciness than desired. Interestingly, some processors prefer a high RH (>85-90%) in order to favor the development of molds on the meat surface that are believed to generate particular flavors (USDA, 2024). The presence of molds will be discussed in the dedicated section.

From a safety point of view, the lower the temperature the lowest is the possibility for pathogenic microorganisms to multiply/survive with a limiting value at the freezing point of meat. High-speed ventilation and low RH strongly contribute to the control of microorganism growth favoring the superficial desiccation but reducing the process yield and increasing the thickness of the crust consequently increasing losses due to trimming.

Meat and Livestock Australia (2016) identified temperature of 0.5-3°C, ventilation to 0.2-0.5 m/s, and 75-85% RH as the most suitable parameters for meat dry aging; while USMEF (2014) indicated 0-4°C, ventilation 0.5-2 m/s, 80-85 % RH, while the Commission Delegated Regulation (EU) 2024/1141 reports surface temperature of - 0.5 to 3.0 °C, with a RH of a maximum of 85 % and an airflow of 0,2 to 0,5 m/s (European Commission, 2024).

Dashdorj *et al.* (2016) reviewed several literature studies and reported that the following ranges are applied to perform dry aging: 0,5-4 °C, airflow 0,5-2,0 m/s, and 61-85% RH. In addition, the authors suggested an approach based on the duration of aging regarding temperature: for long-term aging the ideal temperature is -0.5±1 °C, while a higher temperature of 2-3°C might be acceptable for shorter (1 to 2 weeks) aging processes.

In our review, we identified four investigations on the process parameters applied by FBO: Rezende de Souza *et al.* (2021) reported that, among Brazilian companies, 78.3% of 37 small producers process dry-aged meat at temperature <4°C, 67,5% at RH<80% and 78,4% at mean or high air speed; the remaining producers's percentage either applied different or did not monitor process parameters. Lancaster *et al.* (2022) investigated 10 commercial aging locations across the U.S. and revealed that 70% processed meat at temperature <4°C (min 0.74 max 5.26), 80% at RH <85% (min 64.87 max 92.21) while all utilised a wind speed > 0.5 m/s (range 0.56-2.03 m/s). Gowda *et al.* (2022) analyzed the process parameters applied by 15 commercial meat companies in Belgium and reported a temperature of processing in the range 1-3°C and RH in the range 40-75%, while air speed was not investigated. EFSA reported that out of 10 respondents to a questionnaire, 90% used a RH <85% and all of them (100%) set the temperature between 0 and 4°C; again, airflow speed was not provided.

The USMEF (2014) suggested applying an airflow range of 0.5-2 m/s to obtain a 0.2 to 1.6 m/s speed over the product which is considered sufficient for a correct maturation.

Despite differences among field investigations and values reported by Agencies/FBO associations, there is a general agreement that low temperatures, low RH and high wind speed represent the correct process parameters for dry aging, with the air speed representing the most underestimated/neglected parameter both by researchers and FBO.

Airflow, together with low temperature and low RH contributes to the formation of a protective layer or “crust” on the meat surface (Rezende de Souza *et al.*, 2021) which has a low water activity compared to the inner part. Lancaster *et al.* (2022) demonstrated that aging losses, that are related to the reduction of water content during processing, are more related to low RH and high air speed rather than to temperature. In this regard, a direct effect of desiccation in the reduction of some microorganisms was described by several authors. Knudsen *et al.* (2011) reported that different levels of desiccation can influence different survival of *Salmonella* spp. on meat during dry aging as well as during cold storage (Scott and Vickery, 1939). A reduced RH and a good air velocity during the dry-aging process lead initially to a quick drying and subsequently reduction of water activity of the surface of the meat that, combined with a low temperature considerably reduces the growth of the microflora (Van Damme, 2022).

Tomasello *et al.* (2021) reported the effect of the environmental conditions in dry aging equipment (temperature 1°C; airflow 1.2 m/s; RH 75%) on selected foodborne pathogens inoculated on stainless steel plates and showed a reduction in 24 hours of 1.11, 2.65, 2.75 and 3.83 Log₁₀ colony-forming units (CFU)/cm² for *S. aureus*, *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* respectively. Besides the impossibility of directly comparing the results obtained on stainless steel plates and those concerning microorganisms naturally present on a biological matrix, the data revealed that the environment within the dry aging cabinets is extremely hostile to microorganisms. Indeed, EFSA suggested that aerobic meat maturation must be performed in equipment designed and certified for this aim, establishing one of the discriminating factors for differentiating dry-aged meat and meat preserved only using low temperature (EFSA BIOHAZ Panel *et al.*, 2023).

Tenderness and flavor improvement of meat are dependent on the degree of alteration and weakening of myofibrillar structures and have been largely attributed to the endogenous proteolytic enzymes. The utmost activity of enzymes is recorded within the first 7 days of aging, leading to the greatest gains in tenderness by 14 days after which the enzymes only decelerate the proteolytic activity over time. Indeed, the authors reported that the longer the aging period, the better the meat tenderness (Dashdorj *et al.*, 2016; Rezende de Souza *et al.*, 2021).

Studies have reported that dry-aged beef flavor begins to develop after 14 days and intensifies thereafter and that the most frequent range for dry-aged subprimal is between 14 and 40 days, which have all appeared effective in producing the desired results of this process (Dashdorj *et al.*, 2016). The development of tenderness and specific taste is time- and temperature-dependent, where the same level of tenderness can be achieved in 4 weeks at -0.5°C or in 2 weeks at 5°C with the highest rate of tenderness improvement achieved during early stages of aging (Dashdorj *et al.*, 2016).

In field studies, Dashdorj *et al.*, (2016) and Rezende de Souza *et al.*, (2021) reported that the most common aging times described by the interviewees ranged from 7 to 8 weeks (28.8%), 4 and 5 weeks (25.8%), and 6 weeks (19.7%); some producers also mentioned aging times shorter than one week (1.5%) and longer than 8 weeks (15.2%). Similarly, Gowda *et al.* (2022) reported that the dry-aging time in Belgium facilities varied greatly between and within the different companies, with a minimum ripening time of 3-4 weeks and a maximum of 10 weeks.

EFSA suggested that 14 days can be considered the minimum period to obtain the typical characteristics of the aged meat, but longer periods are usually applied by food business operators (EFSA BIOHAZ Panel *et al.*, 2023). Similarly, USDA (2024) defines dry aged as the beef products (carcass or cuts) maintained in a fresh unfrozen state for a minimum of 14 days from the day of slaughter, and the USMEF (2014) reports an indication for dry aging time from 14 to 70 days.

According to the scientific literature, national and international agencies and field investigations on FBO practices, it appears that the limit of 14 days can be set to discriminate between fresh preserved meat and dry-aged meat. On the other hand, the time between slaughtering and the start of maturation can have a great variation in practices. Considering that dry aging is defined as a process in controlled conditions of temperature, RH, and airflow, the starting point of 14 days should be set at the moment in which meat is placed under controlled conditions in specifically designed equipment.

Given the importance of the process parameters in relation to the safety and quality of dry-aged meat the dry aging process should be performed into aging equipment that has been designed and certified for the intended use, thus including the possibility to manage and control temperature, RH and air speed and with an appropriate configuration of the airflow and pertaining components such as fans, evaporators, condensators *etc.* Besides, specific indications should be given to producers concerning, among others, the maximum amount of meat to be loaded and its positioning into the cabinet as well as details on the most suitable process parameters for the production of the desired characteristics. Process parameters must be set considering food safety as the priority, and the following parameters, according to Dashdorj *et al.* (2016) can be suggested to accomplish the goal: temperature $1\pm 2^{\circ}\text{C}$, RH 75-85% air speed 0.5-2.0 m/s.

The pH of meat immediately after slaughtering is neutral or slightly alkaline but already after 1-4 hours, it starts to decrease reaching values of about 5.5-5.8 in 24 hours with the resolution of rigor mortis.

A high pH indicates the potential of DFD (dark, firm and dry) condition (Khazzar *et al.*, 2023). In addition, meat with higher pH shows reduced conservability and early alteration that usually starts when the pH is higher than 6.4 and becomes manifest by a value of 6.8.

In most of the studies considered in this review, the pH remained almost unchanged or showed a moderate increase during maturation (Table 1) (Ahnström *et al.*, 2006; DeGeer *et al.*, 2009; Shi *et al.*, 2020; Li *et al.*, 2013; Gudjónsdóttir *et al.*, 2015; Berger *et al.*, 2018; Kozyrev *et al.*, 2018; Da Silva *et al.*, 2019; Smaldone *et al.*, 2019; Bernardo *et al.*, 2020; Di Paolo *et al.*, 2023; Khazzar *et al.*, 2023).

Da Silva Bernardo *et al.* (2020) demonstrated that starting from pH 5.3, the application of different combinations of freezing and thawing before aging resulted in pH values of 5.51-5.59 after 21 days. Interestingly Smaldone *et al.*, (2019), who evaluated the longest aging period, observed a pH increase from 5.7 to about 6 in 290 days: the higher initial value, in comparison with other studies, and the longer maturation time may have influenced the higher final pH.

The stability of pH during aging is an important factor not only from an organoleptic point of view but also in assuring food safety. Van Damme *et al.* (2022) reported an increase in *L. monocytogenes* count during aging of a loin with a starting pH of 6.21 reaching values of 6.49 after 42 days. On the contrary, *L. monocytogenes* counts decreased in 7 loins with a starting normal mean pH of 5.50 (5.34-5.68) and a mean pH of 5.77 (5.60-5.99) at the end of the process.

From an organoleptic point of view Colle *et al.* (2016), observed that meat pH value slightly increased, by about 3% from 5.50 to 5.66 during 35 days of dry aging, reaching values that favor the activation of calpain by releasing Ca^{2+} ions from the sarcoplasmic reticulum and mitochondria when the level of ATP is practically zero. The pH plays a pivotal role in maintaining the activity of other different enzymes as elucidated by other authors (Colle *et al.*, 2016; Álvarez *et al.*, 2021; Kim *et al.*, 2022) such as proteases, including cathepsins, caspases and various N- and C-esopectidases, that could then promote the genesis of bioactive peptides. Dashdorj *et al.* (2016) suggested that meat for dry aging should be selected from carcasses with an ultimate pH between 5.4 and 5.7. Overall, the data extracted from the literature showed that pH along with the other process parameters should be monitored during aging and the range of acceptability should be between 5.4 to 5.8.

Microbial community of dry-aged meat

Meat is normally expected to be contaminated with a range of bacteria and moulds which include both spoilage and pathogenic organisms. The initial contamination level of the carcasses, hygienic practices during the production and subsequent meat storage conditions affect the levels of microorganisms during the dry aging process (Ahnström *et al.*, 2006; Cherroud *et al.*, 2014; Gowda *et al.*, 2022). In addition, the microbial community structures of dry-aged meat are highly dependent on the processing facilities' characteristics as demonstrated by Capouya *et al.* (2020). Usually, the most represented bacteria on the meat are mesophilic, psychotropic and lactic acid bacteria (LAB)

(Hulánková *et al.*, 2018), with bacterial species diversity increasing during the process (Ryu *et al.*, 2020).

In the dry aging process, the evaporation of moisture dries the meat surface making it inedible. Many microbes colonize the meat's surface during aging and the composition of the microbial community changes continuously (Di Paolo *et al.*, 2023).

No regulatory limits have been laid down for microbiological characteristics/criteria, but from a legislative point of view pathogenic microorganisms must be considered together with toxin-producing molds, microbiological process hygiene criteria, and spoilage microorganisms that may make the meat unfit for human consumption. The potential for biogenic ammine formation should be also evaluated.

Food safety criteria are listed in Regulation (EC) No 2073/2005 for different types of foodstuff (Commission of the European Communities, 2005); however, any criteria have been set either for dry-aged meat or for fresh meat. Process hygiene criteria are available for “carcasses of cattle, sheep, goats and horses” namely aerobic colony count, Enterobacteriaceae and *Salmonella*. The aerobic colony count/total bacterial count (TBC) is usually used as process hygiene criteria in most of legislation to assess the general contaminations caused by food processing, together with indicators of fecal contamination such as *E. coli*/Enterobacteriaceae/fecal coliforms/coliforms enumeration.

The pathogenic bacteria relevant in dry-aged meat, for which food safety criteria should be set, include *Salmonella* spp., pathogenic *E. coli* and *L. monocytogenes* included by EFSA BIOHAZ Panel *et al.* (2023) and the Meat and Livestock Australia (2016) guidelines among the pathogenic bacteria of primary importance in the dry aging of beef along with *Staphylococcus aureus*, *Campylobacter* spp. and *Clostridium* spp. As far as *Yersinia enterocolitica* is concerned, it is only considered relevant in pork meat, but it can be occasionally isolated also from beef meat and should be considered in beef dry aged meat (Jaballah *et al.*, 2022).

Evolution of total bacteria count, Enterobacteriaceae and coliforms during meat dry aging

The results of longitudinal studies performed in commercial facilities are available in the literature and show the evolution of microorganisms' count during the dry aging process at different time points but mostly at the end of the process. The studies considered for this review are listed in *Supplementary Table 1*. Most of them report the bacterial populations in the external part of the dry-aged meat, usually defined crust, and the results are expressed both as CFU per g or cm². Some studies report sampling and counting of the external part of the meat, differentiating lean from fat parts. Moreover, in some studies, deep samples are also performed.

Smaldone *et al.* (2019) published a study where they determined high counts of TBC in the meat before the beginning of dry aging and reported a slight decrease of TBC (from 6.82 to 6.13 Log₁₀ CFU/g) during 294 days of maturation. On the contrary, most of the authors witness an increasing trend of the counts on the meat surface, even though variations are recorded among settings. *Supplementary Table 1* shows that the TBC values before the ageing ranged from < 10 Log CFU/g to 4.10 Log CFU/g (Di Paolo *et al.*, 2023) while, generally, a final value of almost 5 Log CFU/g is reached on the crust as reported by different authors at the end of the aging period ranging between 8 to 60 days. Exceptions are represented by the study of DeGeer *et al.* (2009) reporting lower counts, specifically 2.89-3.51 Log CFU/g after 28 days of ageing, and Shi *et al.* (2020) showing high initial bacterial count (4.62 Log CFU/g) that grew in 21 days to 7.53 Log CFU/g.

Interestingly a much higher surface count, namely 9.47 Log CFU/g is described by Bernardo *et al.*, (2021) after 21 days at 2°C and 2.5 m/s air speed with a high (85%) RH, and a lower colony count (4.12 Log CFU/g) with RH set to 65%. Incubation at 85% RH was characterized by high a_w both externally and internally (0.994 and 0.990 respectively) indicating a failure of the drying process, accompanied by signs of spoilage and bad odor; the author concluded that a RH lower than 85% can be suggested to keep microbial counts below the deterioration level.

The TBC trend during time is different among studies: Hulánková *et al.* (2018) reported an increase of TBC in the surface samples during the first 15 days of aging followed by a decline after 2 weeks of storage ($1\pm 1^{\circ}\text{C}$, $85\pm 2\%$ RH, airflow 0.5 ± 0.2 m/s); Shi *et al.* (2020) only evaluated 14 days of aging recording an increase from 4.62 to 7.53 Log CFU/g (2°C , 85% RH, airflow 1.5 m/s); similarly Di Paolo *et al.* (2023) in steaks after trimming reported an increase of the TBC up to 5.45 Log CFU/g after 15 days followed by a stabilization up to 60 days of aging ($1\pm 1^{\circ}\text{C}$; $78\pm 10\%$ RH, airflow 0.5 m/s;) while growth of total bacteria decreased after 50 days in the study of Ryu *et al.* (2018) at $1-4^{\circ}\text{C}$, 80-90% RH (ventilation applied but not quantified) in surface samples. Campbell *et al.* (2001) reported an increase in TBC during the first week of aging in lean and fat surfaces, followed by a period of up to 21 days of aging in which the TBC remained substantially stable (2°C , 75% RH). Few authors investigated the fate of fecal indicators (*i.e.*, *E.coli/Enterobacteriaceae/coliforms*) on the surface of dry-aged meat during aging: Gowda *et al.* (2022) collected samples obtained in 15 dry-aged meat producing companies showing that *Enterobacteriaceae* were under the limit of detection during aging, only in 4/13 loins at the end of aging a maximum load of 4.3 CFU/cm² was evidenced but much higher counts (6.4 Log CFU/cm²) were also recorded. Lancaster *et al.* (2022) tested the microbiological characteristics of steak from loins aged for 45 days in commercial facilities and did not detect *E. coli* while the coliform count ranged from 0.59 to 4 Log CFU/g. Mikami *et al.* (2021) detected 3.10 Log₁₀ CFU/cm² coliforms in surface samples of meat aged for 35 days. Van Damme *et al.* (2022), in experimental tests, showed variable results reporting *Enterobacteriaceae* count in surface samples ranging from <0 to 0.4 Log₁₀ CFU/cm² and from <0 to 1.6 Log₁₀ CFU/cm² in lean and adipose tissue, respectively, during 42 days of aging at different conditions of temperature and RH. The *Enterobacteraceae* count is generally reported to be low but Ryu *et al.* (2018) and Hulánková *et al.* (2018) failed to detect *Enterobacteriaceae*, coliforms and *E. coli* on the surface of samples obtained from dry-aged beef. On the contrary, Da Silva Bernardo *et al.* (2020) observed a high *Enterobacteriaceae* count on meat aged at relatively high RH (85%) for 21 days. Smaldone *et al.* (2019) observed a moderate decrease (2.58 to 2.08 CFU/g) of *Enterobacteriaceae* count during 277 days of aging, while on the contrary Khazzar *et al.* (2023) observed a moderate increase (from 1 to 1.7 log CFU/g) of *Enterobacteriaceae* count after 230 days of aging, and Di Paolo *et al.* (2022) a similar increase of coliforms and *Enterobacteriaceae* (from 3.08 to 3.93 and from 1.78 to 1.92 log₁₀ CFU/g respectively) from the second to the 60th day of aging; in the same study a reduction from 1.33 log₁₀ CFU/g to not detectable count was observed for *E. coli*. Regarding the contamination of the inner parts of the meat, Mikami *et al.* (2021) reported the presence of 5.2, 2.02, 2.20 and 1.97 Log₁₀ CFU/g for TBC, LAB, coagulase negative *Staphylococci* and coliforms respectively. Moreover, Gowda *et al.* (2022) investigated the internal load of samples from commercial facilities and detected aerobic and anaerobic psychrotrophic bacteria up to 3.5 Log₁₀ CFU/g. At slaughtering the microbial contamination should be limited to the meat surface of the meat, unless bacteraemia or spread during slaughtering results in the contamination of the deep parts of the muscle. However, these two cases should be uncommon in commercial conditions. The contamination of the inner parts caused by bacteria originating from the surface of the meat is a condition that should be investigated also in consideration of the cracking that sometimes happens in the dry-aged meat following the dissection of the surface. The presence of an unhomogeneous surface may determine an incorrect drying of the meat since the airflow cannot reach the most internal areas where a_w is higher, representing a favorable substrate for bacteria replication, thus, the microbiology of the deep parts of dry aged meat should be further investigated (Gowda *et al.*, 2022). In general, experimental studies indicate that, in controlled conditions of temperature, RH and ventilation, an increase of the surface TBC happens during aging up to 5-6 Log CFU/cm² and in some cases, higher microbial counts are reported; similar values were detected in steaks and in samples collected in commercial facilities or in samples at retail with the main increase of TBC occurring usually in the first aging period. Generally, off-flavors resulting from spoilage in meat, can be

observed from a bacterial count $> 7 \text{ Log}_{10} \text{ CFU per cm}^2$ or g, (Gowda *et al.*, 2022). None of the authors reported spoilage of the internal part of the meat. Therefore, it can be concluded that prolonged dry aging in controlled conditions can result in meat of acceptable microbiological and organoleptic quality; the total bacterial count is expected to be higher in dry-aged meat than in meat before aging.

Based on the literature results, the dry ageing process is generally considered hostile to Enterobacteria. Specifically, when present at the beginning of the process, the count show only a moderate increase ($<1 \text{ Log}_{10} \text{ CFU}$) during time, while on the contrary *E. coli* count decreases. In comparison to not aged meat a lower count of *E. coli* and a comparable count of Enterobacteriaceae is then expected in dry-aged meat. Therefore, Dashdorj *et al.* (2016) proposed a critical limit of 1000 CFU/g and 10 CFU/g for *Enterobacteriaceae* and *E. coli* respectively for the validation of the dry aging process.

Evolution of psychrotrophic bacteria, lactic acid bacteria and Pseudomonas spoilage bacteria

Bacteria are the most important factor driving the meat spoilage process in meat (Shao *et al.*, 2021). Spoilage signals such as off-flavor can be detected in meat when bacteria counts reach around $7 \text{ Log}_{10} \text{ CFU/g}$ Hulánková *et al.* (2018).

According to EFSA, the meat spoilage bacteria include *Pseudomonas* spp., *Lactobacillus* spp., *Enterococcus* spp., *Weissella* spp. and *Brochothrix* spp., *Leuconostoc* spp., *Shewanella* spp., *Clostridium* spp., *Hafnia* spp., LAB, *Micrococcus* spp., *Bacillus* spp. and *Providencia* spp., with differences depending on the method used to meat aging (*i.e.*, aerobic or under vacuum) (EFSA BIOHAZ Panel *et al.*, 2023).

The data available in the literature regarding meat spoilage bacteria during dry aging were mostly collected by culture-based methods and are summarized in *Supplementary Table 2*. During the dry aging process, a similar trend was observed for TBC, psychrotrophic bacteria, and LAB by Hulánková *et al.* (2018) during aging for 42 days, with psychrotrophic increasing up to $>5 \text{ Log CFU/cm}^2$ and up to $>4 \text{ Log CFU/g}$ in the surface and the deep part of the meat, respectively, and LAB to about 1.5 Log CFU/cm^2 both in the samples collected in the external and internal part of the meat. Similar increasing trends during the process were observed for *Pseudomonas* spp by Di Paolo *et al.* (2023) and Campbell *et al.* (2001) and for LAB by Di Paolo *et al.* (2023), Campbell *et al.* (2001), Ryu *et al.* (2018), Khazzar *et al.* (2023), DeGeer *et al.* (2009), Li *et al.* (2013), Ahnström *et al.* 2006, and Gudjónsdóttir *et al.* (2015), with small differences in enumeration results during aging. High counts of psychrotrophic bacteria were observed by Da Silva Bernardo *et al.* (2020) in spoiled meat aged for 21 days at 2°C , 85% RH, 2.5 m/s air speed. Gowda *et al.* (2022) investigated microbiological traits of meat aged in commercial facilities and detected a large variation in the count of *Pseudomonas* spp. ($<1.0\text{-}8.8 \text{ Log CFU/cm}^2$), *B. thermosphacta* ($<1\text{-}7.4 \text{ Log}_{10} \text{ CFU/cm}^2$) and psychrotrophic LAB ($<1.0\text{-}7.3 \text{ Log CFU/cm}^2$). Starting from a median of $2.1 \text{ Log}_{10} \text{ CFU/cm}^2$ of *Pseudomonas* on fat and $<1 \text{ Log}_{10} \text{ CFU/cm}^2$ on lean tissue, no variation was evidenced on the latter (final value $<1 \text{ Log}_{10} \text{ CFU/cm}^2$), while counts on fat tissue were up to $7.4 \text{ Log}_{10} \text{ CFU/cm}^2$ with a mean value of $4.0 \text{ Log}_{10} \text{ CFU/cm}^2$ after 42 days of aging (Van Damme *et al.*, 2022). However, other authors as Hulánková *et al.* (2018) described levels of *Pseudomonas* spp. below the limit of detection both at the beginning and the end of the aging period (12-36 days).

Pseudomonadaceae are a group of psychrotrophic bacteria that can grow with temperatures as low as -6°C , constituting the major components of the aerobic spoilage microbiota in food and appear to have a preponderant importance in dry-aged meat (EFSA BIOHAZ Panel *et al.*, 2023).

Capouya *et al.* (2020) using a next-generation sequencing approach reported that operational taxonomic units (OTUs) identified in greater than 75% of samples belonged to the *Pseudomonas* genus. Variable numbers of *Pseudomonas* spp. between different loins are reported also by Gowda *et al.* (2022) with results from <1.0 to $8.8 \text{ Log}_{10} \text{ CFU/cm}^2$. Lee *et al.* (2019) observed that *Pseudomonas* spp. become the prevalent bacteria, starting from 42.7% of the relative abundance of the microbial

population and increasing to 84.2-92.7% after 28 days of dry aging and a decrease of *Lactobacillus* spp. and *Flavobacterium*. The air speed seemed to influence the relative abundance of different bacterial populations.

At a species level, up to 90 of OTU identified in 5 commercial dry-aging facilities after 45-day aging period were *P. fragi*, with a relative abundance in each of the five sampled location of at least 28.5% (Capouoya *et al.*, 2020). Alongside Ryu *et al.*, (2020) report *Pseudomonas psychrophila* as the major representative among *Pseudomonas* spp, describing an oscillatory behaviour with appearance at day 30, reduction at day 70, and reiterate appearance at day 160. Lick *et al.* (2021) performed isolation of a novel strain, named *P. paraversuta*.

A reduction of LAB during ageing was observed by Ryu *et al.*, (2020), with *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* being the most abundant bacteria at the beginning of the dry ageing process and reducing their relative abundance up to 160 days of aging. Lee *et al.* (2019) reported changes in the relative abundance during aging for 28 days with an increase mainly in *Pseudomonas* spp. and *Enterobacterium*.

An increase in the relative abundance of *Pseudomonas* spp. during dry aging seems to be a common feature of dry-aged meat.

Da Silva Bernardo *et al.* (2020) investigated an interesting practical application and studied the effect of freezing and thawing of meat before dry aging on the microbiological quality of meat aged for 28 days in comparison to unfrozen dry-aged meat; they showed that freezing and fast or slow thawing didn't influence the final TBC, the *Enterobacteriaceae* count, psychrotrophic count and yeast count, but had an influence LAB count that ranged from <1.53 Log CFU/g of meat directly aged to 2.56 Log CFU/g of meat that underwent freezing and slow thawing.

Pathogenic bacteria

Foodborne pathogens carried by cattle or man or present in the processing environment may be transferred to carcasses during dehiding and evisceration (Beach *et al.*, 2002; Brichta-Harhay *et al.*, 2008; Rhoades *et al.*, 2009) and among them, *L. monocytogenes* poses concerns in the process environment of the meat industry and the final product due to its ability to grow at refrigerated temperature (Buchanan *et al.*, 2017).

L. monocytogenes has not been detected on dry-aged meat by means of classic microbiology (Gowda *et al.*, 2022) for aging lasting up to 10 weeks.

In the EFSA scientific opinion on the microbiological safety of aged meat, the use of models (EFSA BIOHAZ Panel *et al.*, 2023), incorporating as input scenario parameters that might be different from those used in commercial conditions, predicted an *L. monocytogenes* median Log₁₀ increase during 77 days of beef dry-aging of 5.1. However, this value was predicted without considering a possible inactivation of the pathogen during the dry-aging process and the competition between *L. monocytogenes* and other microorganisms. Considering these two last conditions and the lack of data collected in commercial settings, the overall conclusion of the scientific opinion was that a maximum of 2 Log pathogen increase is likely to occur in both beef dry-aged for 35 days at 3°C and standard fresh meat matured under vacuum up to 14 days at refrigeration temperature.

However, direct inoculation of *L. monocytogenes* resulted in the inactivation of the pathogen with a maximum of -0.07 Log₁₀ CFU/day except for one loin with pH>6.0 at the end of dry-aging in which it was evidenced a 1 Log₁₀ CFU/cm² increase after 42 days at 2°C and 85% RH (Van Damme *et al.*, 2022). Inoculation of *L. innocua* as a surrogate of *L. monocytogenes*, demonstrated that the behavior of the microorganism was influenced by the temperature, specifically after 42 days there was a higher reduction on the beef surface aged at 8°C (3.37 Log CFU/g) rather than at 2°C (2.38 Log CFU/g) (da Silva *et al.*, 2019). A reduction of *L. monocytogenes* counts in permissive foods when stored at relatively higher temperatures was previously observed in several studies when a competitive microflora was present; in addition, it is well known that in dry aged meat increasing the temperature results in an increase of the evaporation helping reduction of the humidity of the surface of the meat,

the formation of the crust and the reduction of a_w . In any case, as previously assessed the temperature during aging should be kept as low as possible above the freezing point of the meat. Further studies are necessary to clarify the fate of *L. monocytogenes* during dry aging and to develop more accurate predictive models that incorporate different variables.

Ryu *et al.* (2018) reported that during 60 days of beef aging, there was neither the growth of *E. coli* O157:H7 nor *Salmonella* sp., same results were confirmed by Gowda *et al.* (2022) who did not detect *E. coli* on the steaks and from Hulánková *et al.* (2018) that reported *E. coli* beneath the limit of detection in both fresh and aged meat.

Ryu *et al.* (2020) reported no development of *Salmonella* sp. during dry aging for 60 days, and other authors report a reduction of the counts during time: experimental inoculation of *Salmonella* (one *S. Thompson* and two *S. Typhimurium* strains) under different temperatures (2°C and 6°C) and RH (75% and 85%) for 6 weeks led to a reduction of at least 3 Log₁₀ CFU/cm² (Van Damme *et al.*, 2022) with a daily reduction variation between -0.07 and -0.14 Log₁₀ CFU/day on adipose tissue and between -0.07 and -0.12 Log₁₀ CFU/day on lean meat. Tittor *et al.* (2011) observed a 4 Log reduction for *E. coli* O157:H7 and *S. Typhimurium* after inoculation, with the highest reduction being at 7 and 14 days respectively. A more comprehensive inoculum, consisting of wild-type strains of eight different serotypes (Knudsen *et al.*, 2011), demonstrated a daily reduction between -0.113 and -0.216 Log₁₀ CFU/cm² over 14 days (at 1°C in a conventional refrigerator with a RH varying between 70 and 100%). Interestingly, the reduction was serotype and even strain dependent, indeed strains of *S. Typhimurium* DT104 and *S. Enteritidis* PT4 and PT8 survived significantly longer than strains of the serovars Dublin, Derby, Infantis and Newport. All studies confirm a reduction of *Salmonella* during aging that is expected to be present in lower numbers than in meat before ageing.

When searched, neither *Bacillus cereus*, nor *S. aureus* were detected by Ryu *et al.* (2018). More in general, coagulase-positive *staphylococci* were detected on two loins and three steaks in low numbers (1.0 Log₁₀ CFU/cm² and ≤2.0 Log₁₀ CFU/cm², respectively) by Gowda *et al.* (2022). Mikami *et al.* (2021) report 3.16±0.50 Log₁₀ CFU/cm² coagulase-negative *staphylococci* on the crust of beef after 35 days of aging while on the inner parts the microorganisms were not detected.

Staphylococcal enterotoxin production is allowed within temperatures between 10°C and 48°C, a pH range of 4-9.6, and NaCl concentrations of 0-10%, thus enterotoxins are not produced during controlled conditions of maturation.

No data are available on the fate of *Yersinia enterocolitica*, *Campylobacter* spp. and *S. aureus*. *Clostridium* spp. were not considered of importance from EFSA given the aerobic aging conditions, but Gowda *et al.* (2022), reported the detection of anaerobic bacteria in internal samples of dry-aged meat; this further underlines that the microbiology of deep parts of aged meat should be better investigated.

Molds and yeasts

Dry aging inhibits bacterial growth while encouraging the growth of molds (Dashdorj *et al.*, 2016) that tolerate a_w values to a minimum of 0.71-0.77 for the most common xerotolerant ones.

Within our analysis, the importance for dry-aged meat was given to the molds listed in the Meat and Livestock Australia (2016) guidelines, able to grow at low temperatures, namely *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp., *Chrysosporium* sp., *Thamnidium* sp., *Rhizopus* sp., *Mucor* sp., *Aureobasidium* sp.

The very first report regarding molds on cold-store meat dates to 1923 (Brooks and Hansford, 1923), and regards the first description of molds, along with yeast, isolated from dry aged beef which have also been reported to possess proteolytic and lipolytic activities and to be able to induce the breakdown of myofibrils.

An increase of yeasts and molds from <1 up to 5.56 Log CFU/g is described by Bernardo *et al.* (2021) on the meat surface after 21 days at 85% RH. A similar situation is reported by Shi *et al.* (2020) with an increase of molds from 1.34 Log CFU/g at 7 to 3.14 Log CFU/g after 14 days at 2°C, 1.5 m/s

airflow and a RH of 85%. Besides Van Damme *et al.* (2022) reported an initial absence of molds, that did not change over time and an initial yeast count of 1.1 Log₁₀ CFU/cm² that reached 3.5 at the end of the process. On the other hand, Smaldone *et al.* (2019) reported a stable trend of molds by 2 Log₁₀ CFU/g and similarly, Di Paolo *et al.* (2023) reported a stable count of yeasts and molds during 60 days of aging, while the yeasts slightly increased during aging from 4.6 Log₁₀ CFU/g to 4.81 Log₁₀ CFU/g.

Low counts were reported by DeGeer *et al.* (2009), Li *et al.* (2013), and Lee *et al.* (2017) after 14-28 days of ageing; Berger *et al.* (2018) after 21 days reported numbers for yeasts and molds of 1.49 and 0.23 Log₁₀ CFU/mL respectively; higher counts were observed by Ahnström *et al.* (2006) at 14 and 21 days of aging with values up to 3.9 and 5.2 CFU/cm²; Gudjónsdóttir *et al.* (2015) reported counts of 3.3×10³ and 1.0×10³ for yeast and molds, respectively. Da Silva Bernardo *et al.* (2020) showed higher counts of yeast and molds on the meat surface aged at 85% RH than in meat stored at 65% RH for 21 days (5.56 versus <2.28 CFU/cm²).

Gowda *et al.* (2022) detected molds on the dried surface of 50% of the sampled loins dry-aged for 19 days or longer, and the numbers were generally low. Contrarily, high numbers of yeasts were found in the majority of the loins.

The process of dry-aging may promote the growth of molds on the external surface of the crust, which have been suggested to contribute to the tenderness and flavor development of dry-aged beef (Campbell *et al.*, 2001; Ryu *et al.*, 2018). In particular, the genus *Thamnidium* is known to be adapted to cooler conditions, it is also notable for its ability to produce collagenolytic enzymes that help break down connective tissue in the meat and create a more tender texture (Capouya *et al.*, 2020). On the other hand, species that may potentially produce mycotoxins have been found on dry-aged meat (Capouya *et al.*, 2020), with particular reference to *Aspergillus* and *Penicillium* spp. Meat and Livestock Australia (2016) revised minimal growth temperatures for growth and mycotoxin production of several fungal species and concluded that *Penicillium* spp. and *Aspergillus* spp. are not capable of producing mycotoxins on dry-aged meat at temperatures between -0.5 and 3°C, a RH of 75-85% and an airflow of 0.2-0.5 m/s. *Rhizopus* and *Mucor* genera are also frequently reported in dry-aged beef; however, they have been associated with human infectious diseases and do not provide any favorable characteristics for aging meat.

Mikami *et al.* (2021) identified *Mucor flavus* and *Helicostylum pulchrum* as the predominant molds on the crust of dry-aged meat after 35 days at 2.9°C and 90% RH, while (Bernardo *et al.*, 2021) report that *Aspergillus sydowii* was the only mold identified in the 33% of samples positive after aging for 21 days.

More details regarding the genera and species are given from Capouya *et al.* (2020) who recorded the presence of a varied composition between sampled facilities, demonstrating that the fungal communities are significantly influenced by the environmental conditions in which they are aged. More in general, notable identified OTUs included *Mucor* sp., *P. polonicum* and *P. bialowiezense*.

By means of microbiome analysis, Oh *et al.* (2019) showed that after 28 days of dry aging, the dominant microorganisms were molds (mostly *Mucoraceae*) and yeasts, identified as *Pilaira anomala* and *Debaryomyces hansenii*. Interestingly, the behavior changed depending on the airflow: the prevalence of *Mucoraceae* decreased in the presence of airflow, while more microbial diversity was evident on meat aged at 2.5 m/s rather than at 0 and 5 m/s, because 2.5 m/s of air flow was appropriate to spread them and provide them with oxygen (Oh *et al.*, 2019). Not only beneficial yeasts and molds have been isolated from aged beef, but also potentially harmful ones such as *Candida* sp., *Cladosporium* sp., *Rhodotorula glutinis*, and *Rhodotorula mucilaginosa* only at ~25 days of dry aging (Ryu *et al.*, 2018).

To ensure compliance with Article 14 of Regulation (EC) No 178/2002 (European Parliament and Council of the European Union, 2002), spoilage moulds that make beef unacceptable to consumers must also be considered. Two recent research papers (Ryu *et al.*, 2018; Mikami *et al.*, 2021) report the study of the fungal community of aged meat, but it should be noted that in both studies meat was

aged at 90% RH; it is known that some producers voluntarily favor the development of molds by storing meat at RH>85-90% (USDA, 2024) that is a range higher than suggested values.

Dashorj *et al.* (2016) suggested that meat products must be tested for mold to validate the procedure in the case of molding of the meat and in the case of positivity confirmation that the mold is *Thamnidium* must be conducted; on the other hand, it was reported that the uncontrolled development of mold represents a failure of the process due to wrong parameters setting of RH, ventilation or positioning of the meat inside the equipment (Meat and Livestock Australia, 2016): meat should be considered unfit for human consumption according to EU Regulation 178/2002 (European Parliament and Council of the European Union, 2002).

Hazard analysis and critical control point, good hygienic practices and good manufacturing practices

Meat safety is assured through the development and implementation of HACCP and prerequisite program activities, including GHP and GMP. HACCP, GMP, and GHP for dry-aged meat have been reviewed by Dashdory *et al.*, (2016), Meat and Livestock Australia (2016), and EFSA BIOHAZ Panel *et al.* (2023). Some of them were received in Commission Delegated Regulation (EU) 2024/1141 (European Commission, 2024). Temperature, RH, airflow, and pH have unanimously been identified to be of critical importance for the achievement of desired meat characteristics to prevent spoilage or uncontrolled molding of the meat and more in general for the accomplishment of safety. Setting parameters to 61-85% RH, 0.5-2.0 m/s, and $-0.5\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for long-term aging was proposed, with acceptable higher temperature of 2 to 3°C in case of 1 to 2 weeks aging processes.

According to the literature, the following parameters can be also suggested: working temperature $1\pm 1^{\circ}\text{C}$, RH 75-80%, air speed 0.5-2.0 m/s, with 3°C, 85% RH as critical limits for the monitoring of CCPs and 0.2 m/s air speed on the product.

The airflow on the product is difficult to be monitored during operative conditions and thus it should be studied, designed, and validated by the manufacturer of the equipment considering air speed and circulation/course in the maturation cabinet and characteristics of the equipment (evaporators, condensators, humidification/dehumidification systems), in particular regarding fans and their positioning into the cabinet, amount of meat that can be aged in the same moment and its organization into the cabinet. The air speed and flow should be kept uniform for the duration of the drying process; nevertheless, the most critical moment is at the beginning of the dry aging process since it favors the crust formation. EFSA suggested that air speed should be higher at the beginning of the process, but this is of difficult application from small producers, like restaurants, in which rather than the adoption of an all in - all out protocol, meat is continuously loaded and unloaded (EFSA BIOHAZ Panel *et al.*, 2023). In correctly designed and well-managed equipment this practice is unnecessary according to the literature.

Figure 1 exemplifies how air speed and design of the facilities, fans' positioning, and meat positioning can influence the air flow.

The primal cuts to be dry aged should be placed fat side down on the shelves while in the case of cuts including bones, the cut should rest on the chine bone. Stainless steel wire racks should be used since perforated shelves cannot guarantee an adequate air circulation. If hanged the meat should not be fixed from the flesh but rather from the bone (Figure 2A and B). Separation between pieces of meat is necessary, usually, 3 cm is considered the minimum, while in case loins are too close air is not allowed to correctly circulate (Figure 2C and D). In addition, the introduction of the meat into the cabinets should be performed starting with the freshest cut on the lower shelves to let them drain and move the meat to the upper ones over time.

Meat characteristics also have an impact when performing dry aging: carcasses should be selected among the ones with ultimate pH between 5.4 and 5.7, in addition the pH should be monitored along with the others during aging, with a range of acceptability from 5.4 to 5.8 and a critical limit of 6.0 that, when exceeded, can be sign of spoilage or needs an investigation.

The presence of the crust, as anticipated, is a key factor in controlling bacterial growth, thus eventual irregularities on the surface may hamper uniform drying favoring the penetration of microorganisms (Figure 3A) and/or increasing the losses due to trimming. As a consequence, cuts with regular surfaces must be preferred for dry aging (Figure 3B and E). This is even more important if we consider that sometimes fissurations or wholes due to desiccation can also appear on a cut with regular surface (Figure 3C). Fissurations should be left drying (Figure 3D and E), and subsequently either curetted or filled with beef tallow in order to reduce the losses at trimming (Figure 3F).

Organoleptic inspection (mainly colour and odor) of meat before trimming and after cutting are key factors for the evaluation of the quality and safety of the product.

Generally bacterial counts on the surface of the meat increase rapidly at the beginning of the process (7-20 days) followed by a growth reduction or inhibition; TBC reaches frequently 5-7 Log₁₀ CFU/g without any sign of spoilage that, on the contrary, can be observed when bacterial count overcome 7 Log₁₀ CFU/cm²; this correlates to high bacterial counts on steaks after trimming. *Pseudomonas* spp. constitute the major components of the aerobic spoilage microbiota in food and appear to have a preponderant importance in dry-aged meat. Some authors showed the presence of bacteria in the internal side of the meat, which is generally considered sterile, after aseptic sampling. It is currently unclear whether the internal bacteria originate from the migration from the surface to the inner side of the loin during the drying process or from the growth of bacteria already present inside the meat at the beginning of the process. Besides, the contamination of the inner parts caused by bacteria originating from the surface of the meat is a condition that should be investigated also in consideration of the fissuration that sometimes happens in dry-aged meat following the desiccation of the surface. Thus, the microbiology of the deep parts of dry-aged meat should be further investigated.

Usually, low counts of *Enterobacteriaceae* are detected, showing only a moderate increase during aging, while a decrease is evidenced for *E. coli* counts. In comparison to not aged meat a lower count of *E. coli* and a comparable count of *Enterobacteriaceae* is then expected in dry aged meat. As a consequence, Dashdorj *et al.* (2016) proposed a shareable limit of 1000 CFU/g and 10 CFU/g for *Enterobacteriaceae* and *E. coli* respectively for the validation of the dry aging process.

About the pathogenic microorganisms of the family *Enterobacteriaceae* (*i.e.*, *Salmonella* and pathogenic *E. coli*), a decrease in number is expected during aging.

In controlled conditions, the only microbiological hazards to be considered in dry-aged meat are *L. monocytogenes* and *Y. enterocolitica* since the other microorganisms are unable to grow. Not only no data are available about *Y. enterocolitica*, but also available predictive models have a very low accuracy given the lack of information on the effect of desiccation due to airflow at low temperatures. To the best of our knowledge, challenge tests are the only tool we have to assess the evolution of populations of these two microorganisms in dry-aged meat.

Molds are frequent contaminants of meat and usually increase their number or relative percentage during aging. Nonetheless, the uncontrolled molding of meat in terms of the presence of visible molds on the surface of the meat, represents a failure of the process connected to the wrong positioning of the meat inside the equipment or process parameters' setting of RH or ventilation (Meat and Livestock Australia, 2016). In any case, moldy meat should be considered unfit for human consumption according to EU Regulation 178/2002 (European Parliament and Council of the European Union, 2002). 14 days of aging in controlled conditions of temperature, RH, and ventilation can be a reasonable limit to discriminate meat preserved at cold temperature from dry-aged meat; indeed, at 14 days of aging, the crust has created a protective layer for meat and is clearly visible. As the time of aging increases the crust becomes thicker, deeper and harder becoming inedible and similar to hide.

Few data are available on the shelf-life of aged meat after trimming; Gowda *et al.* (2022) reported that in commercial facilities in Belgium the median reported shelf-life for trimmed steaks was 4 days for unpacked steaks (2-10 days; 11 FBOs) and 18 days for vacuum packed steaks (5-30 days; 11 FBOs) or 5 days for modified atmosphere packaging (1 FBO). A much shorter shelf-life, namely of

2 to 3 days, is suggested by Dashdorj *et al.* (2016) who underlines the high perishability of dry-aged meat, thus encouraging trimming just before sale. At the Department of Veterinary Medicine and Animal Production University of Naples "Federico II", a challenge test was performed by inoculating about 2 CFU/cm² of *L. monocytogenes* strains (ATCC7644 and EURL 12M0B098LM) in trimmed steak of water buffalo stored at 2°C and 4°C after aging at 1°C, 78% RH, airflow 1,2 m/s for 40 or 60 days; in addition in uninoculated meat the total bacterial count and the count of LAB and *Brochothrix* spp. was performed together with pH and aw measurement. Table 2 reports the results of the evolution of the spoilage bacteria populations. Results showed a decrease of *L. monocytogenes* during 21 days of storage at 2°C from 1.90 ±0.05 to 0.67 ±0.58 Log₁₀ CFU/g; on the contrary, an increase from 2.17 ±0,13 to 5,1 ±0,03 Log CFU/g was observed after storage at 4°C. Spoilage populations increased to 6.30 and 7.73 Log₁₀ CFU/g for LAB and *Brochothrix* spp. respectively in meat stored at 4°C and to 4.38 and 7.57 Log₁₀ CFU/g in meat stored at 2°C. TBC increased to >9 Log₁₀ CFU/g after 21 days both at 4°C and at 2°C; an increase in the count of TBC and spoilage bacteria was accompanied by an increase in pH that resulted >6.0 after 5 days of storage and >7.5 after 21 days.

Interestingly, the a_w increased from normal values for dry-aged meat (0.981-0.984) to values comparable to meat not aged (0.991-0.998); these results may have favored the growth of both *L. monocytogenes* and spoilage bacteria and underlines the opportunity of trimming meat immediately before selling and maintaining it untrimmed in the dry aging cabinet in controlled conditions of temperature, ventilation and RH.

Considering 6.5-6.6 of pH and a TBC of 7-8 Log CFU/g as the cutoff values, based on our results, a shelf-life of 5-10 days can be proposed depending on the temperature of storage. Vacuum packing can further increase the shelf-life also reducing the increase of a_w that happens during storage in unpacked meat.

Conclusions

Concerns have been expressed on the microbiological safety of dry-aged meat because the aging process is not only carried out by professional food businesses, but new trends in meat consumption show also the interest of the consumers of meat, with 'dry-aging at home' advertisements.

Knowledge of the microbiological quality and safety of commercially produced dry-aged meat must be evaluated. Moreover, a shared definition of dry-aged meat that allows to set the conditions for identifying hygienic criteria should be reached. In Regulation (EC) No 853/2004, 'fresh meat' is defined 'as meat that has not undergone any preserving process other than chilling, freezing or quick-freezing' (European Parliament and Council of the European Union, 2004). Thus, the term 'fresh' when applied to meat as compared to other food commodities may lead to confusion.

Besides, no identification of the use that this meat is intended for is to date available, eventually posing threats for human health if consumed raw.

Dry aging is defined as a process in controlled conditions of temperature, RH and air flow thus aging must be performed in equipment designed and certified for this aim, representing the discriminant to differentiate dry-aged meat from meat preserved by cold temperature.

Process parameters must be set considering food safety as the priority; for this purpose temperature, RH and air flow were identified as critical and equipment should be able to manage and control these parameters; the following parameters can be suggested to accomplish the goal: working temperature 1±1°C, RH 75-80%, air speed 0.5-2.0 m/s, with 3°C, 85% RH as critical limits for the monitoring of CCPs and 0.2 m/s air speed on the product. The airflow on the product is difficult to be monitored during operative conditions and thus it should be studied and validated by the manufacturer. In addition, the pH should be monitored along with the other parameters during aging, with a limit of about 6.0 that, when exceeded, can be a sign of spoilage and needs an investigation.

In terms of time, 14 days of aging in controlled conditions of temperature, RH, and ventilation can be considered a reasonable limit to discriminate meat preserved with cold temperature from dry-aged meat.

An increase in the surface TBC usually happens during aging up to 5-6 Log₁₀ CFU/cm² and in some cases, higher microbial counts are reported without sign of spoilage. This correlates to high bacterial counts on steaks after trimming. *Pseudomonas* spp. constitute the major component of the aerobic spoilage microbiota in food and appear to have a preponderant importance in dry-aged meat.

In comparison to not aged meat, a lower count of *E. coli* and a comparable count of *Enterobacteriaceae* is expected in dry-aged meat and a shareable limit of 1000 CFU/g and 10 CFU/g for *Enterobacteriaceae* and *E. coli* respectively was proposed.

Despite molds can be detected in dry-aged meat and can increase their number during aging, molding of meat represents a failure of the dry ageing process and mouldy meat should be considered unfit for human consumption according to EU Regulation 178/2002 (European Parliament and Council of the European Union, 2002).

Organoleptic inspection (mainly color and odor) of meat before trimming and after cutting are key factors for the evaluation of the quality and safety of the product.

In controlled conditions, the only microbiological hazards to be considered in the dry-aged meat are *L. monocytogenes* and *Y. enterocolitica* since the other microorganisms are unable to grow. No data are available in the literature on *Y. enterocolitica* and further studies are necessary to clarify the fate of *L. monocytogenes* during dry aging. To the best of our knowledge, challenge tests are the only tool available to assess the evolution of populations of these two microorganisms in dry-aged meat.

Regarding pathogenic microorganisms of the family *Enterobacteriaceae* (*i.e.*, *Salmonella* and pathogenic *E. coli*) a decrease in number is expected during aging. Few data exist on the fate of other pathogenic microorganisms. The microbiology of deep parts of aged meat should be better investigated in relation to the presence of anaerobic microorganisms.

A shelf-life of 5-10 days for trimmed meat can be proposed depending on the temperature of storage. Vacuum packing can further increase the shelf life.

From a research point of view, a protocol with standardized extrinsic parameters for maturation would help to compare results obtained within challenge studies and better understand the behavior of microorganisms.

References

- Ahnström ML, Seyfert M, Hunt MC, Johnson DE, 2006. Dry aging of beef in a bag highly permeable to water vapour. *Meat Sci* 73:674-9.
- Álvarez S, Mullen AM, Hamill R, O'Neill E, Álvarez C, 2021. Dry-aging of beef as a tool to improve meat quality. Impact of processing conditions on the technical and organoleptic meat properties. *Adv Food Nutr Res* 95:97-130.
- Beach JC, Murano EA, Acuff GR, 2002. Prevalence of *Salmonella* and *Campylobacter* in beef cattle from transport to slaughter. *J Food Prot* 65:1687-93.
- Berger J, Kim YHB, Legako JF, Martini S, Lee J, Ebner P, Zuelly SMS, 2018. Dry-aging improves meat quality attributes of grass-fed beef loins. *Meat Sci* 145:285-91.
- Bernardo APDS, Da Silva ACM, Ferreira FMS, Do Nascimento MDS, Pflanzler SB, 2021. The effects of time and relative humidity on dry-aged beef: traditional versus special bag. *Food Sci Technol Int* 27:626-34.
- Brichta-Harhay DM, Guerini MN, Arthur TM, Bosilevac JM, Kalchayanand N, Shackelford SD, Wheeler TL, Koohmaraie M, 2008. *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Appl Environ Microbiol* 74:6289-97.

- Brooks FT, Hansford CG, 1923. Mould growths upon cold-store meat. *Trans Br Mycol Soc* 8:113-42.
- Buchanan RL, Gorris LGM, Hayman MM, Jackson TC, Whiting RC, 2017. A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control* 75:1-13.
- 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 and Muscle Biology* 4.
- Cherroud S, Cachaldora A, Fonseca S, Laglaoui A, Carballo J, Franco I, 2014. Microbiological and physicochemical characterization of dry-cured Halal goat meat. Effect of salting time and addition of olive oil and paprika covering. *Meat Sci* 98:129-34.
- Colle MJ, Richard RP, Killinger KM, Bohlscheid JC, Gray AR, Loucks WI, Day RN, Cochran AS, Nasados JA, Doumit ME, 2016. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the biceps femoris and semimembranosus. *Meat Sci* 119:110-7.
- Commission of the European Communities, 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. In: *Official Journal*, L 338/1, 22/12/2005.
- da Silva ACM, de Oliveira Pena P, Pflanzler 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.
- da Silva Bernardo AP, da Silva ACM, Francisco VC, Ribeiro FA, Nassu RT, Calkins CR, da Silva do Nascimento M, Pflanzler SB, 2020. Effects of freezing and thawing on microbiological and physical-chemical properties of dry-aged beef. *Meat Sci* 161:108003.
- Dashdorj D, Tripathi VK, Cho S, Kim Y, Hwang I, 2016. Dry aging of beef; review. *J Anim Sci Technol* 58:20.
- DeGeer SL, Hunt MC, Bratcher CL, Crozier-Dodson BA, Johnson DE, Stika JF, 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. *Meat Sci* 83:768-74.
- 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.
- EFSA BIOHAZ Panel, 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.
- European Commission, 2024. Commission Delegated Regulation (EU) 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*, L 2024/1141, 19/04/2024.
- European Parliament, Council of the European Union, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. In: *Official Journal*, L 031, 01/02/2002.
- European Parliament, Council of the European Union, 2004. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. In: *Official Journal*, L 139/55, 30/04/2004.
- 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.
- Gudjónsdóttir M, Gacutan MD, Mendes AC, Chronakis IS, Jespersen L, Karlsson AH, 2015. Effects of electrospun chitosan wrapping for dry-ageing of beef, as studied by microbiological, physicochemical and low-field nuclear magnetic resonance analysis. *Food Chem* 184:167-75.
- Hulánková R, Kameník J, Saláková A, Závodský D, Borilova G, 2018. The effect of dry aging on instrumental, chemical and microbiological parameters of organic beef loin muscle. *LWT* 89:559-65.
- Jaballah S, Fhoula I, Boumaiza M, Najjari A, Mhajbi N, Boudabous A, Klibi N, Ouzari H, 2022. Prevalence and risk factors of potential pathogenic *Yersinia enterocolitica* in Tunisian frozen ground beef through a shelf-life monitoring protocol validation. *Food Process Preserv* 46:e16360.
- Khazzar S, Segato S, Riuzzi G, Serva L, Garbin E, Gerardi G, Tenti S, Mirisola M, Catellani P, 2023. Influence of ageing time and method on beef quality and safety. *Foods* 12:3250.
- Kim S, Kim G, Moon C, Ko K, Choi Y, Choe J, Ryu Y, 2022. Effects of aging methods and periods on quality characteristics of beef. *Food Sci Anim Resour* 42:953-67.
- Kozyrev I, Kozyrev I, Mittelshtein T, Mittelshtein T, Pchelkina V, Pchelkina V, Kuznetsova T, Kuznetsova T, Lisitsyn A, Lisitsyn A, 2018. Marbled beef quality grades under various ageing conditions. *Foods and Raw Materials* 6:429-37.
- Knudsen GM, Sommer HM, Sørensen ND, Olsen JE, Aabo S, 2011. Survival of *Salmonella* on cuts of beef carcasses subjected to dry aging: *Salmonella* survival on beef carcasses. *J Appl Microbiol* 111:848-54.
- Lancaster JM, Smart JH, Van Buren J, Buseman BJ, Weber TM, Insausti K, Nasados JA, Glaze B, Price WJ, Colle MJ, Bass PD, 2022. Assessment of dry-aged beef from commercial aging locations across the United States. *Int J Gastron Food Sci* 27:100466.
- Lee HJ, Choe J, Kim KT, Oh J, Lee DG, Kwon KM, Choi YI, Jo C, 2017. Analysis of low-marbled Hanwoo cow meat aged with different dry-aging methods. *Asian-Australas J Anim Sci* 30:1733-8.
- 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.
- Li X, Chen H, Kingsley DH, 2013. The influence of temperature, pH, and water immersion on the high hydrostatic pressure inactivation of GI.1 and GII.4 human noroviruses. *Int J Food Microbiol* 167:138-43.
- Meat and Livestock Australia, 2016. Dry aged beef – design and good manufacturing practices review. Available from: https://www.mla.com.au/contentassets/e4b4dbda8b5645f8b7d584700f2e332e/p.psh.0679_final_report.pdf. Accessed on: 09/05/2024.
- Mikami N, Toyotome T, Yamashiro Y, Sugo K, Yoshitomi K, Takaya M, Han K-H, Fukushima M, Shimada K, 2021. Dry-aged beef manufactured in Japan: microbiota identification and their effects on product characteristics. *Food Res Int* 140:110020.
- Oh H, Lee HJ, Lee J, Jo C, Yoon Y, 2019. Identification of microorganisms associated with the quality improvement of dry-aged beef through microbiome analysis and dna sequencing, and evaluation of their effects on beef quality. *J Food Sci* 84:2944-54.
- Rezende de Souza JH, Cardello FAB, De Paula APM, Ribeiro FA, Calkins CR, Pflanzler SB, 2021. Profile of producers and production of dry-aged beef in Brazil. *Foods* 10:2447.
- Rhoades JR, Duffy G, Koutsoumanis K, 2009. Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: A review. *Food Microbiol* 26:357-76.

- 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.
- Ryu S, Shin M, Cho S, Hwang I, Kim Y, Oh S, 2020. Molecular characterization of microbial and fungal communities on dry-aged beef of hanwoo using metagenomic analysis. *Foods* 9:1571.
- Scott WJ, Vickery JR, 1939. Investigations on chilled beef. Part II. Cooling and storage in the meatworks. In: Empey WA, ed. *Investigations on chilled beef*. Government Printer, Melbourne, Australia.
- Shao L, Chen S, Wang Haodong, Zhang J, Xu X, Wang Huhu, 2021. Advances in understanding the predominance, phenotypes, and mechanisms of bacteria related to meat spoilage. *Trends Food Sci Tech* 118:822-32.
- Shi Y, Zhang W, Zhou G, 2020. Effects of different moisture-permeable packaging on the quality of aging beef compared with wet aging and dry aging. *Foods* 9:649.
- Smaldone G, Marrone R, Vollano L, Peruzy MF, Barone CMA, Ambrosio RL, Anastasio A, 2019. Microbiological, rheological and physical-chemical characteristics of bovine meat subjected to a prolonged ageing period. *Ital J Food Saf* 8:8100.
- Tittor AW, Tittor MG, Brashears MM, Brooks JC, Garmyn AJ, Miller MF, 2011. 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* 74:289-93.
- Tomasello F, Pollesel M, Mondo E, Savini F, Scarpellini R, Giacometti F, Lorito L, Tassinari M, Cuomo S, Piva S, Serraino A, 2021. Effectiveness of alkaline electrolyzed water in reducing bacterial load on surfaces intended to come into contact with food. *Ital J Food Saf* 10:9988.
- USDA, 2024. Food standards and labeling policy book. Available from: <https://www.fsis.usda.gov/sites/default/files/import/Labeling-Policy-Book.pdf>.
- USMEF, 2014. Guidelines for U.S. dry-aged beef for international markets.
- Van Damme I, Varalakshmi S, De Zutter L, Vossen E, De Smet S, 2022. 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. *Food Microbiol* 104:104000.

Online supplementary material:

Supplementary Table 1. Data of total bacteria count, *Enterobacteriaceae* and coliforms including *Escherichia coli* retrieved from the literature and dry-aging duration in days, relative humidity in %, air ventilation in m/sec, meat type, time points, type of sampling, pH, and water activity.

Supplementary Table 2. Data on psychrotrophic bacteria, lactic acid bacteria, and *Pseudomonas* spp. retrieved from the literature and dry-aging duration in days, relative humidity in %, air ventilation in m/sec, meat type, time points, type of sampling, pH, and water activity.

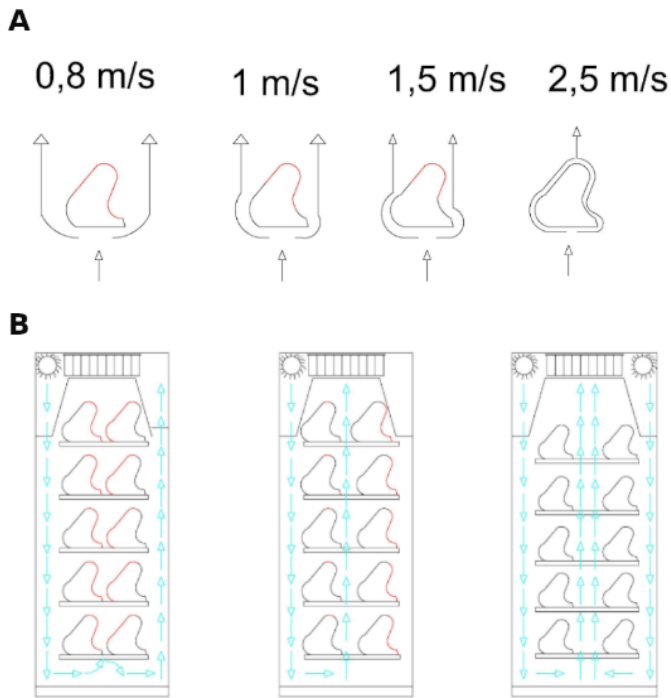


Figure 1. A) Air speed influencing the drying of meat; B) different meat and fan positioning within cabinets can affect dry aging.



Figure 2. A,B) Beef hanging from the bones when is not directly positioned on the shelves; C) correct positioning of ribs: a distance of usually 3 cm is considered the minimum among ribs; D) ribs are too close to one another and do not allow correct circulation of airflow.



Figure 3. A) Meat with deep spoilage; before trimming the meat showed a fessuration with a humid apperance; after cutting meat showed areas of discoloration (green) with off odor that increased after cooking; B) meat with regular surface: a uniform crust can be observed; C) meat with regular surface, but with fessurations on the surface; D) meat with irregular surface: the crust is irregular and several fessuration can be observed; E) meat with regular surface and with regular and homogeneous crust; F) dry-aged meat with beef tallow applied on fessurations.

Table 1. Summary of the pH values registered in literature at the beginning and at the end of the aging period.

Author	Duration (days)	Starting pH value	End pH value
Shi <i>et al.</i> , 2020	14	5.41	5.61
Khazzar <i>et al.</i> , 2023	18	5.54	5.60
Gudjonsdottir <i>et al.</i> , 2015	21	5.62	5.60-5.65
Ahnstrom <i>et al.</i> , 2006	21	5.4	5.7
Li <i>et al.</i> , 2013	21	5.57	5.62
Bernardo <i>et al.</i> , 2020	21	5.39	5.51
DeGeer <i>et al.</i> , 2009	28	5.51	5.49
Kozyrev <i>et al.</i> , 2018	28	5.74	5.62
Berger <i>et al.</i> , 2018	18	5.79	5.79
Da Silva <i>et al.</i> , 2019	42	5.5	5.5
Di Paolo <i>et al.</i> , 2023	60	5.7	5.72
Smaldone <i>et al.</i> , 2019	290	5.7	6.0

Table 2. Evolution of spoilage bacteria population in trimmed steak of water buffalo stored at 2°C and 4°C after aging at 1 °C, 78% relative humidity, airflow 1,2 m/s.

T	Day	pH	a _w	<i>Listeria monocytogenes</i> * (log10 CFU/g)	<i>Listeria monocytogenes</i> * SD	TVC	LAB	<i>Brochothrix thermosphacta</i>
4°C	0	5.99	0.984	2.17	0.13	7.46	3.95	3.61
	1	5.90	0.986	2.44	0.29	7.34	4.18	3.48
	2	5.91	0.986	2.22	0.09	7.48	4.54	3.65
	3	5.88	0.984	3.31	0.04	8.68	5.62	5.54
	4	5.90	0.983	4.11	0.03	8.72	5.75	6.11
	5	6.20	0.985	4.21	0.06	8.98	5.93	6.43
	6	6.57	0.985	4.28	0.06	9.23	5.96	6.76
	8	6.29	0.987	4.29	0.10	9.23	5.96	6.68
	10	6.83	0.985	4.44	0.11	9.38	6.38	7.59
	13	7.41	0.999	4.92	0.07	9.51	6.86	7.76
	16	7.54	0.999	5.16	0.06	9.59	6.40	7.61
21	7.58	0.998	5.18	0.03	9.60	6.30	7.73	
2°C	0	5.96	0.981	1.90	0.05	5.40	4.34	0.00
	1	5.87	0.982	2.39	0.04	3.93	3.80	0.00
	2	5.90	0.988	2.44	0.01	3.87	3.72	0.00
	3	5.89	0.994	2.41	0.05	4.34	3.62	0.00
	4	5.80	0.996	2.26	0.18	4.76	3.53	3.56
	5	6.06	0.994	2.32	0.13	4.96	3.59	4.70
	8	5.73	0.990	2.23	0.29	5.94	4.18	4.59
	11	6.03	0.993	2.05	0.17	7.85	4.00	6.67
	14	6.18	0.990	1.84	0.20	8.32	4.32	6.76
	18	6.89	0.994	1.10	0.17	9.28	4.28	7.48
	21	7.25	0.991	0.67	0.58	9.18	4.38	7.57

T, temperature; a_w, water activity; SD, standard deviation; TVC, total viable count; LAB, lactic acid bacteria.