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

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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 hygiene 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 hygiene evaluation of the production process, a count no higher than that of unmatured 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_{10} 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 matured 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 Europe (EFSA BIOHAZ Panel *et al*., 2023). The process is carried out in aerobic conditions by hanging beef carcasses or placing subprimal or 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 airflow.

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 particularly important since the recent European Food Safety Authority (EFSA) opinion on the microbiological safety of aged meat (EFSA BIOHAZ Panel *et al*., 2023) stated that aging 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, which 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 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, the environment, and equipment. During dry-aging, the contamination can also be advantaged by the continuous loading of loins into cabinets, which is adopted by most 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, a specific HACCP plan and good manufacturing practices (GMP)/good hygiene practices (GHP) for dry-aging should be applied to control the aging process. Guidelines for the safe production of aged meat have been drafted by Meat Livestock Australia (Meat and Livestock Australia, 2016) and the U.S. Meat Export Federation (USMEF, 2014) to help FBO produce safe and standardized dry-aged meat. In addition, the information available in the literature was recently revised by the EFSA BIOHAZ Panel *et al*. (2023). According to the Commission Delegated Regulation (EU) No. 2024/1141 (European Commission, 2024), meat subject to dry-aging must be stored at a surface temperature of -0.5-3.0°C, with an 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 equivalent guarantees 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, along 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, microbiological hygiene criteria, and GMP 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 took into consideration the 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 and maturation, are used to describe the meat process. From the regulatory point of view, Commission Delegated Regulation (EU) No. 2024/1141 defines "dry-aging" 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, the 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 critical importance for the achievement of desired meat characteristics, as their increase is 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 (Dashdorj *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%) 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 lower 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 superficial disseccation but reducing the process yield and increasing the thickness of the crust, consequently increasing losses due to trimming.

Meat and Livestock Australia (2016) identified a temperature of 0.5-3°C, ventilation of 0.2-0.5 m/s, and 75-85% RH as the most suitable parameters for meat dry-aging; the USMEF (2014) indicated a temperature of 0-4°C, ventilation of 0.5-2 m/s, 80-85% RH; while the Commission Delegated Regulation (EU) No. 2024/1141 reports a surface temperature of -0.5 to 3.0 °C, with an RH of a maximum of 85% and 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 into 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 <4°C, 67.5% at RH<80%, and 78.4% at mean or high air speed; the remaining producers's percentage either applied differently 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 <4°C (min 0.74 max 5.26), 80% at RH<85% (min 64.87 max 92.21), and all utilized 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 an 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 and 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 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 low a_w 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 than to temperature. In this regard, a direct effect of disseccation on the reduction of some microorganisms was described by several authors. Knudsen et al. (2011) reported that different levels of disseccation can influence the 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 a reduction of a_w on the surface of the meat, which, 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 Log_{10} colony-forming units (CFU)/cm^2 for S. aureus, Salmonella spp., Listeria monocytogenes, and Escherichia coli, respectively. Despite the impossibility of directly comparing the results obtained on stainless steel plates with 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 from meat preserved only at low temperatures (EFSA BIOHAZ Panel et al., 2023).

Tenderness and flavor improvement in meat are dependent on the degree of alteration and weakening of myofibrillar structures and have been largely attributed to 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 the 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 1 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 FBO (EFSA BIOHAZ Panel et al., 2023). Similarly, the USDA (2024) defines dry-aged beef products (carcass or cuts) as those 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 in 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 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 a priority, and the following parameters, according to Dashdorj *et al*. (2016), can be suggested to accomplish the goal: temperature 1±2°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, 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 for 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; 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; Shi *et al*., 2020; Bernardo *et al*., 2021; Di Paolo *et al*., 2023; Khazzar *et al*., 2023).

Da Silva Bernardo *et al*. (2020) demonstrated that starting at 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 ensuring food safety.

Van Damme *et al*. (2022) reported an increase in *L. monocytogenes* count during the aging of a loin, with a starting pH of 6.21 and 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 values 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 0. 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-esopeptidases, 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 and 5.8.

Microbial community of dry-aged meat

Meat is normally expected to be contaminated with a range of bacteria and molds, which include both spoilage and pathogenic organisms. The initial contamination level of the carcasses, hygienic practices during 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

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 *et al*., 2020	14	5.41	5.61
Khazzar *et al*., 2023	18	5.54	5.60
Gudjónsdóttir *et al*., 2015	21	5.62	5.60-5.65
Ahnström *et al*., 2006	21	5.4	5.7
Li *et al*., 2013	21	5.57	5.62
Bernardo *et al*., 2020	21	5.39	5.51
DeGeer *et al*., 2009	28	5.51	5.49
Kozyrev *et al*., 2018	28	5.74	5.62
Berger *et al*., 2018	18	5.79	5.79
Da Silva *et al*., 2019	42	5.5	5.5
Di Paolo *et al*., 2023	60	5.7	5.72
Smaldone *et al*., 2019	290	5.7	6.0

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 foodstuffs (Commission of the European Communities, 2005); however, no 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) are usually used as process hygiene criteria in most 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 the 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 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 as 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 Log_{10} CFU/g) during 294 days of maturation. On the contrary, most of the authors witness an increasing trend in the counts on the meat surface, even though variations are recorded among settings. *Supplementary Table 1* shows that TBC values before the aging ranged from <10 Log_{10} CFU/g to 4.10 Log_{10} CFU/g (Di Paolo *et al*., 2023) while, generally, a final value of almost 5 Log_{10} CFU/g is reached on the crust as reported by different authors at the end of the aging period, ranging from 8 to 60 days. Exceptions are represented by the study of DeGeer *et al*. (2009) reporting lower counts, specifically 2.89-3.51 Log_{10} CFU/g after 28 days of aging, and Shi *et al*. (2020) showing a high initial bacterial count (4.62 Log_{10} CFU/g) that grew in 21 days to 7.53 Log_{10} CFU/g.

Interestingly, a much higher surface count, namely 9.47 Log_{10} 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_{10} 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 authors concluded that an RH lower than 85% can be suggested to keep microbial counts below the deterioration level.

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±1°C, 85±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_{10} 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_{10} CFU/g after 15 days followed by a stabilization up to 60 days of aging (1±1°C; 78±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°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 on lean and fat surfaces, followed by a period of up to 21 days of aging in which 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 from 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_{10} 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_{10} CFU/g.

Mikami *et al*. (2021) detected 3.10 Log_{10} 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 Log_{10} CFU/cm² and from <0 to 1.6 Log_{10} CFU/cm² in lean and adipose tissue, respectively, during 42 days of aging at different conditions of temperature and RH. *Enterobacteriaceae* 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 Khazzar *et al*. (2023) observed a moderate increase (from 1 to 1.7 Log_{10} CFU/g) of *Enterobacteriaceae* count after 230 days of aging, and Di Paolo *et al*. (2022) observed a similar increase of coliforms and *Enterobacteriaceae* (from 3.08 to 3.93 and from 1.78 to 1.92 Log_{10} CFU/g respectively) from the 2nd to the 60th day of aging; in the same study, a reduction from 1.33 log_{10} CFU/g to a 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 Log_{10} 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 Log_{10} CFU/g. At slaughter, the microbial contamination should be limited to the meat surface, unless bacteriaemia or spread during slaughtering results in 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 also be investigated in consideration of the cracking that sometimes happens in dry-aged meat following surface dissection. The presence of an inhomogeneous 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_{10} 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 at retail, with the main increase in TBC occurring usually in the first aging period. Generally, off-flavors resulting from spoilage in meat can be observed from a bacterial count > 7 Log_{10} CFU per cm² 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 with TBC is expected to be higher in dry-aged meat than in meat before aging.

Based on the literature results, the dry-aging process is generally considered hostile to Enterobacteria. Specifically, when present at the beginning of the process, the count shows only a moderate increase (<1 Log_{10} CFU) during time, while on the contrary, the E. *coli* count decreases. In comparison to not-aged meat, a lower count of E. *coli* and a comparable count of *Enterobacteriaceae* are 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 factors 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 Log_{10} 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 for 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 Log_{10} CFU/cm² and up to >4 Log_{10} CFU/g in the surface and deep parts of the meat, respectively, and LAB increasing to about 1.5 Log_{10} CFU/cm² both in the samples collected in the external and internal parts 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, and 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. (<1.0-8.8 Log_{10} CFU/cm²), *B. thermosphacta* (<1-7.4 Log_{10} CFU/cm²), and psychrotrophic LAB (<1.0-7.3 Log_{10} CFU/cm²). Starting from a median of 2.1 Log_{10} CFU/cm² of *Pseudomonas* on fat and <1 Log_{10} CFU/cm² on lean tissue, no variation was evidenced on the latter (final value <1 Log_{10} CFU/cm²), while counts on fat tissue were up to 7.4 Log_{10} CFU/cm² with a mean value of 4.0 Log_{10} CFU/cm² after 42 days of aging (Van Damme *et al.*, 2022). However, other authors, such 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 also reported by Gowda *et al.* (2022), with results ranging from <1.0 to 8.8 Log_{10} CFU/cm². Lee *et al.* (2019) observed that *Pseudomonas* spp. became the prevalent bacteria, starting at 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 in *Lactobacillus* spp. and *Flavobacterium*. The air speed seemed to influence the relative abundance of different bacterial populations.

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

A reduction of LAB during aging was observed by Ryu *et al.* (2020), with *Lactobacillus*, *Bifidobacterium,* and *Streptococcus* being the most abundant bacteria at the beginning of the dry-aging process and reducing their relative abundance up to 160 days of aging. Lee *et al.* (2019) reported changes in 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 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 did not influence the final TBC, the *Enterobacteriaceae* count, the psychrotrophic count, and the yeast count, but had an influence on the LAB count that ranged from <1.53 Log_{10} 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 humans 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). 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 temperatures (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 Log_{10} 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 conditions and the lack of data collected in commercial settings, the overall conclusion of the scientific opinion was that a maximum of 2 Log_{10} pathogen increase is likely to occur in both beef dry-aged for 35 days at 3°C and standard fresh meat matured under vacuum for 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 Log_{10} CFU/day except for one loin with a pH>6.0 at the end of dry-aging, in which it was evidenced a 1 Log_{10} 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_{10} CFU/g) rather than at 2°C (2.38 Log_{10} 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 in evaporation, helping to reduce 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. The same results were confirmed by Gowda *et al.* (2022), who did not detect *E. coli* on the steaks, and by Hulánková *et al.* (2018), who reported *E. coli* below 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 reported 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 Log_{10} CFU/cm² (Van Damme *et al.*, 2022), with a daily reduction variation between -0.07 and -0.14 Log_{10} CFU/day on adipose tissue and between -0.07 and -0.12 Log_{10} CFU/day on lean meat. Tittor *et al.* (2011) observed a 4 Log_{10} 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 Log_{10} CFU/cm² over 14 days (at 1°C in a conventional refrigerator with an 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 aging.

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 Log_{10} CFU/cm² and ≤2.0 Log_{10} CFU/cm², respectively) by Gowda *et al.* (2022). Mikami *et al.* (2021) report 3.16±0.50 Log_{10} 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. was not considered of importance by the 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 of dry-aged meat was given to the molds listed in the Meat and Livestock Australia (2016) guidelines that are able to grow at low temperatures, namely *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp., *Chrysosportim* sp., *Thamnidium* sp., *Rhizopus* sp., *Mucor* sp., and *Aureobasidium* sp.

The very first report regarding molds on cold-stored meat dates back to 1923 and regards the first description of molds, along with yeast, isolated from dry-aged beef (Brooks and Hansford, 1923), which has 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_{10} 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 in molds from 1.34 Log_{10} CFU/g at 7 to 3.14 Log_{10} 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 Log_{10} 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 Log_{10} 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 Log_{10} CFU/g to 4.81 Log_{10} 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 aging; Berger *et*

al. (2018) after 21 days reported numbers for yeasts and molds of 1.49 and 0.23 Log_{10} 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 $3.3×10^3$ and $1.0×10^3$ 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 has 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 by 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 generally, 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 molds 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), which is a range higher than the 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 the wrong parameter 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 No. 178/2002 (European Parliament and Council of the European Union, 2002).

Hazard analysis and critical control points, 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) No. 2024/1141 (European Commission, 2024). Temperature, RH, airflow, and pH have unanimously been identified as being 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±1°C for long-term aging was proposed, with an acceptable higher temperature of 2 to 3°C in case of 1 to 2 weeks of aging processes.

According to the literature, the following parameters can be also suggested: 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 monitor 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 the characteristics of the equipment (evaporators, condensators, humidification/dehumidification systems), in particular regarding fans and their positioning into the cabinet, the amount of meat that can be aged at the same time, 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 crust formation. EFSA suggested that air speed should be higher at the beginning of the process, but this is of difficult application to small producers, like restaurants, where, rather than adopting 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 the air speed and design of the facilities, fans' positioning, and meat positioning can influence airflow.

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 adequate air circu-

lation. If hanged, the meat should not be fixed to the flesh but rather to the bone (Figure 2 A,B). Separation between pieces of meat is necessary; usually, 3 cm is considered the minimum. However, in cases where the loins are too close, the air is not allowed to correctly circulate (Figure 2 C,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 an 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 a 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 3 B,E). This is even more important if we consider that sometimes fissurations or wholes due to desiccation can also appear on a cut with a regular surface (Figure 3C). Fissurations should be left drying (Figure 3 D,E) and subsequently either curetted or filled with beef tallow to reduce the loss-
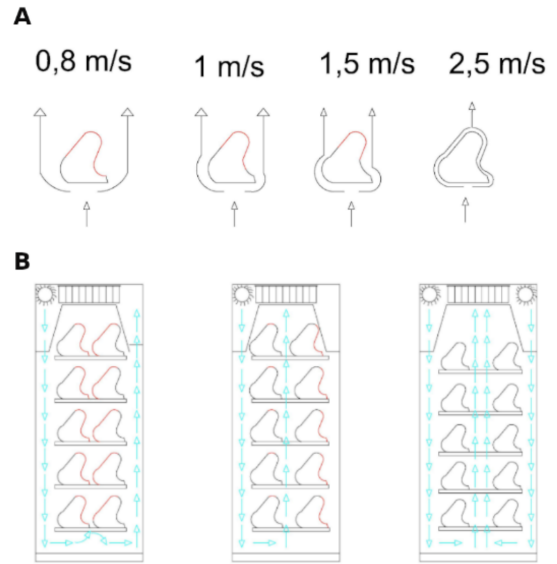

Figure 1. A) Air speed influencing the drying of meat; **B)** different meat and fan positioning within cabinets can affect dry-aging.

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.

Table 2. Evolution of spoilage bacteria population in trimmed water buffalo steak 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	*Listeria monocytogenes* (log$_{10}$ CFU/g)	*Listeria monocytogenes* SD	TVC	LAB	Brochothrix thermosphacta
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; aw, water activity; SD, standard deviation; TVC, total viable count; LAB, lactic acid bacteria.

Figure 3. A) Meat with deep spoilage; before trimming the meat showed a fessuration with a humid appearance; 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 fissurations.

es at trimming (Figure 3F).

Organoleptic inspection (mainly color and odor) of the 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 frequently reaches 5-7 Log_{10} CFU/g without any sign of spoilage, which, on the contrary, can be observed when bacterial counts exceed 7 Log_{10} 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 on 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 also be investigated, considering 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* are 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 the available predictive models have 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 in meat and usually increase in 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 No. 178/2002 (European Parliament and Council of the European Union, 2002). Fourteen 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), 18 days for vacuum-packed steaks (5-30 days; 11 FBOs), and 5 days for modified atmosphere packaging (1 FBO). A much shorter shelf-life, namely 2-3 days, is suggested by Dashdorj *et al.* (2016), who underline the high perishability of dry-aged meat, thus encouraging trimming just before sale. At the Department of Veterinary Medicine and Animal Production of the 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, TBC and the count of LAB and *Brochothrix* spp. were performed together with pH and a_w 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 Log_{10} CFU/g; on the contrary, an increase from 2.17±0.13 to 5.1±0.03 Log_{10} CFU/g was observed after storage at 4°C. Spoilage populations increased to 6.30 and 7.73 Log_{10} CFU/g for LAB and *Brochothrix* spp., respectively, in meat stored at 4°C and to 4.38 and 7.57 Log_{10} CFU/g in meat stored at 2°C. TBC increased to >9 Log_{10} 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 in >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 underline 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 a pH of 6.5-6.6 and a TBC of 7-8 Log_{10} 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 while also reducing the increase of a_w that happens during storage of unpacked meat.

Conclusions

Concerns have been expressed about 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 also show the interest of 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 for the identification of hygiene 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 to human health if consumed raw.

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

Process parameters must be set considering food safety as a

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 $1\pm1°C$, 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 monitor during operative conditions, so 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 distinguish meat preserved at a cold temperature from dry-aged meat.

An increase in the surface TBC usually happens during aging up to 5-6 Log_{10} CFU/cm^2 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* are 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 the fact that molds can be detected in dry-aged meat and can increase in number during aging, the molding of meat represents a failure of the dry-aging process, and moldy meat should be considered unfit for human consumption according to EU Regulation No. 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.

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Online supplementary material:

Supplementary Table 1. Data on 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.

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