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Behaviour of *Listeria monocytogenes* and *Yersinia enterocolitica* during beef dry-aging up to 60 days

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Abstract

The Commission delegated Regulation (EU) 2024/1141 states that beef dry aging can be performed in a dedicated cabinet, at a surface temperature of -0.5 to 3°C, maximum relative humidity of 85%, air speed of 0.2 – 0.5 m/s and for a maximum of 35 days. Food business operators can apply different process parameters, even longer aging times, as long as they demonstrate to the competent authority that the aged meat is microbiologically safe. To support food business operators in the implementation of longer dry aging processes, in this study we investigated the behaviour of *Listeria monocytogenes* and *Yersinia enterocolitica* during beef dry aging in a patented cabinet up to 60 days. Beef loins were experimentally spiked with a mean load of 4.4 and 3.9 Log_{10} CFU/g of *Listeria monocytogenes* and *Yersinia enterocolitica* respectively and stored at 1°C, relative humidity 78% and ventilation 2.0 m/s for 60 days. *L. monocytogenes* counts did not significantly change during the dry aging process, while *Y. enterocolitica* significantly increased up to 3 Log_{10} CFU/g during the aging process and then tended to decrease at 60 days. The results of this study showed that beef dry aging at 1±2°C, relative humidity 78±7%, ventilation 2.0 m/s for 60 days does not support

the growth of *L. monocytogenes.* On the contrary, the same process supported the growth of *Y. enterocolitica* up to 40 days in two of the three tested batches, showing that the impact of dry aging on the behaviour of this pathogen deserves further studies.

Keywords

microbiological safety; challenge test; dry aging.

1. Introduction

The market for dry aged meat is quickly increasing in the European Union but there is a lack of information on the conditions applied by the food business operators, including restaurants, for the dry aging process. In a recent scientific opinion, the European Food Safety Authority (EFSA) addressed the microbiological safety of beef dry aging (2023). In the document it has been concluded that aging under categorized pH, water activity (a_w) and temperature conditions can result in loads of microbiological hazards and spoilage bacteria comparable to those reached in standard fresh meat preparation. Following the publication of the EFSA scientific opinion, specific requirements were laid down in Regulation (EC) No 853/2004 through the Commission delegated Regulation (EU) 2024/1141. Within the latter, dry aging is defined as “the storage of fresh meat in aerobic conditions of hanging carcasses or cuts either unpacked or packed in bags permeable to water vapour in a refrigerated room or cabinet and left to age for several weeks at controlled environmental conditions of temperature, relative humidity and airflow”. In addition, the Regulation clarifies that “such meat is placed on the market as fresh meat or, *e.g.,* by the adding of ripening cultures during dry-aging to fresh meat, as meat preparation”.

The EFSA scientific opinion on microbiological safety of aged meat shows that many pathogenic bacteria may be present on dry-aged beef. However, only *Listeria monocytogenes* and *Yersinia enterocolitica* can grow on the meat surface under chilled (0–4°C) conditions. Indeed, the minimal growth temperature for *L. monocytogenes* ranges from 0° C to 2° C while

Y. enterocolitica growth can also take place at temperatures of −2° C with a faster multiplication than *L. monocytogenes* at chill temperatures.

L. monocytogenes is a microorganism that is widely distributed in the environment and can be isolated from a variety of sources such as soil, water, sewage, animal feeds, silage, farm environments, abattoirs (Demaître et al., 2021) and food-processing environments (Jordan et al., 2018). The pathogen usually contaminates meat products due to extensive manipulation (Currie et al., 2015; Kvistholm Jensen et al., 2016) and processing. *L. monocytogenes* is among the zoonotic agents included in the Directive 2003/99 for compulsory annual monitoring in the EU while *Y. enterocolitica* is monitored according to the epidemiological situation. In the last EU One Health zoonoses report, referred to the year 2022 (EFSA & ECDC, 2023), listeriosis and yersiniosis were the fifth and third most commonly reported zoonosis in humans, respectively. A total of 2738 confirmed invasive human cases of listeriosis resulted in 1330 hospitalisations and 286 deaths, making listeriosis one of the most serious foodborne agents in Europe. The confirmed cases of yersiniosis were 7919 corresponding to a notification rate of 2.2 cases per 100,000 population, corresponding to an increase of 16.3% compared with 2021. No deaths from yersiniosis were reported in 2022 and the few data available indicate a hospitalization rate around 30%. *Yersinia enterocolitica* is the species reported in the majority (98.7%) of human cases. *Yersinia enterocolitica* is usually associated with pork meat due carcasses contamination in slaughterhouses, with prevalence on surface ranging from 0.3% (Gürtler et al., 2005) to 39.7% post-evisceration (Van Damme et al., 2015) being pigs healthy carriers (De Boer & Nouws, 1991; Doyle & Beuchat, 2007). Beef is generally less contaminated by *Y. enterocolitica* than pork, indeed while domestic pigs could be the principal reservoir, cattle may act as occasional hosts (Liang et al., 2015).

The Commission delegated Regulation (EU) 2024/1141 states that dry aging of beef can be performed at a surface temperature of -0.5 to 3°C, maximum relative humidity (RH) of 85%, air speed of 0.2 – 0.5 m/s in a dedicated cabinet for a maximum of 35 days starting at the end of the stabilisation period upon slaughter. Food business operators can perform dry aging with different process parameters or for longer time, but they must demonstrate to the competent authority that their meat is microbiologically safe. To support food business operators in the implementation of longer dry aging processes, in this study we investigated the behaviour of *L. monocytogenes* and *Y. enterocolitica* during beef dry aging in a patented cabinet up to 60 days.

2. Materials and methods

2.1 Study design

A total of 12 loins (*longissimus dorsi*) weighing 3.5-5.5 kg obtained from Holstein male beef cattle carcasses of approximately 360 kg and mean age of 23 months were purchased from a commercial slaughterhouse and tested in this study. After slaughtering, the half carcasses were cold stored (0 – 3 °C) for 4 days, then the loins were removed from each carcass and subsequently vacuum packed for 8 days. Three batches (labelled as B1, B2 and B3), each one consisting of 4 randomly selected loins were subjected to dry aging for 60 days within a dedicated cabinet, where the process parameters were set as follows: temperature of 1±2°C, RH 78±7%, ventilation 2.0 m/s, following the manufacturers' indications for beef aging. Each loin, of normal bloomed beef colour and without quality defects, was considered a sample unit. The cabinet was a Stagionello® Meat Curing Device (Crotone, Italy), an equipment designed and patented (European Patent No. EP 2769276B1; Canadian Patent No. CA2852650) for meat dry aging.

On each loin, areas of 10 cm^2 were identified using the guide of a sterile stainless-steel shape (5×2 cm) where at the corners, sterile toothpicks were inserted in the lean meat. Identified areas were either experimentally inoculated with the selected pathogens or used for Lactic Acid Bacteria (LAB), Enterobacteriaceae (EB) and Total Bacteria (TBC) enumeration. Samplings were performed at T0 and after 13, 27, 40 and 60 days (T13, T27, T40 and T60) respectively. Physicochemical parameters (*i.e.*, pH and a_w) were collected at each timepoint.

The sampling of the contaminated areas was performed following the BS EN ISO 20976-2:2022, dedicated to challenge tests. The ISO method specifies that a minimum of 3 batches have to be included in the challenge test, with a minimum of 5 sampling points, comprising T0 and Tend. The Welch's t-test was performed to determine the statistical significance of differences in microbiological counts and physicochemical parameters between sampling times.

2.2 Experimental contamination of the loins

The experimental contamination of the loins was performed at T0 with three different *L. monocytogenes* and three different *Y. enterocolitica* strains (Table 1) that were spiked on the lean surface following the EURL *Lm* (2021) and the ISO 20976-2:2022.

Pathogen species	**Strain ID**	**Origin**	**Isolation source**
Listeria monocytogenes	12MOB045LM	ANSES*	Meat
	12MOB085LM	ANSES*	Meat
	12MOB089LM	ANSES*	Meat
Yersinia enterocolitica	ATCC° 9610	ATCC	-
	SEFA A2 IN	Wild strain	Dry aged beef
	SEFA 63737	Wild strain	Rabbit carcass

Table 1: *Listeria monocytogenes* and *Yersinia enterocolitica* strains experimentally spiked on the tested meat. * French Agency for food, environmental and occupational health & safety. °American Type Culture Collection (ATCC). Both A21N and 63737 selected field strains belonged to biotype 1A (BT1A), while with respect to the serotype they were not-typable (O: ; NT) and O:5.

Concerning *Y. enterocolitica* strains SEFA A2 IN and SEFA 63737, the first isolation was performed in our laboratory on dry aged beef and on a rabbit carcass respectively. Both strains have been bio- and sero- typed. Biotyping was performed in accordance with ISO 10273:2017, while serotyping was carried out using commercially available sera from Biolife Italiana, (Milan, Italy). The serotype results were confirmed through molecular serotyping. Additionally, the chromosomal genetic markers *ail (*attachment and invasion locus) and *ystB* (stable yersinia toxin B) were evaluated using SYBR Green real-time PCR (Ventola et al., 2023). Preparation of the inoculum was performed following the EURL *Lm* technical guidance document. Prior to use in the study, each strain was cultured in a 9 mL tube of Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. Two successive subcultures of each strain were prepared and adapted to grow in chilled conditions. When an approximate concentration of 10^4 CFU/g for each microorganism was reached, the subcultures were equivalently mixed to obtain the mixed culture of each pathogen, from where 50 µl were used for inoculation of the meat 10 cm^2 surface.

2.3 Physicochemical analyses

At each sampling point, measurements of pH and a_w were performed on each sample that had to be sampled in that specific timepoint, as well as on the ones to be sampled in the following timepoints.

The pH was measured on the surfaces of lean meat where the pathogens were not experimentally spiked, following the non-destructive protocol. After excision of the surface by means of a sterile scalpel, insertion of the electrode was performed directly into the meat (ISO 2917:1999) at a depth of 2 cm using a pH meter equipped with an insertion electrode (Delta-OHM HD 2105.2).

Water activity was measured at 25 °C by means of the a_w recorder AquaLab, series 3, Model TE (Decagon Devices, Inc., Pullman, USA) in accordance with ISO 18787: 2017. Samples for measurement of a_w consisted of 2mm thick slices of the crust that were collected with a sterile scalpel running parallel to the surface, thus avoiding the collection of internal portions of meat. Before proceeding with the a_w measurement the slices of crust were chopped and homogenously distributed on the surface of the sample tray.

2.4 Microbiological analyses

To verify that *L. monocytogenes* and *Y. enterocolitica* were not present in the experimentally spiked meat both pathogens were tested in a 25 g pool sample representing each of the 4 loins of each batch. *L. monocytogenes* was tested following BS EN ISO 11290-1:2017. Briefly, 25 g of the pooled beef were enriched into 225 ml of Half Fraser Broth (Thermo Fisher Scientific Basingstoke, UK) followed by Fraser Broth (Thermo Fisher Scientific Basingstoke, UK) and streaked on Brilliance Listeria Agar Base (CM 1212 Thermo Fisher Scientific Basingstoke, UK) and ALOA (Agar Listeria According to Ottaviani and Agosti 4016052 Biolife, Milan, Italy). *Y. enterocolitica* was tested following the BS EN ISO 10273:2017. Briefly, 25 g of the pooled beef were enriched into 225 ml PSB and subsequently processed following Annex A.

At each sampling point, 10 grams of the surface sample where the pathogens were experimentally spiked were aseptically collected with a sterile scalpel and diluted in 90 mL of sterilized Buffered Peptone Water (PW, CM0009, Thermo Fisher Scientific Basingstoke, UK), placed in a sterile stomacher bag and homogenized for 2 minutes using a stomacher (BagMixer®, Interscience, St Nom, Francia). Subsequently, ten-fold serial dilutions of each homogenate were prepared in PW, followed by spread plating in duplicate in selective agar plates. Enumeration of *L. monocytogenes* was performed following the BS EN ISO 11290-2:2017 on Brilliance Listeria Agar Base (CM 1212 Thermo Fisher Scientific Basingstoke, UK) incubated at 37°C for 24-48 hours, while for *Y. enterocolitica*, since no ISO is available for enumeration, analyses were performed on *Yersinia* Selective Agar Base (CM0653 Thermo Fisher Scientific Basingstoke, UK) incubated at 32°C for 24 hours. In addition, at each sampling point, ten grams of the surface sample where the pathogens were not experimentally spiked were tested for TBC on Plate Count Agar (CM0325 Thermo Fisher Scientific Basingstoke, UK) incubated at 30° for 72 h, EB on Violet Red Bile Glucose Agar (CM0485 Thermo Fisher Scientific Basingstoke, UK) incubated at 37°C for 24 hours and LAB on De Man Rogosa Sharpe agar (CM1153 Thermo Fisher Scientific Basingstoke, UK) incubated at 30°C for 72 hours following the ISO 4833-2:2022, 21528-2:2017 and 15214:1998, respectively. The *Y. enterocolitica* colonies showed a typical red bull's-eye surrounded by a transparent border. To confirm that the colonies identified based on morphology were indeed *Yersinia*, analysis was performed by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (Biotyper, Bruker Inc., USA) on 5 colonies at each sampling point from each plate where colonies were counted.

3. Results and discussions

3.1 Physicochemical parameters

The initial mean pH value measured in all tested loins (N=12) was 6 (± 0.24) that is higher than what generally reported in literature at the beginning of dry aging. The pH of the loins showed a high variability growth throughout the experiment starting from T0, throughout aging, reaching 6.45 (± 0.15) at T60.

The mean initial a_w value of 0.993 (± 0.003) drastically decreased ($p=0.045$) to 0.937 (± 0.030) already after 13 days of aging. No significant difference was observed at T27 (0.942 ± 0.020) followed by a significant reduction at T40 (0.849 ± 0.020) vs T27 ($p=0.00$) and T60 (0.874 ± 0.030) vs T40 ($p=0.00$).

The a_w value is a crucial parameter because, along with pH, it strictly affects the bacteria ability to grow on the meat. Nevertheless, few information is available in the literature about the a_w profile of the meat surface along the process (EFSA 2023; Savini et al., 2024) probably because the a_w measurement need a disruptive protocol and it is not cost effective in commercial conditions. Moreover, the few studies in which a_w results are reported do not provide information regarding the sampling method, including the thickness of the meat surface tested which largely affect the a_w result. To fill this gap, we described the sampling procedure we used for a_w measurement that can be used as reference in future studies.

3.2 Microbiological parameters

L. monocytogenes and *Y. enterocolitica* were not detected before the experimental contamination. Therefore, the enumeration results refer to the behaviour of the spiked cells only. The mean initial contamination of experimentally spiked areas resulted of 4.4±0.41 and 3.9±0.45 Log_{10} CFU/g for *L. monocytogenes* and *Y. enterocolitica* respectively.
Results of *L. monocytogenes* and *Y. enterocolitica* Log_{10} CFU/g, pH and a_w at each sampling point and their mean ± standard deviations are reported in Table 2.

Batch	Timepoint	Days	Log_{10} CFU/g LM	Log_{10} CFU/g YE	pH	a_w
B1	T0	0	3.96	3.46	5.71	0.993
	T13	13	4.50	6.70	6.46	0.969
	T27	27	4.21	5.20	6.70	0.950
	T40	40	5.90	7.25	6.57	0.881
	T60	60	3.74	3.65	6.47	0.879
B2	T0	0	4.67	4.33	5.76	0.997
	T13	13	4.10	5.61	6.65	0.970
	T27	27	4.71	7.28	6.29	0.952
	T40	40	5.04	7.08	6.32	0.862
	T60	60	4.64	1.30	6.54	0.875
B3	T0	0	4.69	4.09	5.84	0.990
	T13	13	4.52	5.63	6.60	0.956
	T27	27	3.94	7.71	6.67	0.923
	T40	40	3.29	2.45	6.04	0.839
	T60	60	3.26	1.30	6.34	0.868
Mean of the 3 batches ±SD	**T0**	**0**	**4.44±0.36**	**3.96±0.37**	**5.77±0.07**	**0.994±0.003**
	T13	**13**	**4.37±0.21**	**5.98±0.62***	**6.57±0.08**	**0.945±0.030**
	T27	**27**	**4.29±0.35**	**6.73±1.20**	**6.55±0.20**	**0.942±0.020**
	T40	**40**	**4.74±1.31**	**5.59±2.72**	**6.31±0.26**	**0.861±0.020**
	T60	**60**	**3.88±0.65**	**2.08±1.17**	**6.45±0.10**	**0.874±0.010**

Table 2: Log_{10} CFU/g of *L. monocytogenes* (LM) and *Y. enterocolitica* (YE) during 60 days of aging in batch 1, 2 and 3 and mean ± standard deviation (SD). * represents difference in Log_{10} CFU/g count of *Y. enterocolitica* in comparison to previous time point.

The study of Van Damme et al., (2022) showed a more extensive reduction of *L. monocytogenes*, namely 1 to 3 Log_{10} CFU/cm^2, already after 42 days on 3 out of 4 tested loins, on the contrary a 1.0 log_{10} CFU increase was registered on a single loin with pH > 6.0 at the end of dry-aging, indicating that this pathogen can potentially grow under certain dry-aging conditions, and this might have also influenced our results. Da Silva et al., (2019) inoculated, on the surface of boneless loins, *L. innocua* as a surrogate of *L. monocytogenes*, that decreased of 2.4 and 3.4 Log_{10} CFU/g within 42 days. The differences in the results achieved in our and other studies reported in the literature can be attributed to the different process parameters set. Indeed, Van Damme et al., (2022) set a 2x2 factorial design for temperature (2 °C and 6 °C) and relative humidity (75% and 85%) but did not specify ventilation while da Silva et al., (2019) set an air velocity of 2 ± 0.5 m/s at 2 ± 1 and 8 ± 1 °C with 75 ± 2% RH. Our results are more similar to the results obtained by Van Damme et al., (2022) in lean meat at 2°C and 85% RH.

In our study pH and a_w parameters should support the growth of *L. monocytogenes* at least until 27 days of aging, but a significant increase of Log_{10} CFU/g count was not observed; it should be noted that, in our study as in others, the pH was measured on the internal portion of the meat, rather than on the surface where the inoculum was performed thus a direct correlation cannot be formulated. Since the measurement of the pH on the surface of the loin cannot be performed following a non-destructive procedure, the only manageable parameter is the internal pH. However, its value in predicting the growth potential of pathogenic bacteria contaminating the loin surface should be further investigated.

In general, temperature and relative humidity values registered among studies show little variation from one another and can be easily controlled within the cabinet. On the contrary, for ventilation, that was demonstrated to affect bacterial growth and survival (Savini et al., 2023), factors such as positioning and loin shape, as well as the design of the ventilation system of the cabinet largely impact on desiccation effectiveness. In this respect, to avoid the influence of fixed locations in maturation cabinet on the pathogen's behaviour, da Silva et al., (2019) randomly relocated the samples every two days inside the chamber. Nevertheless, we simulated a real commercial scenario where loins are moved only in case fresh meat is introduced in the cabinet where other meat is already under aging.

In the EFSA scientific opinion, the application of predictive models showed a *L. monocytogenes* increase ranging from 1 to 2 Log_{10} based on the aging process parameters that were applied based on different realistic cases of dynamic profiles of aw, pH and temperature during dry-ageing of beef under specific target temperature (from 0 to 3 °C) and RH (from 60 to 78%) conditions retrieved from the scientific literature. However, these

predictions might be overestimated because both rate of meat surface drying and impact of competitive exclusion with meat microorganisms were not considered in the models.
Yersinia enterocolitica showed a higher growth variability than *L. monocytogenes* among the three batches (Table 2). Differently from the other batches, in Batch 1 a decrease of Log_{10} CFU/g of *Y. enterocolitica* was shown between T13 and T27, followed by an increase of about 2 Log_{10} CFU/g. One possible explanation for this decrease is that in Batch 1 between T13 and T27 the a_w reached values not supporting the growth of *Y. enterocolitica*. Moreover, cabinet and the biological variability among the tested loins should be also considered.
In general, counts reported a significant increase of about 2 Log_{10} CFU/g ($p=0.01$) in the first phases of the aging, followed by a decrease that started after T27 for batch 3 and afterT40 for the other two batches. These decreasing trends resulted not statistically significant due to high variability between batches within the same time point.
The minimum pH for *Y. enterocolitica* growth has been reported between 4.2 and 4.4 (Bari et al., 2011) much lower than the values detected in our study; *Y. enterocolitica* can grow at temperatures as low as -1.15°C (Alber and Schaffner, 1992) and is reported to multiply faster than *L. monocytogenes* at chill temperatures (Gill & Reichel, 1989). On the other side, *Y. enterocolitica* that displays a minimum a_w of 0.96 (Stern et al., 1980) for growth has a lower resistance to a_w reduction (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2023) if compared with *L. monocytogenes.* In our test a_w decreased at values <0.96 starting from T13. As a consequence, when a_w values are higher (T0 to T13), a more pronounced and significant growth of *Y. enterocolitica* is displayed, as well as a more evident decreasing trend can be observed later on, within each batch, when a_w values tend to lower, while *L. monocytogenes* is less affected by the a_w variations. Further research is needed to better understand the behaviour of *Y. enterocolitica* during aging. All our results refer to the behaviour of reference and field *Y. enterocolitica* strains belonging to biotype 1B and 1A but additional biotypes should be also tested since they might display different characteristics in term of survival and growth in beef during the dry aging process. We decided to experimentally spike the meat with strains belonging to the biotype 1A because its role as foodborne pathogen has been recently highlighted (Palau, 2024). The absence of a standard method for the enumeration of the pathogen could have had an influence on the results as it is established that there are considerable difficulties associated with isolating *Y. enterocolitica* from clinical, food, and environmental samples (Bari et al., 2011).

Batch	Timepoint	Day	Log_{10} CFU/g TBC	Log_{10} CFU/g EB	Log_{10} CFU/g LAB
B1	T0	0	3.32	1.81	<1
	T13	13	4.54	<1	<1
	T27	27	6,01	<1	1.78
	T40	40	6.67	<1	<1
	T60	60	6,06	<1	2.91
B2	T0	0	3.16	<1	<1
	T13	13	7.31	2.95	3.47
	T27	27	8.22	<1	<1
	T40	40	8.14	<1	<1
	T60	60	8.87	<1	<1
B3	T0	0	2.42	<1	<1
	T13	13	5.62	<1	2,06
	T27	27	8.30	<1	2.60
	T40	40	8.56	<1	<1
	T60	60	3.86	<1	1.30

Table 3: Log_{10} CFU/g of Total Bacteria Count (TBC), Enterobacteriaceae (EB) and Lactic Acid Bacteria (LAB) during 60 days of aging in Batch 1, 2 and 3.

TBC count on the surface of the samples at T0 showed similar values in the three tested batches, with a mean of 3.32 Log_{10} CFU/g, rapidly increasing in the initial time points (T13 and T27) to reach values comprised between 6.1 to 8.3 Log_{10} CFU/g in different batches to 6.06 – 8.87 Log_{10} CFU/g at T60 (Table 3). Literature reports variable initial TBC values on raw beef ranging from <10 CFU/g up to 4.10 Log_{10} CFU/g (Di Paolo et al., 2023) with counts generally increasing during aging. Our results further confirm the increase of TBC within the first phases of dry aging resulting in meat without sign of spoilage. Generally, an off-flavour, which is a result of meat spoilage, can be observed in meat with a bacterial count of around 7 log_{10} CFU/cm^2, although some negative changes can be observed much earlier with bacteria counts of between 5 and 6 Log_{10} CFU/cm^2 (Feiner, 2006). Nevertheless, in our case we did not observe modifications related to spoilage defects.

The LAB were generally very low, initially near the quantification limit of 10 CFU/g without a clear trend during time with the highest value of 3.47 Log_{10} CFU/g at T13 of B2 and a maximum final count of 2.91 Log_{10} CFU/g in B1(Table 3). Indeed, LAB grow in meat under anaerobic conditions (Gram et al., 2002; Huis In'T Veld, 1996) where are known to compete with other microorganisms and produce compounds with antimicrobial activity (Leroy & De Vuyst, 2004; Tomé et al., 2006). In addition, many strains are capable of producing bacteriocins in foods (Cotter et al., 2005; Panesar et al., 2007) that may inhibit the growth of pathogenic Gram-positive bacteria, such as *L. monocytogenes*. In dry aged meat a scarce concentration of LAB is usually reported at the beginning of the process, followed by a limited growth, reported by metagenomic (Ryu et al., 2020; Savini et al., 2024) and culture methods (Campbell et al., 2001; da Silva et al., 2019; Hulánková et al., 2018) in line with our results. As a consequence, their importance on this specific product can be considered minor.

EB counts were always under the limit of quantification (*i.e.,* <10 Log_{10} CFU/g). Our results are in line with others that report that when present, Enterobacteriaceae count decrease (Li et al., 2013; Smaldone et al., 2019) during time.

4. Conclusions

Dry aging at 1°C, relative humidity 78% and ventilation 2.0 m/s for 60 days does not support the growth of *L. monocytogenes.* On the contrary, the same process supported the growth of *Y. enterocolitica* up to 40 days in two of the tested batches, showing that the impact of dry aging on the behaviour of this pathogen deserves further studies. However, it is worth to highlight that at the end of the aging process *Y. enterocolitica* largely decreased in comparison to initial concentration. This is the first study showing the behaviour of *Y. enterocolitica* experimentally spiked in dry-aged beef. Further trials will be organised testing different aging conditions and other *Y. enterocolitica* biotypes.

Glossary

Meat dry aging: the storage of fresh meat in aerobic conditions of hanging carcasses or cuts either unpacked or packed in bags permeable to water vapour in a refrigerated room or cabinet and left to age for several weeks at controlled environmental conditions of temperature, relative humidity and airflow (COMMISSION DELEGATED REGULATION (EU) 2024/1141 of 14 December 2023).

Process parameters: the parameters that are set and should be monitored and controlled during aging, namely temperature, relative humidity and ventilation.

Cabinet: the equipment within which the dry aging takes place and that encompasses monitoring and control of process parameters

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