Preliminary data on the microbial profile of dry and wet aged bovine meat obtained from different breeds in Sardinia

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Abstract

This study aimed to evaluate the influence of dry and wet aging on microbial profile and physicochemical characteristics of bovine loins obtained from four animals of two different breeds, namely two Friesian cull cows and two Sardo-Bruna bovines. During dry and wet aging aerobic colony count, *Enterobacteriaceae*, mesophilic lactic acid bacteria, *Pseudomonas*, molds and yeasts, *Salmonella enterica*, *Listeria monocytogenes* and *Yersinia enterocolitica,* pH and water activity (a_w) were determined in meat samples collected from the internal part of the loins. Moreover, the microbial profile was determined with sponge samples taken from the surface of the meat cuts. Samples obtained from Friesian cows were analyzed starting from the first day of the aging period and after 7, 14, and 21 days. Samples obtained from the Sardo Bruna bovines were also analyzed after 28 and 35 days. Wet aging allowed better control of *Pseudomonas* spp. during storage that showed statistically lower levels (P>0.05) in wet-aged meats with respect to dry-aged meats during aging and particularly at the end of the period (P>0.01) in both cattle breeds. At the end of the experiment (21 days), aerobic colony count and *Pseudomonas* in Fresian cows' dry-aged meats showed mean levels >8 log, while lactic acid bacteria mean counts >7 log were detected in wet-aged meats of both cattle breeds. In meats submitted to dry aging, pH was significantly higher (P<0.01) with respect to wet-aged meats at all analysis times and in both cattle breeds. A_w showed a stable trend during both dry and wet aging without significant differences. These preliminary results highlight the critical importance of the strict application of good hygiene practices during all stages of production of these particular cuts of meat intended for aging.

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Introduction

The post-slaughter aging is a long-established preservation method that consists of meat storage at refrigerated temperatures for a variable period, to allow the action of natural enzymatic activity and thus the development of flavor characteristics, increased tenderness and overall acceptance (Li *et al*., 2014; Ha *et al*., 2019; Álvarez *et al*., 2021). Dry-aging is a traditional process of aging bovine carcasses or primal/sub-primal cuts under controlled environmental conditions of temperature, relative humidity, and ventilation, usually without protective packaging (Kim *et al*., 2016). As dry-aged meat is exposed to the air flowing during the entire period, its surface undergoes drying and crust development (Mikami *et al*., 2021) which implies trimming of spoiled or oxidized external layer before selling, with consequent weight losses and a rise in prices (Juarez *et al*., 2011; Smaldone *et al*., 2019). In addition, the exposure of meat to the environment enhances the risk of secondary microbial contamination and increases surface microbial development. On the other hand, drying of the surface with the consequent crust formation allows the growth of molds and yeasts with proteolytic and lipolytic activity that, within the action of endogenous enzymatic activity in muscle, lead to the tenderness of dry-aged meat (Mikami *et al*., 2021). Wet aging is the aging of meat in vacuum packaging stored at constant tempera-

tures between −1 and +3°C. The microflora is mainly represented by lactic acid bacteria due to the anaerobic conditions (Bischof *et al*., 2020). Wet aging was introduced in the 1970s (Terjung *et al*., 2021) and since then, it has become the prevailing packaging/aging method in the meat industry, because of its advantages regarding low losses and convenience during storage and transport (Li *et al*., 2013). Moreover, this technique requires less space, is adaptable to automation, and can ensure a longer shelf-life compared to dry aging due to the possibility of better control of microbial development (Terjung *et al*., 2021). Despite these advantages, wet-aged beef can be associated with negative taste attributes, such as acidic or bloody (Ramanathan *et al*., 2020; Bischof *et al*., 2020).

During the last decades, the application of dry aging has been limited because of its mentioned low yield and consequent high price (Kim *et al*., 2019). However, in recent years, consumption of dry-aged meat has been increasing worldwide and consumers are willing to pay more when they get used to its unique flavor (DeGeer *et al*., 2009; Li *et al*., 2013; Álvarez *et al*., 2021; Gowda *et al*., 2022).

Several factors influence the quality of aged beef and a clear picture of their effect seems to be difficult, especially for dry-aged meat. Among the most important factors, the quality of meat before the beginning of aging depends on the proper application of hygienic and manufacturing procedures during slaughtering and the subsequent production steps, most of all during sectioning. These factors, along with storage conditions, particularly humidity, temperature, air flow parameters, and storage duration, will determine the microbial quality of aged beef (Ahnström *et al*., 2006; Gowda *et al*., 2022). The importance of microbial control during aging has been reported in different studies under controlled laboratory conditions (Li *et al*., 2013, 2014; Kim *et al*., 2019; Smaldone *et al*., 2019; Van Damme *et al*., 2022). Other authors showed that microbiological counts on dry-aged beef meat varied greatly under field conditions, reflecting the varieties of the applied processes (Lancaster *et al*., 2022; Gowda *et al*. 2022). Microorganisms most commonly responsible for meat spoilage during storage are *Pseudomonas* spp., *Brochotrix thermosphacta*, lactic acid bacteria, *Enterobacteriaceae,* and yeasts (Ercolini *et al*., 2011; Piras *et al*., 2013). Moreover, pathogens like *Salmonella* spp., *Listeria monocytogenes,* and *Escherichia coli* can be transferred to carcasses during slaughtering operations and consequently to meat during the following production steps (Lee *et al*., 2017; Van Damme *et al*., 2022).

The objective of this study was to evaluate the influence of dry and wet aging on microbial profile and physiochemical characteristics of beef loins obtained from two different cattle breeds.

Materials and Methods

Tested animals

Experiments were conducted on four animals belonging to two different bovine breeds: two Friesian cull cows (F, age 60 and 64 months) and two Sardo-Bruna bovines (SB, age 10 months).

Measurement and microbiological analysis at the slaughterhouse

All animals were slaughtered at a commercial slaughter plant according to their standard routines.

At the slaughterhouse the following evaluations were carried out: i) live weight: animals were weighted before slaughtering, after 12 hours of fasting; ii) dressing percentage: weight of the carcass 1 hour after slaughtering/weight of live animal *100; iii) cooler shrink percentage: weight of the carcass after 24 hours of cold storage/weight of live animal *100; iv) carcass pH and temperature: pH and temperature were continuously monitored starting from one hour after slaughtering and during the following 24 hours [ultimate pH and T (pHu, Tu)] in *M. longissimus dorsi* between the 4th and the 7th lumbar vertebra, using a portable pH-meter, equipped with a pH-electrode fixing and a temperature probe (Crison GLP21; Crison Instruments, Alella, Spain); v) carcass superficial contamination: on each carcass, after dressing and before chilling, non-destructive samplings were performed (ISO, 2015; European Commission, 2005) with a hydrated-sponge (pre-moistened with 10 mL Buffered Peptone Water Broth, 3 M Health Care, Milan, Italy) on four areas of 100 cm^2 each. The selected areas were the most representative of carcass contamination: shoulder, brisket, rump, and loin sites. All the sponge samples were analyzed for enumeration of aerobic colony count (ACC) at 30°C (ISO, 2013), *Enterobacteriaceae* (ISO, 2017a), *Pseudomonas* spp. (ISO, 2009), mesophilic Lactic Acid Bacteria (LAB) (ISO, 1998), and yeast and molds (ISO,2008). Moreover, *Salmonella enterica* (ISO, 2017b), *Listeria monocytogenes* (ISO, 2017c), and *Yersinia enterocolitica* (ISO, 2003 with some modifications) presence/absence was determined.

Measurement and microbiological analysis during ageing processes

The half carcasses were cold stored (0±3°C) for 48 hours at the slaughterhouse and then transported to a local butchery. Each of the half carcasses was cut in loins that comprised the six lumbar vertebrae and the last seven costs.

Dry aging: loins from one of the half carcasses were placed uncovered in a dry-aging chamber (Armadio Factotum FCT1400) under the following conditions: temperature 3±1°C, humidity 75%, air flow 1±0.5 m/s.

Wet aging: on the same day, loins from the contralateral half carcass were deboned, and packaged under vacuum in 90 µm thick plastic bags (Plastar PAK s.r.l., Milan, Italy), with a permeability to O_2 of 67 cm^3/m^2, 24 hours bar at 23°C and 0% relative humidity, and stored at 4±1°C. On loins obtained from F cows, the analytical determinations were performed at the following intervals: the day of the beginning of the aging (T0), then after seven (T7), fourteen (T14), and twenty-one (T21) days. Considering that loins intended to be submitted to wet aging were cut deboned and packaged on the same day of loins submitted to dry aging, samples collected from wet-aged meats were analyzed starting from T7. Based on the microbiological results obtained, SB loins were also analyzed 28 (T28) and 35 (T35) days after the beginning of the aging.

Table 1 shows the number of samples and type of analysis performed at each sampling time by breed and aging technology.

Microbial profile

Surface microbial profile

Microbial profile of the meat cuts submitted to dry and wet aging was determined by sampling the whole surface with a hydrated sponge (pre-moistened with 10 mL Buffered Peptone Water Broth, 3 M Health Care, Milan, Italy).

Internal parts microbial profile. From each loin, 10 g of meat was aseptically sampled. As regard meat cuts submitted to dry aging, the most superficial layer of the surface, about 3 mm thick, was removed before sampling using sterile scalpel and forceps. For both surface and internal microbial profile, the same microbial

groups/microorganisms considered for carcasses were evaluated.

Water activity and pH. For samples collected from the internal parts of the loins, pH was determined with pH-meter GLP 22 (Crison Instruments SA, Barcelona, Spain). Moreover, water activity (a_w) analysis was conducted at +25°C, using an Aqualab CX3 (Decagon, Pullman, Washington, USA).

Statistical analysis

Differences among average pH and a_w and microbiological group counts (log_{10} cfu/cm^2 or g) over time, among breeds and treatments, were compared using Fisher's least significant difference test. Statistical analyses were performed with Statgraphics Centurion XIX software (Stat Point Technologies, Warrenton, VA, USA).

Results

Measurements and microbiological analysis at the slaughterhouse

Live weight was 640 and 550 kg for F cows and 590 and 612 kg for SB bovines. The dressing percentage ranged between 52.5-54.7% for F cows that showed a cooler shrink of 1-1.2%. For SB beefs a dressing percentage between 53.9-57.8% was measured, while the cooler shrink was 1.5-2%. Muscular pH showed a regular trend in all the animals with levels [±standard deviation (SD)] measured 1 hour after slaughtering of 6.7±0.1, and 5.5±0.1 at 24 hours after slaughtering. Temperature decreased from 30.8±0.5°C to 5.4±0.5°C, respectively 1 hour and 24 hours after slaughtering. As regards carcasses microbial profile (±SD; log_{10} CFU/cm^2), ACC showed levels of 1.07±0.9 and 0.95±0.14 for F and SB carcasses, respectively. Levels of *Pseudomonas* spp. were 0.59±0.83 in F and 0.19±0.26 in SB carcasses. *Enterobacteriaceae*, LAB, yeasts and molds, *Salmonella enterica*, *L. monocytogenes,* and *Y. enterocolitica* were not detected.

Measurement and microbiological analysis during ageing processes

Microbial profile

Surface microbial profile. Table 2 shows the microbial profile of the surface of the loins (log_{10} CFU/cm^2; ±SD) during dry and wet aging. In F dry-aged meat, ACC initial mean levels (3.95±0.30) showed a progressive increase during dry aging with a significant change between T0 and T7 ($P<0.01$), reaching levels >8 log at the end of the aging process (T21). In SB meats lower ACC mean levels were observed in comparison to F meats, with significant differences ($P<0.05$) starting from T14 and final values of *ca.*

Table 1. Type of analysis, testing time, performed during the experiment on the number of samples.

	Aging	Breed	Sampling time						Total
			T0	T7	T14	T21	T28	T35	
pH	D	F	8	8	8	8	-	-	32
	W		-	8	8	8	-	-	24
a_W	D	SB	8	8	8	8	8	8	48
	W		-	8	8	8	8	8	40
Total			16	32	32	32	16	16	144

a_w, water activity; F, Friesian; SB, Sardo Bruna; D, dry aging; W, wet aging; -, not tested; T0, first day of aging; T7, T14, T21, T28, T35, respectively 7, 14, 21, 28, 35 days after start of aging. Microbial profile: total bacterial count, *Enterobacteriaceae*, mesophilic lactic acid bacteria, *Pseudomonas* spp., yeasts, molds, *Salmonella* spp., *L. monocytogenes*, *Y. enterocolitica.*

Table 2. Microbial profile (log_{10} CFU/cm^2; $\bar{x}$ ± standard deviation) of loins surface during dry and wet aging of the two cattle breeds.

Microbial group	Aging	Breed	Sampling time					
			T0	T7	T14	T21	T28	T35
Aerobic mesophilic bacteria	D	F	3.95±0.30^{a,x}	7.16±0.36^{b,x}	8.65±0.59c,x	8.29±0.47c,x	-	-
		SB	4.44±0.47^{a,x}	5.96±1.35^{a,x}	6.28±1.01^{a,y}	5.95±0.35^{a,y}	3.58±1.82b	5.96±0.33^{a}
	W	F	-	7.32±0.23^{a,x}	8.88±0.16^{b,x}	8.72±0.23^{b,x}	-	-
		SB	-	7.27±0.25^{a,x}	7.73±0.16^{a,y}	8.37±0.28^{b,x}	6.29±0.14c	8.28±0.20^{b}
Enterobacteriaceae	D	F	1.24±0.34 (2/4)^{a,x}	4.21±0.54^{b,x}	3.86±0.95b,x	4.09±2.09 (3/4)^{b,x}	-	-
		SB	1.15±0.21 (2/4)^{a,x}	4.51±0.94 (3/4)^{a,x}	4.24±0.35^{a,x}	4.09±0.57^{a,x}	3.18±0.75a	4.30±0.77^{a}
	W	F	-	3.12±0.24^{a,x}	5.55±0.18^{b,x}	4.98±0.47^{b,x}	-	-
		SB	-	4.63±0.33^{a,y}	5.06±0.43^{a,x}	5.07±0.30^{a,x}	5.36±0.27a	4.49±0.29^{a}
Mesophilic lactic acid bacteria	D	F	3.53±0.18a^{a,x}	5.20±0.77^{b,x}	5.69±0.70^{b,x}	5.45±0.76^{b,x}	-	-
		SB	2.99±0.62^{a,x}	3.18±0.51^{a,y}	0.00^{b,y}	3.64±0.23^{a,y}	0.00b	4.01±0.38^{a}
	W	F	-	5.64±0.24^{a,x}	7.50±0.18^{b,x}	8.08±0.47^{b,x}	-	-
		SB	-	6.38±0.33^{a,y}	7.01±0.43^{a,x}	7.88±0.30^{b,x}	7.69±0.27b	8.04±0.29^{b}
Pseudomonas spp	D	F	3.60±0.32^{a,x}	6.39±0.12^{b,x}	8.85±0.20^{c,x}	8.95±0.43^{c,x}	-	-
		SB	4.52±0.29^{a,y}	6.06±1.80^{a,x}	6.66±0.87^{a,y}	5.77±0.68^{a,y}	4.00±1.21a	6.15±0.46^{a}
	W	F	-	5.57±0.36^{a,x}	6.81±0.48^{b,x}	7.07±0.49^{b,x}	-	-
		SB	-	5.97±0.27^{a,x}	6.39±1.21^{a,x}	6.18±0.89^{a,x}	4.01±0.44b	7.05±0.65^{a}
Molds and yeasts	D	F	2.44±0.31^{a,x}	2.83±0.55^{a,x}	3.66±0.76^{a,x}	3.90±1.16^{a,x}	-	-
		SB	1.65±0.49 (2/4)^{a,y}	1.15±0.21 (2/4)^{a,y}	2.63±0.32^{b,y}	1.65±0.49 (2/4)^{a,y}	1.72±0.69^{a}	2.26±0.15^{a}
	W	F	-	3.94±0.00 (1/4)^{a,x}	2.82±1.07^{a,x}	3.02±0.00 (1/4)^{a,x}	-	-
		SB	-	0.00^{a,x}	2.26±0.24 (3/4)^{a,x}	0.00^{a,x}	1.30±0.00 (1/4)^{a}	2.11±1.57 (2/4)a

F, Friesian; SB, Sardo Bruna; D, dry aging; W, wet aging; -, not tested; T0, first day of aging; T7, T14, T21, T28, T35, respectively 7, 14, 21, 28, 35 days after start of aging. Means in the same row with different letters were significantly different ($P<0.05$); means in the same column among aging techniques with a different superscript number were significantly different ($P<0.05$). Values within brackets indicate the prevalence of positive samples.

6 log (T35). As regards *Enterobacteriaceae,* similar values were observed in dry-aged meats of the two breeds, without significant differences (P>0.05) and final levels of *ca.* 4 log. In F dry-aged meats, a significant increase between T0 and T7 was observed for LAB (P<0.05) and *Pseudomonas* spp. (P<0.01), with a more stable trend for the remaining aging period. In SB meats, LAB showed significantly lower values with respect to F meats starting from T7 (P<0.05). *Pseudomonas* spp. showed a regular increase during aging, with significantly lower levels (P<0.05) with respect to F starting from T14, and final values of *ca.* 6 log. Molds and yeasts mean values in F meat were comprised between 2 and 4 log during the aging period, while in SB meats were detected more sporadically and with significantly lower mean values (P<0.05) with respect to F. As for meat submitted to wet aging, ACC showed similar mean values between F and SB, without significant differences (P>0.05), and remained stable during the whole period with final counts of ca. 8.5 log. *Enterobacteriaceae*, LAB, and *Pseudomonas* spp. showed a similar trend in F and SB wwet-agedmeats, without significant differences(P>0.05) and final values of *ca.* 5, 8, and 7 logs, respectively for *Enterobacteriaceae,* LAB, and *Pseudomonas* spp. Lastly, molds and yeasts were sporadically detected without significant differences (P>0.05) between the two breeds.

Salmonella enterica, *L. monocytogenes,* and *Y. enterocolitica* were never detected.

Internal parts microbial profile. Table 3 shows the microbial profile of the internal part of loins (log_{10} CFU/g; ±SD) during dry and wet aging.

In F dry-aged meats, ACC, *Enterobacteriaceae,* and LAB showed a regular increase during the experiment with significant changes between T0 and T7 (P<0.01) and T14 and T21 (P<0.05) and during all analysis times for *Enterobacteriaceae* (P<0.05). A similar trend was observed for ACC and *Enterobacteriaceae* in SB dry-aged meats but without any significant changes during the period (P>0.05). In F dry-aged meats, LAB mean counts showed significant changes between T0 and T7 (P<0.01) and T14 and T21 (P<0.05). *Pseudomonas* spp. mean levels showed a progressive increase during the aging of F meats with significant changes at all analysis times (P<0.05) and final levels >9 log. *Pseudomonas* spp. showed lower mean levels in SB dry-aged meats in comparison to F with significant differences at T14 (P<0.05) and T21 (P<0.01). Molds and yeasts showed significantly higher levels in F meats if compared to SB meats starting from T7.

In meats submitted to wet aging, ACC, *Enterobacteriaceae*, LAB, and *Pseudomonas* spp. showed a stable trend and similar values in both breeds, with final values of 7 log for ACC and LAB, 5 log for *Pseudomonas* spp., and 3 log for *Enterobacteriaceae*. No significant differences were observed between the two breeds (P>0.05). Molds and yeasts were sporadically detected, particularly after T21. As far as the comparison between the two aging methods is concerned, in dry-aged meats (internal parts) obtained from the two breeds, higher mean counts of Pseudomonas were observed in comparison to wet-aged meats, with significant differences (P<0.01) starting from T7 and for the remaining aging period. On the contrary, wet-aged meats of both cattle breeds showed significantly higher levels of lactic acid bacteria (P<0.01) starting from T14 and until the end of the period.

Salmonella enterica, *L. monocytogenes,* and *Y. enterocolitica* were never detected.

Water activity and pH. In dry aging, pH (±SD) showed initial mean levels of 5.52±0.01 in F meats and 5.54±0.03 in SB meats. During dry aging, an increase in pH values was observed in meats obtained from both breeds, with significant changes (P<0.01) during all analysis times for F; for SB meats a significant increase was detected between T7 and T14 (P<0.05) and between T21 and T28 (P<0.01). During wet aging, an initial pH of 5.50±0.01 and 5.49±0.01 was observed respectively in F and SB meats. Afterward, a decrease of mean levels was observed with significant changes between T7 and T14 (P<0.05) for F, between T21 and

Table 3. Microbial profile (log_{10} CFU/g; $\bar{x}$ ± tandard deviation) of internal loins during dry and wet aging of the two cattle breeds.

Microbial group	Aging	Breed	Sampling time					
			T0	T7	T14	T21	T28	T35
Aerobic mesophilic bacteria	D	F	1.59±0.12^{a,x}	6.51±0.74^{b,x}	7.33±0.60^{b,x}	8.76±0.22^{c,x}	-	-
		SB	3.29±0.77^{a,y}	5.24±2.84^{a,x}	5.29±1.12 a, y	5.40±1.69^{a,y}	4.67±0.99^{a}	5.07±2.30^{a}
	W	F	-	6.02±0.37^{a,x}	7.50±1.40^{b,x}	7.45±1.21^{b,x}	-	-
		SB	-	6.23±1.10^{a,x}	6.89±1.07^{a,x}	7.23 0.76^{a,x}	7.05 ±0.33^{a}	7.11±1.49^{a}
Enterobacteriaceae	D	F	0.00^{a}	3.97±0.59^{b,x}	3.34±0.00 (1/4)^{c,x}	4.58±0.57d,x	-	-
		SB	0.00^{a}	0.00a,y	0.00^{a,x}	5.46±1.05 (2/4)^{a,x}	2.73±1.17 (2/4)^{a}	5.87±1.26 (3/4)^{b}
	W	F	-	2.52±0.38^{a,x}	4.24±0.52^{a,x}	3.33±0.65^{a,x}	-	-
		SB	-	3.84±0.64 (2/4)^{a,x}	4.35±0.20^{a,x}	4.68±0.33^{a,x}	4.48±0.49^{a}	2.71±0.57^{a}
Mesophilic lactic acid bacteria	D	F	1.57±0.23 (3/4)^{a,x}	5.01±0.30^{b,x}	4.61±1.38^{b,x}	6.66±0.33^{c,x}	-	-
		SB	2.26±0.92 (3/4)^{a,x}	5.18±0.28 (2/4)^{a,x}	0.00a,y	2.55±0.59 (3/4)^{a,y}	0.00^{a}	1.87±0.12 (2/4)^{a}
	W	F	-	5.73±0.45^{a,x}	6.98±1.31^{b,x}	6.90±0.72^{b,x}	-	-
		SB	-	5.42±0.73^{a,x}	6.40±0.82^{a,x}	6.45±0.51^{a,x}	6.74±0.92^{a}	7.08±0.57^{a}
Pseudomonas spp	D	F	0.00a	6.17±0.20^{b,x}	7.63±0.80^{c,x}	9.12± 0.26^{d,x}	-	-
		SB	2.89±0.27(2/4)^{a}	6.54±2.58 (2/4)^{a,x}	6.58±0.00 (1/4)^{a,y}	4.81±2.19^{a,y}	4.03±0.83^{a}	5.73±2.01^{a}
	W	F	-	4.18±0.45^{a,x}	5.81±1.31^{a,x}	5.02±0.72^{a,x}	-	-
		SB	-	4.99±0.73^{a,x}	5.06±0.82 (2/4)^{a,x}	5.04±0.51^{a,x}	4.44±0.92^{a}	4.93±0.57^{a}
Molds and yeasts	D	F	2.00±0.00 (1/4)^{a,x}	3.18±0.79^{a,x}	4.21±0.74 (3/4)^{a,x}	4.59±0.53^{a,x}	-	-
		SB	1.84±0.48 (3/4)^{a,x}	2.28±0.46 (2/4)^{a,y}	2.50±0.71 (2/4) a, y	1.95±0.62 (3/4)^{a,y}	1.00 ±0.00 (1/4)^{a}	3.44±0.15 (2/4)^{a}
	W	F	-	3.48±0.00 (1/4)^{a,x}	3.79±0.74 (2/4)^{a,x}	0.00^{a}	-	-
		SB	-	2.00±0.00 (1/4)^{a,x}	3.24±0.34 (2/4)^{a,x}	0.00^{a}	0.00^{a}	1.00±0.00 (1/4)^{a}

F, Friesian; SB, Sardo Bruna; D, dry aging; W, wet aging; -, not tested. T0, first day of aging; T7, T14, T21, T28, T35, respectively 7, 14, 21, 28, 35 days after start of aging. Means in the same row with different letters were significantly different (P<0.05); means in the same column among aging technique with different superscript numbers were significantly different (P<0.05). Values within brackets indicate the prevalence of positive samples.

T28 (P<0.01), and at the end of the aging (P<0.01) for SB. Ph was significantly higher (P< 0.01) in meats submitted to dry aging with respect to meats submitted to wet aging at all analysis times and in both cattle breeds.

A_w showed a stable trend during both dry and wet aging without significant differences.

Table 4 shows results about pH and a_w (±SD) evaluated on loins during dry and wet aging.

Discussion

The present study provides preliminary data about the influence of the aging technique (dry and wet) on the microbial profile and the physicochemical characteristics of bovine meats obtained from two different breeds. Evaluations were also conducted at the slaughterhouse on live animals and carcasses before the beginning of the aging period.

As regards, measurement and analysis conducted at the slaughterhouse, the dressing percentages were comprised in the expected range for the considered cattle categories (Coyne *et al*., 2019).

During the transformation of muscle in meat, the evolution of temperature and pH values in the post-mortem period is of paramount importance for meat shelf life and plays a critical role in the degradation of muscle proteins during meat storage allowing the development of the standard flavor and taste in meat (Álvarez *et al*., 2021; Barrasso *et al*., 2022). At the moment of slaughter, normal muscle pH is ~7.0, and this value tends to decrease in meat. Normally, pHu drops to a range between 5.4 and 5.8, when the temperature is gradually decreased to 10-15°C during the first 12 hours and then to 2-4°C (Hamoen *et al*., 2013). In our study, pHu at *longissimus dorsi* level was comprised between 5.5 and 5.6 (Tu 5.4±0.5°C), thus indicating correct muscle acidification (Collins and Huey, 2015).

ACC and *Enterobacteriaceae* mean levels detected on carcasses at the end of slaughtering were in accordance with the criteria set by EC Reg. n. 2073/2005 (European Commission, 2005). Also, the others investigated microbial groups showed levels <0.5 log ufc/cm^2 or were not detected, thus highlighting the correct application of slaughtering and hygiene practices.

As regards the microbial profile of aged meats, a general increase of all microbial groups was observed during both dry and wet aging in accordance with most of similar studies conducted under controlled conditions (Li *et al*., 2013, 2014; Kim *et al*., 2019; Gowda *et al*., 2022; Van Damme *et al*., 2022). *Pseudomonas* spp., *Brochotrix thermosphacta,* and LAB are the predominant microbial groups in meats submitted to dry aging (Parrish *et al*., 1991). In the present study, higher mean counts of total mesophilic aerobic bacteria, *Pseudomonas* spp., and LAB have been found in dry-aged meats obtained from F cull cows at the end of the ripening process, if compared to loins obtained from SB bovines at the corresponding analysis times; in particular, these differences were significant (P<0.05) for *Pseudomonas* spp. starting from T14. Moreover, at the end of aging, the final levels of all investigated microorganisms' groups were lower in SB than in F meats, also considering the longer duration of the experiment in this breed (35 *vs* 21 days). The choice to stop the duration of the aging period at 21 days for F cull cows was made based on the microbiological results obtained. Dry-aged meats obtained from F cows showed mean levels >8.5 log ufc/g and >9 log ufc/g respectively for CCA and *Pseudomonas* spp. after 21 days. It is known that the contamination level of the carcass may determine the microorganism counts during the production process (Cherroud *et al*., 2014). As we said, levels of contamination in carcasses at the end of slaughtering referred to total mesophilic aerobic bacteria and *Pseudomonas* spp. were higher, although not significantly (P>0.05), in F carcasses if compared to SB, which could partially explain the subsequent trend of the different microbial groups during aging. Wet-aged meats were characterized by high levels of LAB (*ca* 5 log in the internal part at the end of the aging period), and these differences were significant (P<0.05) starting from T14. This result was expected, as it is known that under conditions of oxygen absence, as occurs with vacuum packaging, bacterial flora is gradually selected towards CO_2-tolerant microorganisms with the prevalent being *Brochothrix thermosphacta,* and LAB, mainly *Lactobacillus*, *Leuconostoc* and *Carnobacterium* (Castellano *et al.*, 2008). Other authors found similar LAB levels in meats submitted to wet aging for 21 days (Kim *et al*., 2019). Moreover, the significantly higher levels of LAB in wet-aged meats with respect to loins submitted to dry aging could explain the difference in pH. In fact, in agreement with Kim *et al*. (2016), Ha *et al*. (2019) and Kim *et al.*, (2020), wet-aged loins showed significantly lower (P<0.01) pH values with respect to meats submitted to dry aging at all analysis times and in both cattle breeds. It is known that lactic acid bacteria produce lactic and acetic acid in meat and this could lead to a decrease in pH (Casaburi *et al*., 2015; Mikami *et al*., 2021). In an investigation by Leisner *et al*. (1995) pH of meat after experimental inoculation with *Lactobacillus sake*, decreased to 5.31 after 10 weeks of under-vacuum storage. pH in meats submitted to dry aging showed mean values in accordance with other studies and a progressive increase during storage (Li *et al*., 2013, 2014; Smaldone *et al*., 2019). This increase is associated with the formation of nitrogen compounds from proteolysis (Aksu *et al*., 2005).

Enterobacteriaceae were detected at levels of *ca.* 4 log on the surface of loins obtained from both cattle breeds at the end of the

Table 4. Physico-chemical parameters during dry and wet aging of meats obtained from the two cattle breeds.

Analysis	Aging	Breed	Sampling time					
			T0	T7	T14	T21	T28	T35
pH	D	F	5.52±0.01^{a,x}	5.58±0.01^{b,x}	5.67±0.03^{c,x}	5.72± 0.02^{d,x}	-	-
		SB	5.54±0.03^{a,x}	5.58±0.02^{a,x}	5.64±0.02^{b,x}	5.67±0.02^{b,y}	5.72±0.01^{d}	5.73±0.01^{d}
	W	F	-	5.50±0.01^{a,x}	5.43±0.04^{b,x}	5.39±0.07^{b,x}	-	-
		SB	-	5.49±0.01^{a,y}	5.45±0.04^{a,x}	5.47±0.01^{a,y}	5.38±0.01^{b}	5.22±0.06^{c}
a_w	D	F	0.992±0.002^{a,x}	0.991±0.001^{a,x}	0.992±0.001^{a,x}	0.991±0.001^{a,x}	-	-
		SB	0.992±0.001^{a,x}	0.992±0.001^{a,x}	0.993±0.001^{a,x}	0.992±0.001^{a,x}	0.991± 0.002^{a}	0.994±0.003^{a}
	W	F	-	0.995±0.001^{a,x}	0.995±0.001^{a,x}	0.994±0.001^{a,x}	-	-
		SB	-	0.993±0.000^{a,x}	0.996±0.001^{a,x}	0.995±0.001^{a,x}	0.995±0.000^{a}	0.994±0.001^{a}

a_w, water activity; F, Friesian; SB, Sardo Bruna; D, dry aging; W, wet aging; -, not tested; T0, first day of aging; T7, T14, T21, T28, T35, respectively 7, 14, 21, 28, 35 days after start of aging. Means in the same row with different letters were significantly different (P<0.05); means in the same column among aging technique with different superscript numbers were significantly different (P<0.05).

two aging methods. Similar levels were found by Mikami *et al*. (2021). *Enterobacteriaceae* are indicators of hygiene during meat processing and such high levels could be an indicator of the not correct application of hygiene and manufacturing procedures particularly during sectioning. However, in the internal part of dry-aged loins, *Enterobacteriaceae* were detected more sporadically, confirming that this ripening method can reduce their growth (Mikami *et al*., 2021). In wet-aged meats, *Pseudomonas* spp. was detected at levels of *ca.* 5 log at the end of ripening. Normally, lower levels of *Pseudomonas* spp. are found in under-vacuum packaged meats as most members of the species are strictly aerobes. However, the growth of *Pseudomonas* spp. in vacuum packaging can occur, due to the presence of residual oxygen, not proper permeability of packaging materials, or the presence of strains capable of growing anaerobically (Xu *et al*., 2022).

There was a general correspondence of mean levels of the different microbial groups between the surface (evaluated by sponge sampling) and the interior, with lower levels in the interior in most cases. A possible explanation for the presence of bacteria in the inner part of the meat, is the high value of a_w (>0.99) that supports the survival and growth of bacteria, presumably after they migrate from the surface to the inner side during the drying process (Gowda *et al*., 2022). Higher levels of yeasts and molds were found on dry-aged meats with respect to wet-aged meats, both on the surface and in the interior. This is in accordance with other studies that showed similar levels (Lee *et al*., 2018; Kim *et al*., 2019; Van Damme *et al*., 2022). The dry aging process can encourage mold growth (Gowda *et al*., 2022).

Conclusions

In the last years, there has been an increasing interest in meat aging, especially with the drying technique. However, there is still limited knowledge about the microbial profile and safety obtained with the aging process. Our study showed that some microbial groups, such as *Enterobacteriaceae* and *Pseudomonas* spp., can be better controlled with wet aging with respect to dry aging which allowed us to obtain a more stable trend of the different microbial groups. However, the finding of high numbers of some bacterial species in both types of aging process, especially spoilage microorganisms such as lactic acid bacteria and *Pseudomonas* spp. in the inner part of the meat, highlights the critical importance of the flawless application of hygiene practices of these particular cuts of meat intended for aging, from slaughtering to cutting/deboning and storage of meats.

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