Reduction of the microbial load in meat maturation rooms with and without alkaline electrolyzed water fumigation

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Abstract

Dry-aging is a process during which meat is stored within maturation chambers at low temperatures and low relative humidity, resulting in improved tenderness and flavor development. The cuts are exposed to the atmosphere by hanging them or setting them on racks in the maturation chamber without any protective packaging. Animals and humans are usually the major sources of bacterial food contamination in the meat industry, but other routes might be involved. Therefore, procedures to reduce or eliminate pathogens from surfaces are crucial for an effective hazard analysis critical control point program in the food industry and other environments.

This study aimed to assess the survival of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* on the inner surface of dry aging chambers. Moreover, we tested the efficacy of alkaline electrolyzed water (REW) for its eventual application within a procedure aimed at reducing foodborne pathogens during meat storage.

Environmental conditions inside the dry aging cabinet determine a reduction of *circa* 3 log CFU/cm^2 of the considered microorganisms on the inner surface in 24 hours. Additionally, the nebulization of alkaline electrolyzed water with the smoking system increased the count reduction in 24 hours due to environmental conditions for *L. monocytogenes* (~1 log CFU/cm^2) and for *S. aureus* (~2 log CFU/cm^2). In this context, the use of REW can be justified for routine cleaning procedures of the surfaces, with the added value of being safe to handle, not containing environmental pollutants, and making it unnecessary to rinse surfaces due to its instability.

Introduction

Meat consumers are demanding products of higher and more consistent quality, with a distinctive flavor and aroma, able to provide a particular sensorial experience during their consumption. Dry-aging is a process in which meat, usually primal or subprimal cuts of beef, is stored at a low temperature and low relative humidity (RH), resulting in improved tenderness and flavor development. During this time, the cuts are hung or placed on a rack in the maturation chamber without protective packaging in order to be exposed to environmental conditions.

Gowda *et al*. (2022) published an interesting investigation on dry-aged meat practices applied by 15 commercial companies in Belgium, demonstrating that maturation was performed only within closed drying chambers and that none used cabinets with air circulation, where temperature conditions ranged from -1 to 3°C and the relative humidity from 40 to 75%. In addition, instead of an all-in/all-out loading system, chambers were filled with continuous addition of pieces of meat. Animals and humans are usually the major sources of bacterial food contamination in the meat industry, but products are often contaminated through contact with equipment surfaces, such as shredders, slicers, and cutting boards (Tomasello *et al*., 2021).

In the dry ager machines managed by a continuous loading system, the environment of the maturation chambers may become a significant source of contamination; thus, procedures aimed at reducing or eliminating pathogens from surfaces are crucial for an effective hazard analysis critical control point program in the food industry and also for controlling food contamination in homes, food markets, restaurants, health facilities, and public areas (Venkitanarayanan *et al*., 1999). Foodborne pathogens like *Listeria monocytogenes*, *Enterohaemorragic Escherichia coli* (EHEC), *Salmonella* spp., and coagulase positive *staphylococci* may be present and multiply during meat storage under particular conditions (Meat and Livestock Australia, 2018; Gowda *et al*., 2022). Meat and Livestock Australia guidelines for the safe production of dry-aged meat (2018) considered that pathogenic bacteria that are reasonably likely to be present in raw meat incoming in the maturation chambers may include *Salmonella* spp., EHEC, *Staphylococcus aureus*, and *L. monocytogenes*. In the few papers available in the literature about the presence of the aforementioned foodborne pathogens in dry-aged meat, the results of the experimental inoculation report similar data, showing either the absence of multiplication or the reduction of their number during aging, with outcomes depending on the aging conditions considered (Knudsen *et al*., 2011; Tittor *et al*., 2011; Muniz da Silva *et al*., 2019; Van Damme *et al*., 2022). Process parameters influencing the evolution of the

microbial population and microbiological quality during meat aging are temperature, airspeed, airflow between the meat cuts, and RH inside the equipment (Meat and Livestock Australia, 2018; Gowda *et al.*, 2022; US Department of Agriculture, 2014). US Department of Agriculture guidelines for US dry-aged beef for international markets (2014) and Meat and Livestock Australia guidelines for the safe production of dry-aged meat (2018) report the process parameters reported in Table 1.

Since no data are reported in the literature regarding the effect of environmental conditions in the dry aging chamber on the survival of foodborne pathogens that might contaminate surfaces, the aim of this study was to assess the survival of 4 selected foodborne pathogens on the inner surface of maturation chambers used for meat dry aging. In addition, we tested the efficacy of alkaline electrolyzed water (REW) for its eventual application within a procedure aimed at reducing foodborne pathogens during meat storage.

Materials and Methods

For the tests, commercial dry aging equipment (Maturmeat®, Arredoinox, Crotone, Italy) furnished with control of temperature, airflow, and a patented system for active control of RH and smoking of meat was used. The system has continuous monitoring of process parameters, which during our experiment were set according to manufacturer instructions as follows: temperature 1°C; airflow 1,2 m/s; RH 75%.

REW was obtained from Aquasol S.r.l. (Bologna, Italy) and generated through an electrochemical process that uses reverse osmosis water and potassium carbonate (K_2CO_3) as electrolytes. REW is made up of 99.83% pure water and 0.17% potassium hydroxide (KOH) with a pH of 12.2-12.5 and an oxidation-reduction potential of -40/-90 mV.

Two tests were performed to verify: i) the reduction of pathogenic bacteria on the inner steel surface of the maturation chamber due to the environmental conditions set for dry aging; ii) the additional reduction due to the REW nebulization by the system during maturation.

For both tests, the experimental inoculation was performed in the same way as previously described by Tomasello *et al.* (2021). Briefly, a mix of three different strains, including one reference strain and two isolates from animal production chains (Table 2), was used for the four bacteria tested, namely *L. monocytogenes*, *E. coli*, *Salmonella* spp., and *S. aureus*. For each test, three repetitions for the four bacterial species were represented by three stainless steel plates, where an area of 100 cm^2 was inoculated, after sterilization to obtain a total concentration of about 10^4 CFU/cm^2. The 100 cm^2 area was divided in two and, after drying, half of the plate was sampled with a sterile sponge to evaluate the pre-treatment microbial load. The stainless steel plates were put in the Maturmeat® (Arredoinox, Crotone, Italy) and subjected to treatment with or without the nebulization of REW during the two tests.

During the first test, to evaluate the reduction of the bacterial load during dry aging processing, the plates were put in the Maturmeat® (Arredoinox, Crotone, Italy) and the dry aging program was started. After 24 hours, the stainless steel plates were sampled through a sterile sponge as above described to evaluate the microbial load after treatment. Sponges were put in a sterile plastic bag containing 100 mL of sterile saline solution. After mixing in a stomacher (BagMixer®, Interscience, St Nom, France), 0.1 mL of the obtained sample and 0.1 mL of four serial 10-fold dilutions were seeded in plastic Petri dishes containing triptone soy agar (Oxoid Ltd., Basingstoke, UK), and incubated at 37°C for 24 hours. After incubation, colonies were identified with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF, Bruker, Massachusetts, USA) and counted.

The second test, aimed at evaluating the bacterial load reduction during the dry aging process with REW nebulization, was performed as the first one with the only modification of filling the water tank of the smoking system of the machine with REW. The REW quantity used in the 24 hours of the test was measured by calculating the consumption after 24 hours.

An additional test was performed to evaluate whether pathogenic bacteria spread due to ventilation and nebulization during machine work. In this test, in order to reduce contamination before the beginning of the trial, the inner surface of the chamber was first sanitized with ethanol, followed by steaming at 65°C for 1 hour by applying a specific program to the machine. At the end of steaming, stainless plates contaminated as previously described were put in the machine and the dry aging program started. After 24 hours, all the inner surfaces of the machine were sampled with two sponges, one was put in a stomacher bag with 100 mL of buffered peptone water (Oxoid, Ltd, Basingstoke, UK), homogenized by stomacher (BagMixer®, Interscience, St Nom, France) and incubated at 37°C for 24 hours. After incubation, 0.1 mL of buffered peptone water was transferred into 9 mL of Rappaport Vassiliadis enrichment broth (Oxoid Ltd., Basingstoke, UK) at 42°C for 24 hours; the other sponge was put into 9 mL of listeria enrichment broth (Oxoid Ltd, Basingstoke, UK) and incubated at 30 °C for 24 hours. After incubation, *Salmonella* spp. and *L. monocytogenes* enrichments were plated on Agar Listeria Ottaviani Agosti (Oxoid Ltd., UK), and xylose lysine deoxycholate agar (Oxoid Ltd., Basingstoke, UK).

Statistical analyses of the differences between bacterial load before and after treatments in the first and second tests were performed by paired T-test, analysis of variance, and tukey post-hoc test ($P \leq 0.01$).

Results

After 24 hours of exposure to contaminated stainless plates, the first test showed a mean reduction of log CFU/cm^2 count of 2.57 (±1.14 standard deviation). The lowest mean reduction was observed for stainless plates contaminated with *S. aureus* (1.11 log CFU/cm^2±0.22) and the highest one (3.83±0.27 log CFU/cm^2) for *E. coli*; in plates contaminated with *Salmonella* spp. and *L. monocytogenes* a reduction of 2.65 log CFU/cm^2 (±0.82) and 2.75 log CFU/cm^2 (±0.88) was observed respectively. Detailed results are summarized in Table 3.

Additional treatment of nebulization of REW by the aromatization control system in the second test resulted in a similar reduction of log CFU/cm^2 count for *E. coli* (3.06±0.14) and *Salmonella* spp. (2.85±0.38), and a higher reduction for *L. monocytogenes* (3.69±0.76) and *S. aureus* (3.33±0.95); details are reported in Table 4.

The consumption of REW by the Maturmeat® (Arredoinox, Crotone, Italy) during 24 hours of treatment was 0.490 kg.

No growth of bacteria was observed from sponges collected from the inner surface of the maturation chamber in the test performed to evaluate the potential spread of bacteria due to airflow and nebulization during machine working.

Discussion

Results showed that environmental conditions inside the dry aging cabinet determined the reduction of the considered bacterial load on the inner surface in a short period of 24 hours. To the best of our knowledge, there is no data available in the literature to compare these results with, but several works report similar data about pathogenic microorganisms on the surface of meat: Tittor *et al*. (2011) showed a *Salmonella* and *E. coli* O157:H7 reduction in beef of about 4.5 log CFU/cm^2 during 28 days of aging; similarly, Knudsen *et al*. (2011) observed a reduction of several *Salmonella* serovars, calculating a D value ranging from 5.25 to 8.07 days and Van Damme *et al*. (2022) reported that *Salmonella* and *E. coli* O157:H7 counts significantly decreased during dry-aging with a daily reduction ranging from 0.07 to 0.14 log CFU and from 0.09 to 0.14 log CFU, respectively. Also for *L. innocua* used as a surrogate of *L. monocytogenes* a 2.38-3.37 log CFU reduction was observed during 42 days of aging; on the contrary, Van Damme *et al*. (2022) observed a not linear reduction of *L. monocytogenes* count in their experiments and an increase of about 1 log CFU/g in one loin characterized by an increase in pH.

Surface desiccation due to RH and airflow at low temperatures seems to play a key role in the reduction of pathogens on the surface of the meat (Knudsen *et al*., 2011). Also, in the European

Food Safety Authority's scientific opinion about the microbiological safety of aged meat (EFSA Panel on Biological Hazards, 2023), the impact of the rate of drying is considered important. However, even if *L. monocytogenes* growth is predicted during dry aging, these predictions are considered overestimations of log increases since the effect of competition and inactivation are not included (EFSA Panel on Biological Hazards, 2023). The same effect can explain the reduction of the selected bacteria on steel surfaces in our study, in which the count reduction was amplified by the absence of protective organic materials, differently from the aged meat scenario. The higher effect of environmental conditions on the decrease of Gram negative bacteria than on Gram positive species is in line with the results of the studies cited above and reflects the general intrinsic greater resistance of Gram positive bacteria to environmental factors.

The efficacy of REW to reduce the count of *Salmonella* spp., *E. coli*, *L. monocytogenes,* and *S. aureus* on steel surfaces was previously demonstrated by direct spray application (Tomasello et al., 2021). In this study, the nebulization of REW with the smoking system increased the count reduction for *L. monocytogenes* and *S. aureus* in 24 hours due to environmental conditions. REW is well known as a detergent, dissolving fats and proteins; moreover, REW is safe to handle and doesn't contain environmental pollutants, and its instability makes rinsing surfaces with water after its use unnecessary, which saves time during working activities. For its characteristics, the use inside the maturation chambers in the presence of meat could be proposed given the usual management of continuous loading of dry agers (Gowda *et al*., 2022). On the other hand, REW is not economically competitive with the most common commercial sanitizers. In our work, high consumption of REW was observed during the 24 hours of the test. In addition, environmental conditions inside the cabinet during aging caused sufficient bacterial load reduction within the range of acceptable contamination conditions that can be easily achieved by standard cleaning procedures.

Conclusions
Our results show that environmental parameters set within the aging cabinet can reduce the microbial load in a short period of 24 hours; in this context, the REW use can be justified for the routine cleaning procedures of the surfaces.

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Table 1. Process parameters suggested by the US Department of Agriculture and Meat and Livestock Australia for dry aging and process parameters used in this work.

	USDA	MLA	This work
Airflow (m/s)	0.5-2	0.2-0.5	1.2
RH%	80-85	75-85	75
Temperature (°C)	0-4	1-2	1

USDA, US Department of Agriculture; MLA, Meat and Livestock Australia; RH, relative humidity.

Table 2. Bacterial strains used for experimental tests and source of isolation.

	Strain	**Source**
Escherichia coli	ATCC 25922	
Escherichia coli	VeLaBac 444	Bovine feces
Escherichia coli	VeLaBac 445	Meat
Salmonella Thyphimurium	ATCC 14020	
Salmonella Thyphimurium monophasic	118174/1	Pork sausage
Salmonella Derby	106463/1	Pork meat
Listeria monocytogenes	ATCC 15313	
Listeria monocytogenes	12MOB085LM	Meat
Listeria monocytogenes	12MOB089LM	Meat
Staphylococcus aureus	ATCC 25923	
Staphylococcus aureus	SA B/122/2	Raw milk
Staphylococcus aureus	SA 22-7-16/2	Goat

Table 3. Log CFU/cm² count before and after 24 hours of exposure to environmental conditions for dry aging in the Maturmeat® (Arredoinox, Crotone, Italy) maturation chamber and estimated log reduction (Δlog CFU/cm²).

	Log CFU/cm² mean (±SD) before treatment	Log CFU/cm² mean (±SD) after treatment	Δ Log CFU/cm² mean (±SD)
Escherichia coli	3.83 (±0.27)	nd*	3.83 (±0.27)
Salmonella spp.	3.53 (±0.07)	0.88 (±0.78)	2.65 (±0.82)
Listeria monocytogenes	3.11 (±0.29)	0.39 (±0.67)	2.75 (±088)
Staphylococcus aureus	5.19 (±0.44)	4.08 (±0.64)	1.11 (±0.22)

SD, standard deviation; *nd, no colonies observed after plating.

Table 4. Log CFU/cm² count before and after 24 hours of exposure to environmental conditions for dry aging in the Maturmeat® (Arredoinox, Crotone, Italy) maturation chamber with nebulization with REW and estimated log reduction (Δlog CFU/cm²).

	Log CFU/cm² mean (±SD) before treatment	Log CFU/cm² mean (±SD) after treatment	Δ Log CFU/cm² mean (±SD)
Escherichia coli	3.29 (±0.26)	0.23 (±0.40)	3.06 (±0.14)
Salmonella spp.	3.08 (±0.12)	0.23 (±0.40)	2.85 (±0.38)
Listeria monocytogenes.	3.69 (±0.76)	nd*	3.69 (±0.76)
Staphylococcus aureus	4.22 (±0.14)	0.88 (±0.92)	3.33 (±0.95)

SD, standard deviation; *nd, no colonies observed after plating.