Preliminary evaluation of the hygienic level of refrigerated vacuum-packed wild boar meat

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Abstract

Wild boar meat is usually available frozen, but the catering industry is also interested in fresh vacuum-chilled meat. This work aims to evaluate the hygienic level of vacuum-packed and refrigerated game meat [*Longissimus dorsi* (LD) muscle] and to investigate the existence of relationships with other parameters measured at the game handling establishment (GHE). The pH of the LD muscle and carcass surface contamination [aerobic colony count (ACC) and *Enterobacteriaceae* (EC)] were measured on 12 wild boar regularly processed at a local GHE. Subsequently, 2 cm-thick steaks were taken from the LD muscle at a cutting plant from the same subjects sampled at the GHE and individually vacuum sealed and stored at 2°C for 1, 7, 14, and 21 days. The meat was analyzed for ACC and EC at each storage time and for *Escherichia coli* β-glucosidase + counts at 21 days. The mean final pH value of the muscle samples was 5.6, and the ACC and EC carcass surface average load was 2.54 and 0.79 Log CFU/cm^2, respectively. Meat preparations at 21 days had ACC, EC, and *E. coli* mean values of 5.80, 3.13, and 2.03 Log CFU/g, respectively. Pearson's analysis showed a significant positive correlation between pH and ACC at day 1 and between EC on carcasses and meat at day 1. The results confirm that if the pH of meat is high, the development of microorganisms is favored, and, therefore, a shorter shelf life is expected. Furthermore, the EC on carcasses seems to be a good index for estimating the hygienic level of the obtained meat.

Introduction

The wild boar population increased in Italy during the last decade, together with problems due to their effects on human activities and farm animal health. The wildlife-livestock interface highlights the risk of contact between them that is responsible for the spreading of animal diseases (Altissimi *et al.*, 2023a), such as African Swine Fever (Brown *et al.*, 2024). This disease has a relevant impact on wild boar health, pig farming, pork meat production, and product export (Brown *et al*., 2021). Despite the improvement of biosecurity measures at the farm level, one of the main actions to combat African Swine Fever in Italy foresees a drastic reduction in the wild boar population over the next 5 years (European Food Safety Agency, 2018; Italian Government, 2023).

The primary control measure for wild boars remains the implementation of hunting activities across the entire national territory. The need for population control is highlighted in different reports, and the national government established that wild boar population reduction can be carried out during non-hunting seasons by shooting or trapping the animals. The derived meat could be self-consumed (including meat given to relatives and friends or sold on the black market) or sold on the market through registered game meat chains. This distribution is heavily shifted toward hunters' self-

consumption, with only a small portion (less than 3%) going to the restaurants (Gaviglio *et al*., 2017). In fact, the Umbria Region (central Italy) alone must hunt and kill (trapping is also allowed) at least 44,000 wild boars per year in the 2023-2028 period (Italian Government, 2023). This circumstance offers the possibility of having a considerable amount of fresh meat on the market for human consumption derived from animals collected outside specific restricted zones. Furthermore, this meat could meet the specific needs of modern meat consumers and therefore is gaining increasing interest (Corradini *et al.,* 2022). Nonetheless, the wild boar meat chain is peculiar and far different from the livestock meat chain because it involves different actors, such as hunters and operators in collection centers, in game handling establishments (GHE), and in cutting plants, and each of them could be responsible for the hygienic level of the final product. Indeed, several factors, such as hunting methods and related practices, greatly affect the hygienic level of wild boar meat, contributing to the variability in carcass and meat hygiene. The factors responsible for the big variability of the microbiological parameters, generally assessed on carcass and in meat, are reported by different authors, highlighting the importance of hunting system choice; animal age and weight; precision of the shot and time between the shot and animal killing; hunters practice on the wildlife after killing (*e.g*., bleeding, evisceration); presence and distance of an available collection centre or a GHE; time and temperature between kill and refrigeration of the carcasses; procedures adopted at GHE or other hunter structures; presence of specific cutting plants and operators training (Mirceta *et al*., 2017; Orsoni *et al.,* 2020; Ranucci *et al.*, 2021; Korkmaz *et al.,* 2022; Abrantes *et al*., 2023). Moreover, the relation between carcass contamination and meat preparation hygiene is not fully investigated. Indeed, wild boar meat is usually commercially available frozen, but the catering industry and restaurants may also be interested in fresh vacuum-chilled meat. Little information is available on the hygienic level of refrigerated wild boar meat, especially when vacuum packaged (Borilova *et al.,* 2016; Kasałka-Czarna *et al.,* 2022; Enkhbold *et al.,* 2024). The aim of the present work is to preliminarily evaluate the hygienic level of vacuum-packed and refrigerated meat [*Longissimus dorsi* (LD) muscle] and to define the existence of possible relationships with other measurable parameters at the GHE level, such as pH and the hygienic status of the carcasses.

Materials and Methods

The study was conducted in a specific game meat chain developed in the Umbria Region (Central Italy), consisting of a hunting wildlife company where no collective hunting is performed and wild boars are both shot or trapped in large corrals (Altissimi *et al.,* 2023b). Animals are killed only with one shot performed at the head or heart level. Animals are quickly (in less than 1 hour) transferred to a nearby collection center, where animals are bled, eviscerated, and refrigerated (4±1°C). The refrigerated, non-skinned carcasses are then transferred within 3 days to a local GHE (less than 40 minutes away from the collection center). The carcasses are then skinned, and a veterinary officer performs the post-mortem inspection of the organs and the carcasses. Then, carcasses are promptly refrigerated (7°C) and transferred to a local registered cutting plant, maintaining the cold chain during transport, where game meat cuts are obtained.

A total of 12 wild boars [average weight: 45.33 kg, standard deviation (SD): 17.26; 5 male and 7 female] treated under the same conditions and randomly selected were considered in the trial, and sampling was performed at the GHE and at the cutting plant level during January 2024 (when a total of 23 carcasses were processed). At GHE, the pH of the LD muscle was collected by inserting a pHmeter probe (Crison, Barcelona, Spain) at the level of the fifth thoracic vertebra immediately after skinning. Each carcass surface was then sampled in 4 areas of 100 cm^2 each (rump, flank, brisket, and foreleg) by a moisturized sponge swab (3M Italia, Milan, Italy), then inserted into a sterile bag and kept at 4°C during transport to the laboratory for the bacteriological determinations. At the laboratory, 10 mL of buffered peptone water (BPW, Biolife Italiana s.r.l., Milan, Italy) was poured into the bag, and samples were homogenized by a stomacher (Stomacher 400 Circulator, Seward, England). The proper serial decimal dilutions were made and then included in plate count agar (PCA, Biolife Italiana s.r.l., Milan, Italy) incubated at 30°C for 72 hours for aerobic colony count (ACC) (ISO, 2013) and in violet bile glucose agar (Biolife Italiana s.r.l., Milan, Italy) incubated at 37°C for 24 hours for *Enterobacteriaceae* count (EC) (ISO, 2017).

Subsequently, 2 cm-thick steaks were taken from the LD muscle at the cutting plant from the same subjects sampled at GHE, which were individually vacuum sealed and stored at 2±1°C for 1, 7, 14, and 21 days (T0, T1, T2, and T3, respectively). At each storage time, the refrigerated meat samples were collected from the cutting plant and transferred to the laboratory for ACC and EC and for positive *Escherichia coli* β-glucosidase + counts at 21 days (on Tryptone Bile X-glucoronide Agar, TBX Biolife Italiana s.r.l., Milan, Italy) incubated at 44°C for 24 hours (ISO, 2018). Then, 10 g of meat was aseptically collected and inserted into a sterile bag. 90 mL of buffered peptone water was then added to the bag, and samples were homogenized (Stomacher 400 Circulator, Seward, England) and serially diluted before plating. Colonies were then counted in both carcass surfaces and meat samples. The colony-forming units (CFU) were expressed as CFU/cm^2 or CFU/g, for sponge swab and LD muscle, respectively, and converted into Log values. For EC values below the level of detection (≤2 Log CFU/400 cm^2 or 2 Log CFU/g), the value was considered as 0.05 Log CFU (Gowda *et al*., 2022). Descriptive statistics (mean and SD) were calculated by the SAS statistical package (SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance model was also used to evaluate the effect of storage time on the microbial growth during cold storage under vacuum using the generalized linear model, followed by Tukey's test to define the significance of the difference ($p<0.5$). Correlations between factors registered at GHE and the storage of meat were also investigated by performing a Pearson's test. Correlations were considered significant only with $p<0.5$.

Results

The main final pH of LD muscle measured at the GHE was 5.61 (±0.23 SD) with a range from 5.38 to 6.27. The levels of ACC and EC on the carcass surfaces are reported in Table 1. ACC ranged from 1.61 to 3.71 Log CFU/cm^2, and EC from 0.24 to 2.76 Log CFU/cm^2 (with 3 samples below the level of detection). The ACC and EC performed on the meat samples are reported in Figure 1. The ACC main value was 3.81 (±0.547 SD), 4.11 (±0.537 SD), 5.61 (±0.798 SD), and 5.80 (±0.810 SD) Log CFU/g at T0, T1, T2, and T3, respectively. The ECs were 2.12 (±0.298 SD), 2.30 (±0.457 SD), 2.47 (±0.639 SD), and 3.13 (±1.094 SD) Log CFU/g at the four different storage times considered. The glucosidase + *E. coli* count after 21 days of storage was 2.03 (±0.230 SD) Log

CFU/g. Pearson's test reveals a significant correlation between factors. The pH value of the meat was positively correlated to the meat ACC at T1 (0.884, $p<0.001$). No correlation was noticed between the ACC of carcass surfaces and all the microbial parameters considered in wild boar meat, regardless of the storage time considered. The EC of the carcass was positively correlated only to EC on the meat at T0 (0.682, $p=0.011$). Correlations were also highlighted between: ACC at T1 and ACC at T3 (0.562, $p=0.045$); ACC at T2 and ACC at T3 (0.847, $p<0.001$); and EC at T0 and EC at T1 (0.723, $p<0.01$).

Discussion

The results reveal that the final pH values of wild game meat are highly variable. Even in a well-defined and structured game meat chain, different factors, including *ante-mortem* stress and proper exsanguinations, could influence this result (Viganò *et al.*, 2020). The altered muscle acidification could be responsible for dark, firm, and dry (DFD) meat with defects in meat quality and an increase in microbial spoilage (Altissimi *et al.*, 2023b). In this study, the positive correlation between pH and meat ACC at T0 could prove the hypothesis that wild boar DFD meat is more prone to bacterial growth. Then, the level of ACC increases during storage time in relation to the starting hygienic level, even if under vacuum-packaging and refrigeration are performed (Altissimi *et al.*, 2024). Further studies are also needed to define if specific spoilage microorganisms grow in these preparations and the effects on their quality (Borilova *et al.,* 2016; Peruzy *et al.,* 2019; Sauvala *et al.*, 2023).

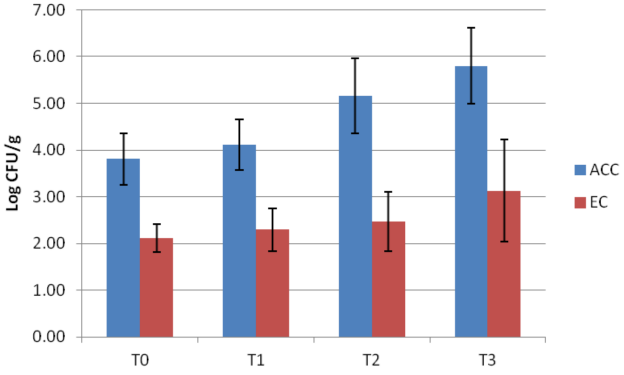

Figure 1. Results of the aerobic colony count (ACC) and *Enterobacteriaceae* count (EC) of the vacuum-packaged wild boar meat samples collected at different storage times at the cutting plant. Values are expressed as Log CFU/g.

Table 1. Results of the aerobic colony count and *Enterobacteriaceae* count of the wild boar carcass surfaces collected at the game handling establishment. Values are expressed as Log CFU/cm².

	ACC	EC
Mean value (Log CFU/cm²)	2.54	0.79
Standard deviation	0.783	0.779

ACC, aerobic colony count; EC, *Enterobacteriaceae* count; n=12 wild boar carcasses.

Other factors concerned with wild boar meat hygiene are related to carcass processing and operators' handling in collection centers and GHEs. In this research, the overall hygienic quality of the wild boar carcasses was fine and similar to those reported by other authors (Stella *et al*., 2018), even if other sampling methods and sampling areas of the carcass surface were selected. In other contests, the ACC and EC in wild boar carcasses were much higher (Mirceta *et al.,* 2017; Orsoni *et al.,* 2020; Gomes-Neves *et al.*, 2021; Peruzy *et al.,* 2022). A correlation between carcass and meat preparation microbial loads was detected only for EC, and that emphasizes the need for a proper operator and hunter formation to avoid contamination with gut content during shooting and evisceration, cross-contamination with dirty fleece or other carcasses, and proper maintenance of cold chains during the different steps of the chain (Mirceta *et al.,* 2017). These factors could also be responsible for the wide variation of EC results detected (Ranucci *et al.,* 2021). Regarding the storage of wild game meat under vacuum in refrigerated conditions, an increase in both the microbiological loads was detected over time, with average values close to 6 Log CFU/g and 3 Log CFU/g after 21 days of storage for ACC and EC, respectively. Similar data were recorded by other authors, even when the starting meat ACC level was lower than that observed in the present study (Enkhbold *et al.*, 2024). The same considerations are for ACC and EC values recorded by other authors after 15 days of refrigerated storage (Ludwiczak *et al.*, 2019). In general, the considered microbial shelf-life limit in meat is when the microbial population (in particular lactic acid bacteria) reaches 7 Log CFU/g and EC reaches 5 Log CFU/g (Rodriguez-Caturla *et al.*, 2023), despite the need for chemical and sensory evaluations for a reliable shelf-life definition (Reitznerová *et al.,* 2023). Furthermore, taking into consideration the limit set by EC Regulation 2073/2005 (European Commission, 2025) for hygienic criteria in meat preparations, that set a satisfactory value below 500 CFU/g and an acceptable range between 500 and 5000 CFU/g in meat preparation from farmed animals, the average value for the wild boar meat regarding *E. coli* counts was in the satisfactory range after 21 days of storage. The results of this study confirm, in this supply chain, the importance of the pH measurements as an early indicator of the hygienic quality of wild boar meat even during the shelf-life. These preliminary results also suggest to the stakeholders that 21 days of shelf-life could be suitable for this product and may be used in the label.

Conclusions

This study highlights that pre-harvest steps and carcass handling could affect wild boar meat quality and hygiene, and these factors (especially pH and carcass hygiene) could influence the hygienic level of vacuum-packed wild boar meat preparations. Especially when the DFD condition occurs, there is a trend in the increase of the microbial loads and possible spoilage of the preparations, even when vacuum packaging is adopted. Further studies are needed to correctly evaluate spoilage microorganism dynamics in wild boar vacuum-packaged meat and related effects on the development of off-odor and off-flavor in the products. Specific shelf-life studies are therefore necessary, also in relation to the structure of the game meat chain and operators' training, to better define the extension of the products and implementation of good handling/processing procedures and technical solutions to improve it.

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