ABSTRACT
The wild boar (Sus scrofa) population in central Italy has strongly increased in the last decades. The meat of the game is characterized by high-quality value and the manufacture of food products from game meat could represent a remarkable added value for the local market promoting local gastronomic specialties and traditions. Adult animals were hunted with the waiting method and the carcasses were processed into the game processing center. Five batches of salami were produced with different amounts of wild boar meat and pork meat. The microbiological, physicochemical, rheological, and sensory evaluations were performed. The microbiological analyses indicated that the salami is safe to consume as Listeria monocytogenes and Salmonella spp. were undetectable in the end products. The Enterobacteriaceae count was below 3 log CFU-g⁻¹ attesting to the adequacy of hygienic characteristics of the process. The chemical composition analyses showed lower lipid content in comparison to pork salami, while the rheological characteristics were equivalent among products. The sensory evaluation highlighted that the consumers’ appreciation of wild boar salami is comparable to that of traditional pork salami.

Keywords: game meat products; fermented meat products; food safety; textural characteristics; wildlife

INTRODUCTION
Wildlife population, especially wild boar (Sus scrofa), has been characterized by a wide geographical and demographical expansion across Europe in the last decades, and although is difficult to precisely esteem boars population density (Fernández-Llario et al., 2004), available data on the hunting bags reveal that in Italy the number of harvested wild boars has grown from less than 50,000 in 1985 to approximately 30,000 in 2015 (Massei et al., 2015). Particularly, in the Umbria region (central Italy) wild boar is the main hunted game species with several 40,000 animals killed per year (Sebastiani et al., 2015). The meat of the game is characterized by very good chemical composition (Šnirc et al., 2017), low-fat content (Pedrazzoli et al., 2017), a favorable ratio of unsaturated/saturated fatty acids (Quaresma et al., 2011), high protein content and good protein composition (Okukhanova et al., 2017), the outstanding value of mineral content (Skobrák et al., 2011) and peculiar taste and aroma (Neethling, Hoffman and Muller, 2016). Multiple factors are responsible for the high quality of game meat, such as the natural and slow growth of animals, wildlife habits, the natural diet, the absence of pharmacological treatments, and farming-related stress conditions (Babcic et al., 2018). Consumers are increasingly interested in healthier food products characterized by a high nutritional value and balanced nutritional profiles; furthermore, their concern about the environmental issues has increased and consequently they tend to prefer low input products, such as organic and natural products (Petrescu, Vermeir and Petrescu-Mag, 2020). All the mentioned aspects have diffusely enhanced interest in game meat (Paleari et al., 2002; Marescotti et al., 2019).

A crucial aspect to consider is represented by the compliance of these food products with the EU food safety regulation: it is essential indeed that game meat products reach quality and safety standards comparable to those of farmed animals produced on an industrial scale (Fettinger, Smulders and Paulsen, 2011). There are several factors potentially affecting the quality and safety characteristics of wild boar meat, like the hunting techniques, the hunter’s ability in terms of precision of the shot, the weather conditions, and the post-mortem activities such as transport and treatments of carcasses, as well as those of storage and meat processing (Ranucci et al., 2019a; Paulsen, Vali and Bauer, 2011). Besides, the game meat plays a key role in the promotion of local gastronomic specialties and traditions, concurring, therefore, to culinary and cultural touristic promotion with a great potential of economic return for the regional economies (Branciari et al., 2020). A growing number of activities have been promoted to strengthen and diversify the “local direct market” of game meat that has a long
tradition in Central Europe: hunters directly supply to consumers or food local businesses (Paulsen, Vali and Bauer, 2011). For this direct supply sector, as well as for game meat companies, the manufacture of food products from game meat could represent a remarkable added value (Paulsen, Vali and Bauer, 2011). In particular, wild boar raw meat marketing can be limited by low color stability and short shelf-life (Neethling, Hoffman and Muller, 2016) and frequently is commercialized underpriced. Therefore, growing attention is focused on the manufacture of wild boar meat products (Paleari et al., 2003; Zochowska-Kujawska et al., 2007). The interest addressed to this meat in central Italy regions is attested by the presence on the market of some game meat-based products and by its presence in the menu of restaurants and local festivals. Despite the significant expansion of wildlife populations throughout Europe, limited literature has been produced concerning the production of wild board meat products and the related safety, quality, and sensory aspects, especially for dry-cured fermented foodstuffs (Zochowska-Kujawska et al., 2007; Schimpl, Bauer and Paulsen, 2010).

Scientific hypothesis
This study aimed to evaluate the microbial, chemical, rheological, and sensory characteristics of experimentally produced salami made with different percentages of pork and wild boar meat. Hygienic characteristics of game meat products are expected to be equivalent to those of products from intensively farmed animals. Furthermore, this approach will likely allow for the profitable use of a frequently undervalued game species and for the production of salami appreciated by consumers.

MATERIAL AND METHODOLOGY
Hunting method
Adult wild boars were hunted from May 2018 to May 2019 in the municipality of Gubbio in the Umbria region (central Italy). The animals were hunted by selection hunters applying the waiting method according to which the hunters stay still in a selected location and wait for the animal to come towards them. This hunting strategy is characterized by a remarkably low level of antemortem stress experienced by animals.

Salami production
After the hunting, animals were promptly exsanguinated and eviscerated on-field then transferred to a collection center for carcass storage and kept at 2 °C for 1 to 6 days (average 4 days). The carcasses were subsequently transferred to a game processing center for skinning and sectioning and then to a local producer for fermented meat (salami) production (Fazi Carni, Gualdo Tadino, Italy). Salami were made following the regional traditional recipe. Firstly, the deboned wild boar meat was combined with swine cuts (belly and shoulder) and ground twice with a 6 mm plate. The meat batter was then added with salt (2.2%), ground black pepper (0.3%), black pepper grains (0.2%), ground garlic (0.05%), and starter cultures (Eurostarter MI Rapid, MEC Import, Roma, Italy; a mix of Staphylococcus xylosus and Staphylococcus carnosus + Lactobacillus sakei in a 2:1 ratio). Antioxidants and preservatives were not used. After resting overnight at 4 °C, the meat batter was stuffed into previously rehydrated dry-salted natural casings (swine intestine) to a final weight of approximately 1 kg for each salami. The products were subjected to a drying process inside controlled hot chambers for 10 days with different temperature (T) and relative humidity (RH) as follows: 22 °C and 62% RH for 48 h, 19 °C and 66% RH for 76 h followed by 1 °C T reduction and 1% RH increase every 24 h until reaching 15 °C and 72% RH. Afterward, the salami were ripened in controlled seasoning rooms at 13 °C and 75% RH for 60 days. The meat products were obtained by mixing wild boar and pork meat in different percentages: 50% wild boar meat and 50% pork meat (high percentage: BH), 40% wild boar meat and 60% pork meat (low percentage: BL), and 100% pork meat (control: C). The trial consisted of five batches for each group of which, to perform all the analytical determination, immediately after stuffing (T0) and after 7 (T1), 14 (T2), and 60 days (T3) samples from each experimental group (BL, BH, C) were collected and transported to the laboratory under refrigeration condition.

Physicochemical analyses
The physicochemical determinations were performed, according to the sampling plan, on two samples per batch. Proximate composition and salt content (% NaCl) were determined according to the AOAC method (AOAC, 2000). Water activity (aw) was measured at 25 °C with the aw recorder AquaLab, series 3, Model TE (Decagon Devices Inc., Pullman, WA, USA) as reported by Ranucci et al. (2019b). pH was measured according to Branciari et al. (2016) using a pH-meter Crison pH25 (Crisone, Barcelona, Spain). The color of meat products was determined on the surface of sample slices using a Minolta Chromameter (CR 400, Minolta, Osaka, Japan). Lightness (L* value), redness (a* value), yellowness (b* value), Chroma (C), and Hue angle (H) were determined according to CIE Lab System (Commission Internationale de l'Eclairage) (CIE, 1976).

Microbiological analysis
At each time of the sampling protocol adopted, three samples for each group were tested in triplicate for Enterobacteriaceae count following the ISO 21528-2 method (ISO 21528-2: 2004); for Enterococcus spp. count by plating diluted samples on Slanetz–Bartley agar (Biolife Italiana, Milan, Italy) and incubated at 37 °C for 48 h; for CNC enumeration by plating diluted samples on manitol salt agar (Biolife Italiana, Milan, Italy) and incubation at 37 °C for 24 h. Furthermore, samples were also analyzed for Lactobacillus spp. counted on De Man, Rogosa, and Sharpe (MRS) agar (Oxoid, Basingstoke, UK) anaerobically incubated at 37 °C for 48 and Lactococcus spp. on M17 agar (Oxoid, Basingstoke, UK) aerobically incubated at 37 °C for 48 h.

Counting results were reported in terms of log CFU.g⁻¹.

The detection of pathogens Listeria monocytogenes and Salmonella spp. were performed according to the ISO 11290-1 (ISO 11290-1:2017) and ISO 6579-1:2017 procedures, respectively.
Texture profile analyses (TPA)

The texture profile analyses of the products belonging to each experimental group were assessed at the end of the ripening time by the excision of three cylinders of height and diameter of 2.5 cm using a core drill as reported. A texture analyzer (TVT 6700, Pertem Instrument, Sweden) was equipped with a cylindrical probe (3 cm in diameter) and set accordingly to a compression rate of 30% and a probe speed rate of 2 mm/s. The parameters considered were hardness, resilience, cohesiveness, gumminess, and chewiness.

Sensory analyses

At the time of the end of ripening the products of the three different groups considered were evaluated through a ranking test according to the ISO methodology 8587 (ISO 8587:2006) set of three samples for general preference. At least 92 untrained panelists were recruited for the execution of the ranking test. For each panelist, samples were assigned random 3-digit numbers and sample order was randomized (Branciari et al., 2017). The lowest rank (1) corresponded to the least preferred, whereas the highest rank (3) corresponded to the most preferred. Data analysis was based on the sum of ranks obtained by each sample.

Statistical Analysis

Analytical results were subject to analysis of variance (ANOVA) (GMP, SAS Institute, Cary, NY, USA), considering the microbiological and physicochemical data assessed over sampling time (T0, T1, T2, and T3) and across different compositions (BH, BL, C). For the analysis performed exclusively on the finished products (T3), a one-way ANOVA was used with the salami composition as a fixed factor (BH, BL, C). Tukey’s post hoc test was used to determine whether the values obtained were different with a significance level of 0.05. The results of the sensory analyses were evaluated using the Friedman Test.

RESULTS AND DISCUSSION

Chemical composition and physicochemical characteristics

The results of the chemical composition of the experimentally produced salami are reported in Table 1. The addition of wild boar meat in the product formulation significantly affected the fat and the ash content (p <0.05). The fat content decreased along with the decrease of the pork meat varying from over 34% for pure swine products to 32% for BH salami. The fat content for all the groups is consistent with the type of product, indeed it has been reported, that fermented meat products should contain fat ranging from 25 to 45% to meet consumer expectations (Yılmaz et al., 2009). The registered fat content is similar to what already found for similar game meat products (mean value 33%) (Ranucci et al., 2019a) and it was lower than that of wild boar salami (48%) registered by Paulsen, Vali and Bauer (2011). Other results in the literature showed that the meat of wildlife is leaner than pork meat due to a more natural diet and periods of prolonged locomotion (Szmanko et al., 2007). Concerning the ash content, the values recorded decreased with the increase of pork meat in the composition of salami, as shown in Table 1. No difference in the values between groups was observed for moisture, protein, and salt content and the data align with those reported by Chakanya et al. (2018) for a similar product produced with different game meats.

The pH and aw values are reported in Figure 1a and Figure 1b, respectively. The pH of all the products decreased as storage progressed from T0 to T1 (p <0.001) followed by a increase in the subsequent sampling times (p <0.001); however, no differences were recorded among experimental groups (p >0.05) indicating that the meat batter composition does not affect such parameter following results reported in the literature by Chakanya et al. (2018) for similar products. This trend in pH was expected, as the bacterial starter culture contained lactic acid bacteria that metabolize carbohydrates into lactic acid lowering pH during the first days of maturation (Fontana et al., 2012). Tissue and microbial (e.g. CNC) proteases are responsible for the subsequent increase of pH values in the latter stages of maturation (Amadoro et al., 2011). It has been reported that antemortem stress can affect pH values hunting strategy using dogs to drive the animals toward the hunters and those in which the hunters stalk the prey can cause high levels of stress for the quarry resulting in meat with higher pH values (pH >6.0) that can frequently be classified as dark, firm and dry (DFD) (Daszkiewicz et al., 2012). However, in the present study, the waiting hunting strategy was adopted causing no stress to the animals and enabling meat to acidify normally.

As shown in Figure 1b, aw values gradually decrease during seasoning time from T0 to T3 (p <0.001), however no significant differences between the experimental group were registered (p >0.05) indicating that aw trend is influenced by the ripening environment regardless the salami composition. This result is under what was reported for similar meat products in previous studies regarding salami produced with different amounts of wildlife meat (Chakanya et al., 2018). The gradual loss of humidity from the salami along the ripening period results in less water available for the microorganism growth improving the safety characteristics of the final product.

The instrumental texture evaluation was conducted on the different salami at the end of the ripening time (T3) testing five different attributes. The results reported in Table 2 show no difference among the experimental groups for the attributes considered. This finding is in contrast with what was reported in a previous study by Ranucci et al. (2019a) for similar products. The author reported that salami with 50% roe-deer and 50% pork meat showed higher values of hardness and gumminess and lower values of chewiness in comparison with 33% roe-deer/ 67% pork salami and 100% pork salami. In the mentioned study, these differences have been related to the differences highlighted in the chemical composition of the three products (Ranucci et al., 2019a; Gómez and Lorenzo, 2013). In the present study, however, such differences in chemical composition have not been recorded, likely explaining the discrepancy in the results for textural determinations.
Figure 1 Values of pH (a) and aw (b) of wild boar and pork salami.
Note: C (control: 100% pork meat); BL (low percentage: 40% wild boar meat and 60% pork meat); BH (high percentage: 50% wild boar meat and 50% pork meat). Different superscripts indicate differences in the mean values during sampling times (p < 0.001).

Table 1 Chemical composition of pork salami and wild boar salami.

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<thead>
<tr>
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<th>Salami formulation</th>
<th>SEM</th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>BH</td>
<td>BL</td>
</tr>
<tr>
<td>Moisture (g.100 g⁻¹)</td>
<td>29.35</td>
<td>29.18</td>
<td>30.35</td>
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<tr>
<td>Fat (g.100 g⁻¹)</td>
<td>34.79b</td>
<td>32.01a</td>
<td>33.28</td>
</tr>
<tr>
<td>Protein (g.100 g⁻¹)</td>
<td>27.36</td>
<td>28.11</td>
<td>27.26</td>
</tr>
<tr>
<td>Ash (g.100 g⁻¹)</td>
<td>8.50a</td>
<td>10.61b</td>
<td>9.11ab</td>
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<tr>
<td>NaCl (g.100 g⁻¹)</td>
<td>4.59</td>
<td>4.66</td>
<td>4.63</td>
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</table>

Note: C (control: 100% pork meat); BL (low percentage: 40% wild boar meat and 60% pork meat); BH (high percentage: 50% wild boar meat and 50% pork meat); SEM (standard error of the mean). Different letters in the same row reveal a difference in the mean values at *p < 0.05.
Concerning the color of salami (Table 3) no significant differences were found between the three experimental groups for a*, b*, C, H indices. However, the values of L* indicate that products containing wild boar meat (BL and BH) show lower levels of lightness in comparison to the 100% pork salami. The inherent darker color of wild boar meat is attributable to a higher content of myoglobin (Young and West, 2001). Wild animals, indeed, have darker muscles than domestic animals due to a higher concentration of red muscle fibres as a result of their intense physical activity (Daszkiewicz et al., 2012).

Generally, fermented meat products are considered microbiologically stable nevertheless, when initial contamination of raw material is too high or production phases are conducted inadequately, the safety of such products can be compromised (Maksimovic et al., 2018). In few cases fermented meat products have been associated with Salmonella spp. and Listeria monocytogenes foodborne outbreaks (Pierre, 2014). The microbiological determination performed on salami revealed the absence of L. monocytogenes (absent in 25 g) in all samples regardless of the product's composition and the sampling time, in compliance with the requirement for food safety criteria fixed by European Regulation (EC) n. 2073 (EC, 2005). Concerning Salmonella spp., the analyses showed the presence of Salmonella enterica serovar Typhimurium in the meat batter and in T1 salami samples of the group BH of batch 1, however, the presence of the pathogen was no longer detected (absent in 25 g) in salami sampled in correspondence of the following sampling times (T2 and T3). Similarly, Salmonella enterica serovar Rissen was found in the meat batter of the BH experimental group of batch 4, nonetheless, the microorganism was undetectable (absent in 25 g) in all the subsequent salami samples (T1, T2, and T3). For both mentioned cases of the presence of Salmonella spp. it was infeasible to determine the specific source of contamination due to the thorough mixing procedure that the pork meat and the wild boar meat were subjected to.

The presence of Salmonella spp. in pork meat dry-cured products has been already reported in the literature (Scavia et al., 2013; D’Ostuni et al., 2016; Bonardi et al., 2017), furthermore pork meat and products were responsible for 4.5% of 1241 food-borne outbreaks caused by Salmonella spp. in 2018, representing, therefore, the meat most frequently related with salmonellosis in humans (EFSA, 2019). On the other hand, Salmonella spp. has also been found in wild boar species in central Italy with an incidence varying from 2.4 to 10.9% (Russo et al., 2017). Conversely, other studies in the literature report the absence of Salmonella spp. in wild boar carcases and meat (Ludwiczak et al., 2019; Stella et al., 2019). However, despite the detection of Salmonella spp. in the meat batter and the T1 salami, it is important to highlight that in the finished products the pathogen was undetectable (absent in 25 g) in compliance with the requirement for food safety criterion of Regulation (EC) n. 2073 (EC, 2005), confirming that the experimentally produced wild boar salami meet the safety standards required for large scaled industrialized products.
A summary of the Log counts of some non-pathogenic microorganisms detected in salami produced with different amounts of wild boar meat and control salami (100% pork meat) were reported in Table 4. The values of Enterobacteriaceae registered at T0 were slightly below 4 log CFU.g⁻¹ for all experimental groups (Table 4). Afterward, the level of such microorganisms decreased by approximately 1 log CFU.g⁻¹ for all products more likely due to the evolution of physicochemical characteristics and to the proliferation of competitive microflora (Paleari et al., 2002). No difference between groups was registered suggesting that the salami formulation had no impact in the presence of such bacteria confirming therefore that the presence of Enterobacteriaceae is principally related to fecal contamination during slaughter operations (Chakanya et al., 2018). These results are in line with Chakanya et al. (2018) who found in wild boar matured salami a level of such bacteria ranging from <1.0 to 3.9 log CFU.g⁻¹. However, it is reported that unacceptable counts of Enterobacteriaceae are found in 33% of wild boar and deer fermented sausages made without the use of starter cultures and nitrites, confirming the importance of adequate production strategies (Maksimovic et al., 2018).

Regarding Lactobacillus spp. count the initial values were around 6 log CFU.g⁻¹ as the result of the addition of the starter culture to the meat batter, subsequently as expected, the values increased significantly during the ripening time similarly for all the salami formulations to a final mean value of 8.62 log CFU.g⁻¹. This evolution of Lactobacillus spp. occurs typically in fermented meat products, such as salami, and it represents a crucial aspect for the safety characteristics of products (Miraglia et al., 2017).

Concerning the evolution of Lactococcus spp., the trend shown by the microorganisms is similar to that reported for Lactobacillus spp. with a constant increase until the

<table>
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<th>Table 4 Microbial counts (log CFU.g⁻¹) of wild boar and pork salami at different times of storage.</th>
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<td>Days of Storage</td>
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<td>0</td>
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<tr>
<td>Enterobacteriaceae</td>
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<td>Lactobacillus spp.</td>
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<td>Lactococcus spp.</td>
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<td>CNC</td>
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<td>Enterococcus spp.</td>
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Note: C (control: 100% pork meat); BL (low percentage: 40% wild boar meat and 60% pork meat); BH (high percentage: 50% wild boar meat and 50% pork meat); SEM (standard error of the mean). Different letters in the same row reveal a difference in the mean values at *p <0.005. T (time); S (samples). CNC = coagulase negative cocci.
last day of sampling (T3) to a final mean value of 8.94 log CFU·g⁻¹. The trend of such microbial population was consistent between experimental groups during the entire ripening period.

The coagulase-negative cocci (CNC) count appeared already high in correspondence of the day of salami production (T0) in all the experimental groups irrespective of the meat batter composition with a mean initial value of 6.53 log CFU·g⁻¹, likely as a consequence of the use of starter culture. Subsequently, the population grew slightly for groups BH (final value 7.58 log CFU·g⁻¹) and BL (final value 7.20 log CFU·g⁻¹) while remaining stable in the control group (C). Similar trends are reported in different traditional Italian salami and mainly in those made with lean meat (Amadoro et al., 2011; Miraglia et al., 2017).

In regards to Enterococcus spp. counts (Table 4), no differences were registered between the samples or along the sampling period, and the registered values ranged between 2.81 and 3.31 log CFU·g⁻¹. The values were lower than those registered in some traditional Italian salami (Branciari et al., 2016).

Concerning the sensory analysis, the ranking test assessed the general preference of assessors regarding the experimental salami investigated in the present study. The sum of ranks attributed to the tested samples was 188 for C salami, 181 and 175 for HB and BL salami, respectively. The results showed no difference (p >0.005) in the appreciation of panelists suggesting that the products containing different amounts of wild boar meat (BH and BL) and traditional 100% pork salami (C) can be similarly valued by consumers.

CONCLUSION

The results exposed in the present study confirm that wild boar meat represents a valuable alternative to meat and meat products obtained from factory-farmed animals. This type of meat not only can attend to the needs of health-conscious consumers attentive to its high nutritional value, but it has also shown to be suitable for dry-cured fermented meat products manufacture. The fat content for the formulation with a higher amount of wild boar meat was 2% lower than that of pure swine products. Both safety and quality standards reached by the two experimental wild boar salamis are comparable to salami produced from intensively farmed animals (pork) and fully comply with EU standards (Salmonella spp. undetectable in 25 g). Therefore, the manufacture of game meat products should be encouraged, pursuing the production of healthier, safe, and sustainable foodstuff characterized by a strong bond with local traditions and that also concur with the correct management of increasing wildlife.

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**Conflict of Interest:**

The authors declare no conflict of interest.

**Ethical Statement:**

This article does not contain any studies that would require an ethical statement.

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