Microbial, chemical-physical, rheological and organoleptic characterisation of roe deer (*Capreolus capreolus*) salami

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Abstract

Game meat and related products are important in the promotion of local economies and rural areas. Microbiological, chemical–physical, rheological and sensory characteristics of fermented meat products (salami) made by different percentages of pork and hunted roe-deer (*Capreolus capreolus*) meat were evaluated. The microbiological determination indicated that the products are safe to eat, as neither *Listeria monocytogenes* nor *Salmonella* spp. was isolated from the samples. The hygienic adequacy of the process was guaranteed, as there was below 3 log CFU/g of *Enterobacteriaceae* level in the final products. The proximal composition analyses showed lower lipid levels in comparison to pork salami. The difference in chemical composition affects the rheological and sensory traits of the final products; the products were harder and with higher gumminess when 50% of roe-deer meat was used. Game meat flavour and odour increased with the increasing percentage of roe-deer meat.

Introduction

Proper management of the wildlife is fundamental in the protection of biodiversity, the promotion of local economies and the defence of urban territories against the increasingly frequent movement of wild ungulates outside of their natural habitats (Ramanzin *et al*., 2010). Game meat plays an important role in enhancing the typicality, tradition and linking of animal production with the territory, and this also allows for touristic promotion, both from a culinary and cultural perspective, which in turn, affects the regional economy (Tomasevic *et al*., 2018). The significant increase in wild ungulate populations in Europe has led to an increase in the hunting season, with a consequent increase in the consumption of meat and meat-derived products, resulting in a conservation and management approach of wildlife rather than protection (Quirós-Fernández *et al*., 2017). Therefore, game meat can not only be a resource to be allocated primarily to self-consumption, but it must be managed in a conscious and shared way through the validation of a structured supply chain to increase its competitiveness and sustainability (Hoffmann and Wiklund, 2006). In this sense, it is of fundamental importance to validate the processes of killing, transport and treatment of carcasses, as well as those of meat processing, through microbiological and chemical–physical analyses, to integrate a scientific and objective approach to the traditional salami making, which does not always allow to obtain a safe and guaranteed product (Kuhn *et al*., 2011).

Although the game meat trade is still very limited, and heavily dependent on the hunting seasons and the established hunting rates, there is an increasing market availability of meat products of the different game species, especially those of wild boar, deer and fallow (Paleari *et al*., 2003). Several studies have been conducted on the microbiological, chemical–physical, rheological and sensory characteristics of game meat products (Capita *et al*., 2006; Chakanya *et al*., 2018; Karwowska and Dolatowski, 2017; Paleari *et al*., 2002, 2003; Soriano *et al*., 2006), but works on roe-deer meat products are scarce.

This paper aims to define the microbiological, chemical centesimal composition, rheological and sensory characteristics of fermented meat products (salami) made with different percentages of pork and roe-deer (*Capreolus capreolus*, Linnaeus 1774) meat.

Materials and Methods

Adult roe-deer males were hunted from April to June 2018 between the municipalities of Gubbio and Gualdo Tardino (Umbria Region, Central Italy). After the hunting, animals were promptly on-field exsanguinated and eviscerated, and then transferred to a collection centre for carcass storage. After resting in a cell at 2°C for between 1 and 6 days (average 4 days), the carcasses were transferred to the local slaughterhouse for skinning and sectioning, and then to a local producer for fermented meat (salami) production (Fazi Carni, Gualdo Tadino, Italy). Salami were made according to the local tradition: the deboned roe-deer meat was combined with pork meat (mainly belly and shoulders) and minced in 6 mm fragments. The meat was then mixed with salt (2.2%), pepper powder and pepper grains (0.2%), 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). Neither antioxidant nor preservatives were added to the minced meat. After storing overnight at 4°C, the meat was stuffed into a previously rehydrated dry-salted natural swine intestine. Following 10 days of drying inside hot chambers (22°C and 62% relative humidity (RH) for 48 h; 19°C and 66% RH for 76 h, followed by a 1°C temperature reduction and 1% increase in the RH each day, so as to reach 15°C and 72% RH within 10 days), the products were ripened in controlled seasoning rooms at 13°C and 75% RH for 60 days. The products were obtained from three different percentages of roe-deer and pork meat: 50% roe-deer meat and 50% pork meat (high percentage: SH), 33% roe-deer meat and 67% pork meat (low percentage: SL), and 100%

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pork meat (control: SC).

The experiment was repeated after 1 month, with the same production technique and meat proportion in the products as indicated above, to obtain a replicate batch.

Immediately after stuffing (T0) and at 7 (T1), 14 (T2), and 60 days (T3), five samples from each product (SL, SH, SC) were collected and transported under refrigeration condition to the laboratory for the analytical determination.

Microbiological analyses

Three samples, for each time considered, were tested in triplicate for: i) *Enterobacteriaceae* count, by following the ISO 21528-2 (ISO, 2004) method; ii) *Enterococcus* spp. count, by plating diluted samples on Slanetz–Bartley agar (Biolife Italiana, Milan, Italy) and incubation at 37°C for 48 h; iii) Sulphite-reducing bacteria, according to the ISO 15123 (ISO, 2003) assay; iv) Lactic acid bacteria (LAB) count, as detailed in the ISO 15124 (ISO, 1998) method; v) *Micrococcaceae* count, by plating diluted samples on mannitol salt agar (Biolife Italiana, Milan, Italy) and incubation at 37°C for 24 h.

The average values of the counts obtained from all the products and at all the times considered were calculated and converted into log colony-forming units (CFU)/g. Furthermore, *Listeria monocytogenes* and *Salmonella* spp. were isolated by using the ISO 11290-1 (ISO, 2017a) and ISO 6579-1 (ISO, 2017b) procedures, respectively.

Chemical-physical analyses

On the other two samples per batch, proximal analyses and salt (NaCl) content were determined, based on the AOAC method (AOAC, 2000). This assay was also performed at the end of the ripening time in all the products, in triplicate.

pH and water activity (a_w) were measured according to Branciari *et al*. (2016), at each sampling time for all the products of the trials, using a pH meter (Crison pH25, Crison, Barcelona, Spain) and hygrometer (AquaLab CX-3, Decagon Pullman, WA, USA), respectively. The International Commission on Illumination (CIE, 1976) *L*a*b** colour space values were recorded using a Minolta C400 chromameter (Minolta Ltd., Osaka, Japan), with six measures taken on the surface of three slides for each sample belonging to each product type considered, at the end of the ripening time.

Rheological analyses

From each product collected at the end of the ripening time, three cylinders with a height and width of 2.5 cm, were removed using a core drill. A texture analyser (TVT 6700, Perten Instrument, Sweden) was equipped with a cylindrical probe (3 cm in diameter), and the relative settings were set. The compression rate used was 30%, with a probe speed rate of 2 mm/s. The parameters considered were hardness, resilience, cohesiveness, gumminess and chewiness, according to user's manual (Perten Instrument Method Description: TVT method 47-01.02).

Sensory analyses

A panel comprising 12 assessors, were trained according to the ISO 8586 (ISO, 2012) criteria. In the same session, the attributes to be evaluated for each sample were defined, and parameters were selected to identify the visual, olfactory, gustative characteristics, consistency and acceptability of the different product types.

The test was repeated on the final products (SH, SL, SC) of the two different batches on different days, using specific questionnaires. To quantify the intensity of each attribute, a 9-point scale was used, in which 0 = minimum intensity and 9 = maximum intensity, as detailed in the ISO 13299 (ISO, 2016). The panel test was conducted as reported by Ranucci *et al*. (2018). The evaluators tasted the products samples that had been sliced to a thickness of 2 mm and pre-equilibrated at room temperature for about 1 h. The samples were served anonymously on white plastic plates and coded with random three-digit numbers. The evaluations were carried out with repeated tastings, and water and crackers were served to allow the assessors to give a more objective judgment, eliminating the flavours deriving from the taste of previous samples.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) (GMP, SAS Institute, Cary, NY, USA), considering, as factors, the microbiological and chemical–physical data monitored over time, the treatment (SH, SL, SC) and the sampling time (T0, T1, T2 and T3). For the other determinations, carried out exclusively on the finished product, a one-way ANOVA was used, with the treatment as a fixed factor. Tukey's *post hoc* test was used to determine if the values obtained were different at a significance level of 0.05.

Results and Discussion

The hygienic-sanitary level of the tested products was adequate, as regards the evaluation of food safety criteria (EC Regulation 2073/2005 and s.m.i.) since in all the samples analysed, regardless of the product type and sampling time, *L. monocytogenes* and *Salmonella* spp. were not detected. The presence of these two pathogens in game meat products are reported in the literature (Cenci Goga *et al*., 2012; Kuhn *et al*., 2011; Lucchini *et al*., 2014) but a frequency variation across populations may be present (Avagnina *et al*., 2012). Besides, the implementation of good hygienic practice throughout the game meat chain could reduce the presence of both pathogens on wild ruminants' carcass (Paulsen *et al*., 2012) and, therefore, in the products. Furthermore, during salami processing, the condition of the production could affect the survival of *Salmonella* spp. in the final products (Cenci Goga *et al*., 2012). The *Enterobacteriaceae* and *Enterococcus* spp. counts are reported in Figure 1.

The level of *Enterobacteriaceae* was quite high in the mixture, with values around 4 log CFU/g in all the products at T0. Subsequently, due to changes in the chemical–physical parameters and the development of competitive flora (Paleari *et al*., 2002) the values decreased to less than 3 log CFU/g. The *Enterobacteriaceae* count tended to be less in the product with 50% roe-deer meat, and this trend was significantly evident at T0, T1 and T2 in these salami ($P<0.05$). Since the presence of *Enterobacteriaceae* is mainly linked to faecal contamination during slaughter operations (Chakanya *et al*., 2018), it is possible to attribute the variation in the *Enterobacteriaceae* counts across the different products to the raw pork meat. Moreover, in pigs, due to the adopted slaughtering system, the hygienic criteria of process hygiene, including the *Enterobacteriaceae* count (EC Regulation 2073/2005 and s.m.i.), are higher than those of other animal species.

No difference in the *Enterococcus* spp. counts ($P>0.05$) were registered between the samples, and the recorded values were maintained at between 2 and 3 log CFU/g over time, in all the samples considered. The values were lower than those registered in some traditional Italian salami (Branciari *et al*., 2016; Ranucci *et al*., 2013). The *Micrococcaceae* and LAB counts are reported in Figure 2. The micrococci count in all the tested groups were already high at the time of the casing (Figure 2), and irrespective of the characteristics of the meat adopted, in view of the use of starter culture directly added to the mix. This population remained stable for the first three sampling times with variation only at T3. Generally, this population tends to fall in the first week of seasoning, concomitantly with the

growth of the LAB (Capita *et al*., 2006) but this behaviour was not apparent in the products under the experiment conditions of the current study. However, in the first week of fermentation, the pH (Figure 3) also maintained rather high values compared with other fermented meat products described in the literature (Zanardi *et al*., 2004). Interestingly, in products with a high percentage of roe-deer meat (SH), the micrococci population tended to remain significantly higher at T3 when compared with SL and SC ($P<0.05$). The presence of such microorganisms is responsible for the increased proteolytic activity, which confers aromatic traits to the product and lowers the acidity (Mauriello *et al*., 2002).

A rapid growth of the LAB occurred, already reaching 8 log CFU/g at 1 week after production. This trend is typical in this kind of meat product (Połka *et al*., 2015) but no difference was found between the different groups.

Sulphite-reducing bacteria (at less than 2 log CFU/g) were found exclusively in the products after casing (T0) and in some salami samples, with no difference between the control and products with roe-deer meat.

As shown in Figure 3, there was a rapid decrease in the pH of the products within the first week of production, followed by an increase, as the seasoning progressed. This phenomenon is attributable to the gradual increase in acidity linked to the fermentative metabolism of the LAB, and then by the onset of proteolytic events due to *Micrococcaceae* (Wang *et al*., 2015). One characteristic of the products is a low–medium acidity (pH >5 after casing and pH around 6 in the final products) and intense proteolysis, typical of various traditional Italian products (Cenci Goga *et al*., 2008; Miraglia *et al*., 2017). This characteristic was most pronounced in both SL and SH, in which, at the end of ageing, statistically higher pH values were observed than in the control ($P<0.05$). Conversely, the counts of *Micrococcaceae*, which are mainly responsible for the proteolytic phenomena (Połka *et al*., 2015), were also higher in SL and SH than the control.

The a_w values are reported in Figure 4. The gradual loss of water from the product during the drying and seasoning steps means less water available for the microorganisms to grow and survive. These data are comparable to those obtained in other experiments (Branciari *et al*., 2016; Capita *et al*., 2006; Soriano *et al*., 2006). No significant differences between the control and treated salami were registered, showing that the product formulation did not influence the a_w level. The low a_w value in the final product contributes to improving product

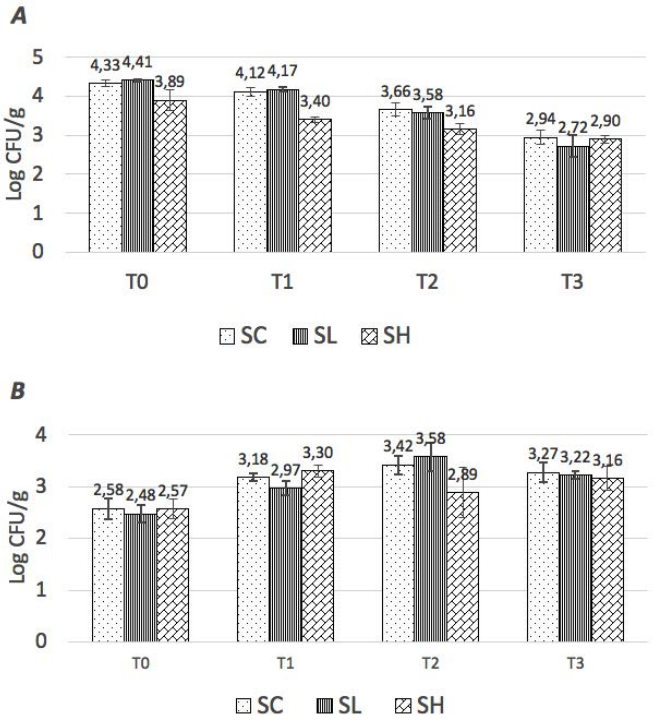

Figure 1. ***Enterobacteriaceae*** **(A) and** ***Enterococcus*** **spp. (B) counts in roe-deer salami and pork salami. SC (control:100% pork meat); SL (low percentage: 33% roe-deer meat and 67% pork meat; SH (high percentage: 50% roe-deer meat and 50% pork meat; T0 (immediately after stuffing); T1 (at 7 days storage), T2 (at 14 days storage); T3 (at 60 days storage).**

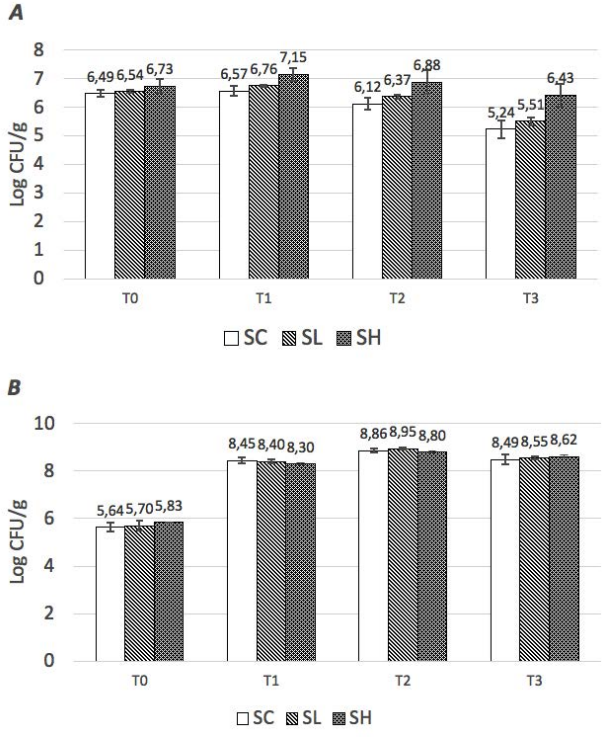

Figure 2. ***Micrococcaceae*** **(A) and lactic acid bacteria (LAB) counts (B) in roe-deer salami and pork salami. SC (control:100% pork meat); SL (low percentage: 33% roe-deer meat and 67% pork meat; SH (high percentage: 50% roe-deer meat and 50% pork meat; T0 (immediately after stuffing); T1 (at 7 days storage), T2 (at 14 days storage); T3 (at 60 days storage).**

safety (Muthukumarasamy and Holley, 2006).

In regards to the products` colour (Table 1), significant differences were found between SL and SH and SC. The unique colour of roe-deer meat, which is darker than pork meat, affected the lightness of the products. Similarly, the red and yellow indices were significantly higher in salami with 50% of roe-deer meat than in SC.

The acceptability of the products heavily depends on their colourimetric characteristics. Several endogenous factors contribute to the colour of meat, particularly the pH, the type of muscle fibre, the presence of antioxidants, lipid oxidation and the mitochondrial activity in the muscles (Mancini and Hunt, 2005). Even the conditions, such as the diet of the wild animals, affect the colour of the meats (Pedrazzoli *et al*., 2017). The presence of roe-deer meat influenced the chemical composition (Table 2) of the products obtained, albeit mediated by the integration of pork meat, with reference to the fat content. The roe-deer meat is leaner than pork meat (Daszkiewicz *et al*., 2012) and SH proved to be less moist and leaner (lower percentage of lipids) than the other salamis, with a protein content between 28 and 29%. Paleari *et al*. (2003) recorded substantially less protein, 18.9-20.4%, in dry-cured game meat products. No differences in the NaCl content were detected between the groups, and the values align with data obtained from other Italian salami (Ranucci *et al*., 2016; Zanardi *et al*., 2010).

Textural profile analyses revealed that SH salamis were harder and gummier but presented less chewiness than the salami belonging to the other two groups (Table 3). The characteristics of the chemical composition of SH, leaner then SL and SC, could be the reason for these findings (Gómez and Lorenzo, 2013). The instrumental texture of the products corroborated the sensory texture (Table 4).

The sensory analyses confirmed the difference in the lightness and redness intensity of the lean meat colour in comparison to pork, as discussed above for the instrumental colour measurements, but also revealed a predominant odour and flavour of game meat that was progressively evident with the increase of roe-deer meat percentage in the salami. These attributes, as well as overall flavour intensity, hardness and chewiness, may act as discriminants for consumer preference between these two products, as further analyses could demonstrate.

Table 1. Colour attributes of roe-deer salami and pork salami.

Parameter	SC	SL	SH	SEM	P
Lightness (L*)	42.38a	39.22b	37.18b	0.91	**
Redness (*a**)	10.46a	11.73ab	13.37b	0.61	*
Yellowness (*b**)	4.82a	4.92ab	5.29b	0.30	*

SC (control:100% pork meat); SL (low percentage: 33% roe-deer meat and 67% pork meat; SH (high percentage: 50% roe-deer 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, **P<0.01.

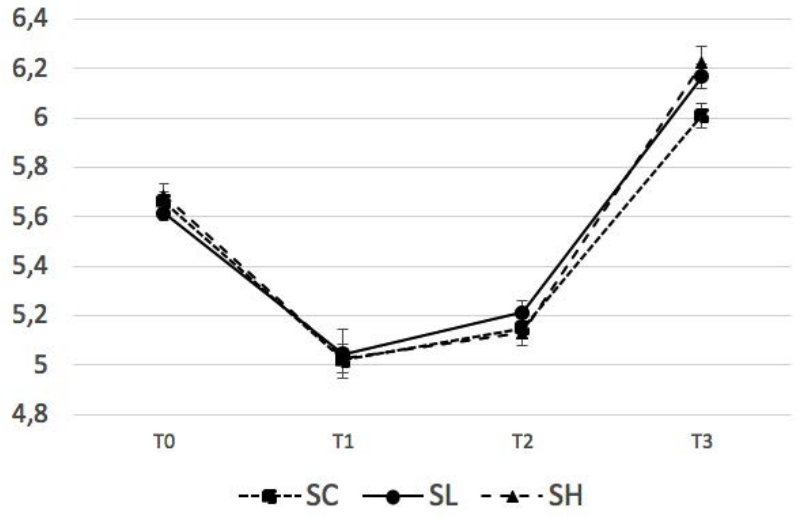

Figure 3. pH values in roe-deer salami and pork salami (mean values and standard deviation). SC (control:100% pork meat); SL (low percentage: 33% roe-deer meat and 67% pork meat; SH (high percentage: 50% roe-deer meat and 50% pork meat; T0 (immediately after stuffing); T1 (at 7 days storage), T2 (at 14 days storage); T3 (at 60 days storage).

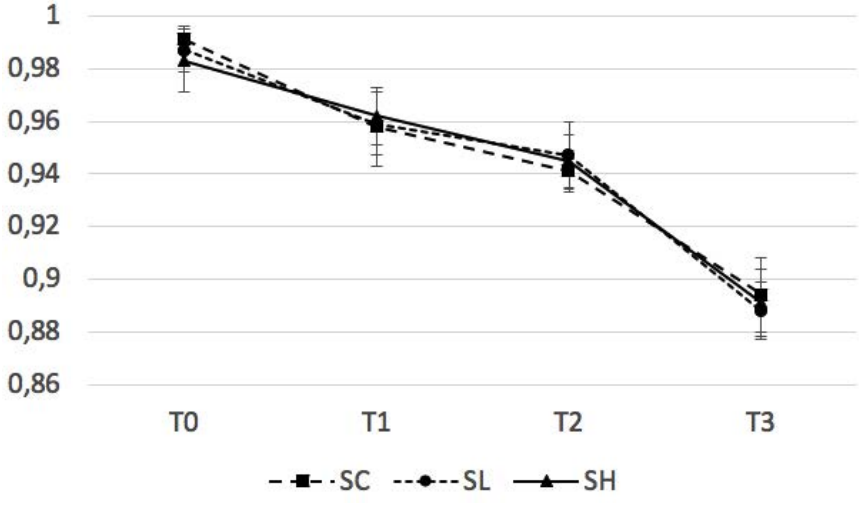

Figure 4. Water activity (a_w) values in roe-deer salami and pork salami (mean values and standard deviation). SC (control:100% pork meat); SL (low percentage: 33% roe-deer meat and 67% pork meat; SH (high percentage: 50% roe-deer meat and 50% pork meat; T0 (immediately after stuffing); T1 (at 7 days storage), T2 (at 14 days storage); T3 (at 60 days storage).

Conclusions

The production of fermented meat products could be a valuable and sustainable strategy for the commercialisation of hunted roe-deer meat, as salami could be appreciated by the consumers. The roe-deer salami could be considered safe if proper management of the production chain, form hunting to processing, is hygienically implemented. Moreover, the percentage of game meat could be calibrated according to the consumers' preference and to provide a product with comparable attributes to the common renown pork products.

Table 2. Chemical composition of roe-deer salami and pork salami.

%	SC	SL	SH	SEM	P
Moisture	29.38b	29.52b	28.00a	0.22	*
Lipid	35.61b	35.15b	30.69a	0.17	*
Protein	27.08a	27.17a	29.26b	0.57	*
Ash	8.03a	9.68b	10.53c	0.30	*
NaCl	4.28	4.30	4.25	0.03	ns

SC (control:100% pork meat); SL (low percentage: 33% roe-deer meat and 67% pork meat; SH (high percentage: 50% roe-deer 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. ns = not significant.

Table 3. Results of texture profile analyses of roe-deer salami and pork salami.

Attribute	SC	SL	SH	SEM	P
Hardness (g)	2034.57a	2215.33a	3270.33b	7.15	***
Resilience	0.63	0.61	0.60	0.01	ns
Cohesiveness	0.61a	0.55b	0.51b	0.01	*
Gumminess (g)	2318.19a	2784.89b	3521.67c	79.27	*
Chewiness (g)	1519.69a	1409.33b	1008.00c	93.42	*

SC (control:100% pork meat); SL (low percentage: 33% roe-deer meat and 67% pork meat; SH (high percentage: 50% roe-deer 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, ***P<0.001. ns = not significant.

Table 4. Sensory descriptive analyses of roe-deer salami and pork salami.

Attribute	SC	SL	SH	SEM	P
Visual examination					
Uniformity of lean	6.08b	5.43a	5.48a	0.03	*
Intensity of lean	4.53a	6.03b	6.35c	0.12	***
Uniformity of the fat	3.30	3.13	3.23	0.04	ns
Intensity of the fat	4.45	4.20	4.48	0.15	ns
Connection between lean and fat	6.55b	5.60a	5.78a	0.05	**
Distribution of the fat	3.56	3.83	3.90	0.08	ns
Odour					
Spicy	4.50	4.48	4.78	0.11	ns
Pepper	4.60c	4.00b	3.65a	0.02	***
Rancid	0.13	0.18	0.23	0.03	ns
Game meat	0.16a	3.95b	5.48c	0.12	***
Mould	0.13	0.15	0.15	0.01	ns
Flavour					
Acid	0.40a	1.05b	1.15b	0.03	**
Game meat	0.10a	2.36b	3.23c	0.08	***
Rancid	0.13a	0.20a	0.40b	0.01	*
Bitter	0.13	0.20	0.18	0.01	ns
Salty	4.53	4.40	4.42	0.04	ns
Mould	0.13	0.13	0.10	0.01	ns
Pungency	1.98b	1.48a	1.57ab	0.01	*
Overall intensity	6.63a	6.68a	7.38b	0.04	*
Texture					
Hardness	4.50a	4.75a	5.95b	0.06	**
Gumminess	4.18a	4.40ab	4.93b	0.04	*
Chewiness	4.55c	4.20b	3.43a	0.03	***
Cohesiveness	1.63a	2.05ab	2.50b	0.06	*
Solubility	1.83	1.93	1.88	0.05	ns
Fattiness	4.83	5.30	4.98	0.12	ns

SC (control:100% pork meat); SL (low percentage: 33% roe-deer meat and 67% pork meat; SH (high percentage: 50% roe-deer meat and 50% pork meat; SEM (standard error of the mean); Different letters in the same row reveal difference in the mean values at *P<0.05, **P<0.01, ***P<0.001. ns = not significant.

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