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Valorization of indigenous dairy cattle breed through salami production

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ABSTRACT

The aim of the research was to produce salami manufactured with meat of three different commercial categories of bovine breed: cow on retirement, beef and young bull. A total of six experimental productions, at small-scale plant, were carried out with and without starter culture inoculums. The evolution of physico-chemical parameters in all trials followed the trend already registered for other fermented meat products. Several LAB species were found during process with different levels of species diversity and frequency of isolation among inoculated (mainly *Pediococcus pentosaceus* and *Staphylococcus xylosus*) and uninoculated (mainly *Enterococcus devriesei*, *Lactobacillus curvatus* and *Lactobacillus sakei*) trials. *Enterobacteriaceae* were found at very low levels during the entire ripening period and no pathogenic bacteria were found in any samples. The multivariate analysis showed that starter inoculums and meat affected significantly the physico-chemical and the microbiological composition of salami. The sensory analysis evidenced the highest overall acceptability was displayed by salami produced with meat from cow on retirement.

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1. Introduction

Salami are generally defined as cured sausages, fermented and air-dried meats obtained from one or more species of animals (Francesca, Sannino, Moschetti, & Settanni, 2013). When this product is partially fermented and requires cooking before consumption, it is called "salsiccia", whereas salami is commonly eaten after a variable period of drying and ripening (Francesca et al., 2013).

Historically, salami are made with meat and fat of swine. In the last years, salami of autochthonous breeds are becoming very appreciated by consumers (Francesca et al., 2013). Salami made also with meat of other animal species (Cenci-Goga et al., 2012; Omer et al., 2015) are gaining importance in several European markets, becoming easily available (Bertolini, Zgrablic, & Cuffolo, 2005; Severini, Stocchi, Cenci-Goga, & Scorciarini Coppola, 1999). Bovine salami are typically produced in northern Italy, but to our knowledge no scientific studies have been carried out to monitor the technological/chemical/microbiological parameters during meat transformation.

Meat quality is influenced by several factors, such as animal breed, feeding and pasture availability, breeding system, and animal activity (Bittante, Andrighetto, & Ramanzin, 2008). All these factors contribute to the chemical-organic composition of meat, as well as to the composition and amount of fat reserve, influencing the sensory quality of the final products.

Sicily hosts several rustic animal breeds and, among them, Cinisara represents an autochthonous cattle breed with prevailing aptitude to milk production, belonging to the group of the "Podoliche" breeds (Liotta & Chiofalo, 2007). Cinisara cows feed mainly on poor natural pasture and are commonly associated to the dairy product Caciocavallo Palermitano cheese (Di Grigoli, Francesca, Gaglio, Guarrasi, & Bonanno, 2015; Settanni et al., 2012).

Recently, the meat of Cinisara cows reared according to the traditional production system has been investigated for its characteristics. The studies showed that this meat is chemically and physically similar to those of the specialized beef breed and possesses a good protein and intramuscular fat amount (Liotta et al., 2011).

During salami production, the microorganisms naturally present on the raw materials and eventually inoculated as starters are responsible for the fermentation process. This process involves a succession of events in which all conditions characterizing the dripping, drying and ripening phases need to be monitored to assure high level of hygienic safety and sensory quality of the final product. The aim of this research was to monitor the production of salami manufactured with bovine meat under different technological and microbiological conditions. To determine the quality of the resulting products, the influence of three different commercial categories (cow on retirement, beef and young bull) on physico-chemical, microbiological and sensory properties of Cinisara salami was studied. This study is part of a project mainly aimed

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to find commercial alternatives for the meat of autochthonous breed appreciated in the market.

2. Materials and methods

2.1. Animals and salami formulations

Fermented bovine salami were produced with meat of the Sicilian bovine breed Cinisara, and the experimental design is shown in Fig. 1. In details, the animals used in this study included retired cows (120 months old), beefs and young bulls. The animals belonging to the categories beef and young bull were of 18 months old but they differed in terms of grazing period. In details, the beefs were breed by grazing until 15 months of age and subsequently they were kept in housing barn until slaughter; on the other hand the young bulls were breed by grazing until slaughter. After slaughter, the carcasses were stored at low temperature (from 4 to 8 °C) for 8 days (aging period); after cutting and deboning the flesh, the meat was cleansed of fat, tendons, and other connective tissues. Per each type of animal categories, the meat was separately mixed with swine fat (20% w/w) of the autochthonous Suino Nero dei Nebrodi swine breed. The mix was minced with plate of 6 mm to obtain the mixture. Subsequently, each mixture was furtherly separated into two batches that differed for the inoculums (with and without) of starter cultures.

The entire salami production was performed two times following the same experimental design (Fig. 1).

By this way, two independent productions were performed, once per week, during February 2014. All salami were manufactured by an experienced craftsman, at the Salumi Lipari sausage factory located in Alcamo (Sicily, Italy).

2.2. Salami manufacturing and sample collection

The experimental salami types were produced as follows: the meat of three animal categories was separately minced and placed into three different stainless steel vats. Subsequently, the content of each vat was divided in two stainless steel vats for a total of six (100 kg each) experimental trials.

Three trials (SP1, SP2, and SP3) were uninoculated with the starter culture preparation since they were spontaneously fermented, while the other three trials (ST1, ST2, and ST3) were added with *Staphylococcus xylosus* and *Pedioccocus pentosaceus* freeze-dried cultures (Tec-AL. s.r.l., Italy) to a final concentration of approximately 10^7 CFU g^{-1}.

After inoculation, the meat of each experimental trial was mixed with the other ingredients into a blender and separately stuffed into natural casings. Each salami sample was formed at approximately 35 cm in length and 7 cm in diameter. Subsequently, the salami were dripped for three days [20 °C and free relative humidity (RH)]. After that they were fermented and dried for a total of seven days in accordance with the following protocol: 20–22 °C and 62–72% RH at day 1; 19–21 °C and 64–74% RH at day 2; 18–20 °C and 66–76% RH at day 3; 17–19 °C and 68–78% RH at day 4; 16–18 °C and 70–80% RH at day 5; 15–17 °C and 72–82% RH at day 6; 14–16 °C and 74–84% RH at day 7; 13–15 °C and 76–86% RH at day 8; 12–14 °C and 78–88% RH at day 9; 12–14 °C and 78–88% RH at day 10. Afterward, samples were fermented and ripened for a total of 45 days (11–13 °C and 80–90% RH).

The starter culture preparations as well as the other ingredients (swine fat, pepper, salt and sugar mixture, and also the gats) were sampled.

Minced meat was sampled before addition of starter cultures and the following samples were collected during production: mixture of meat and other ingredients at time 0 (meat mixture just after stuffing), day 10 (end of drying process and fermentation phase), day 25 (ripening and fermentation phases) and day 45 (end of ripening process). At each sampling time, five salami for trial (starter and control) were collected, 3 subsamples per each salami were analyzed.

All samples were collected in triplicate, placed into sterile containers, immediately refrigerated and transported at controlled temperature (with a portable fridge) to the laboratories of Agricultural Microbiology (University of Palermo) and to the Instituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" (Palermo, Italy).

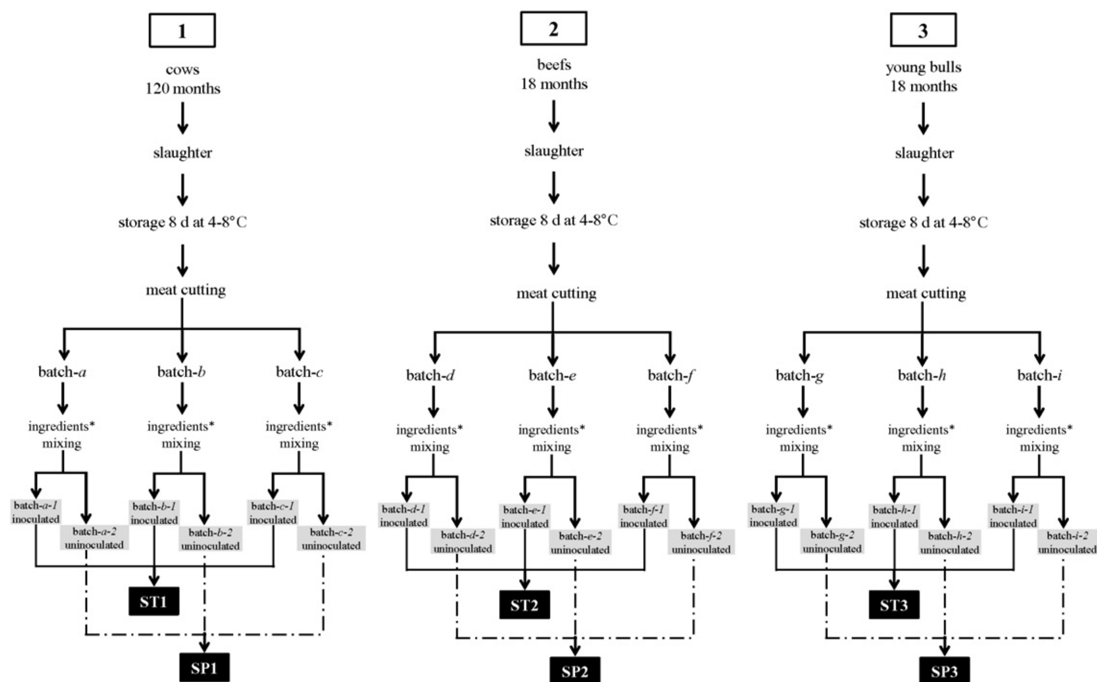

Fig. 1. Experimental design of bovine salami production. 1, cows at 120 months old; 2, beefs at 18 months old; 3, young bulls at 18 months old. Abbreviation: codes of batches from *a*-1 to *i*-2 refer to replicates within each trials; codes from ST1 to ST3 refer to trials inoculated with starter cultures; codes from SP1 to SP3 refer to trials spontaneously fermented. *NaCl (2.5% w/w); sucrose, dextrose and malt dextrin and sodium ascorbate (160 ppm); sodium nitrate (100 ppm) and sodium nitrite (100 ppm).

2.3. Physical and chemical parameters

All samples were analyzed for pH with the pH-meter BASIC 20+ (Crison Instrument S.A., Barcelona, Spain). All samples (10 g) were diluted with distilled H_2O and homogenized by means of a stomacher (LAB Blender 400; Seward Medical, London, UK) at the maximum speed for 2 min. The meat samples stuffed into casings were peeled before dilution. Water activity (a_w) was measured with a dew-point hygrometer HygroLab 3 (Rotronic, Huntington, NY, USA). Calibration was performed using five saturated solutions of known a_w. Weight loss was expressed as difference from the initial weight.

The measurement of colorimetric parameters was performed with a Chroma Meter (CR-300; Minolta, Osaka, Japan) using the illuminant C, the calibration is based upon a white standard as $L^* = 100$ (equivalent to BaSO4) and aperture size ø 8 mm. Results were expressed as lightness (L^*), redness (a^*), and yellowness (b^*), according to the International Commission on Illumination (CIE) $L^*a^*b^*$ system (Menegas et al., 2013). pH, a_w and colorimetric parameters were measured at time 0 and, subsequently, at day 10, 25 and 45 of production.

At the end of ripening, the texture of salami was analyzed by Instron Universal Testing Machine (Instron 5564 tester, Trezzano sul Naviglio, Milano, Italy). Samples were subjected to a load cell force of 50 kg and to record strain to compression to 40% at the end of the run. The results were expressed as maximum resistance to compression (compressive stress, N mm^{-2}).

Chemical composition of salami samples at the end of process (day 45) was determined according to AOAC standards methods (1995). All analyses were carried out in triplicate per each sample at each time.

2.4. Microbiological analyses

Microbiological analyses were carried out to evaluate the main microbial groups associated to salami production. Decimal dilutions of minced meat (25 g), starter culture (25 g), ingredients (25 g) and salami (25 g) samples were prepared in Ringer's solution (Sigma-Aldrich, Milan, Italy) and homogenized as reported above.

The counts for total mesophilic and psychrotrophic bacteria, *Enterobacteriaceae*, enterococci, pseudomonads, coagulase-positive staphylococci (CPS) and coagulase-negative cocci (CNC), mesophilic rod and coccus LAB, as well as yeasts were determined as described by Francesca et al. (2013). All counts were performed in triplicate. All samples were also investigated for the presence of *Salmonella* spp. and *Listeria monocytogenes* (Francesca et al., 2013). All media were purchased from Oxoid (Basingstoke, UK).

2.5. Isolation, grouping and genotypic differentiation of LAB and staphylococci

After growth, colonies of various shapes (at least 3 with identical morphology) were picked up from count plates used for LAB (MRS, M17) and staphylococcus (BP) enumeration. The isolates from MRS and M17 were transferred to the corresponding broth media, while the isolates from KAA into M17 broth. The isolates were purified by successive sub-culturing and the purity of the isolates, as well as the cell-morphology were checked microscopically. Gram-positive [Gregersen KOH method (Gregersen, 1978)] and catalase-negative (determined in the presence of H_2O_2 5%, v/v) were stored in glycerol at −80 °C until further experimentations. Phenotypic characterization of LAB was carried out as reported by Gaglio et al. (2014) based on cell morphology, growth at 15 and 45 °C, resistance at 60 °C for 30 min, NH_3 production from arginine, aesculin hydrolysis, acid production from carbohydrates, and CO_2 production from glucose. Cocci were also grouped for their growth at pH 9.2 and in the presence of 6.5 g L^{-1} NaCl to separate enterococci from other dairy cocci.

The CNC cultures were also picked up from BP agar and inoculated into Nutrient Broth (NB) medium, purified to homogeneity and controlled for their Gram-positive and catalase-positive character. Those isolates were grouped after the phenotypic characterization as described by Fiorentini et al. (2009).

The DNA from salami isolates was extracted using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. Strain differentiation of LAB and staphylococci was performed by random amplification of polymorphic DNA-PCR (RAPD-PCR) analysis in a 25 μL reaction mix using single primers M13 (Settanni et al., 2012). Amplifications were performed by means of T1 Thermocycler (Biometra, Göttingen, Germany) applying the conditions reported by Settanni et al. (2012). RAPD profile were analyzed on agarose gel 1.5% (w/v) in 1X TBE buffer and visualized as above. One representative culture for each cluster was identified by 16S rRNA gene sequencing as described by Settanni et al. (2012).

2.6. Volatile organic compounds (VOCs)

Volatile compounds were determined on salami samples at day 45 by Solid Phase Micro-Extraction technique in Head Space followed by Gas Chromatography/Mass Spectrometry (HS-SPMEGC/MS) (Pawliszyn, 1999).

Samples of homogenized salami (0.50 g) were transferred into 2-mL vials with pierceable silicone rubber septa coated with polytetrafluoroethylene (PTFE) film. 50 μL of 2-pentanol-4-methyl methanol solution (0.981 μg mL^{-1}) was used as internal standard. A Supelco SPME (Bellefonte, PA) holder and fiber was coated with divinylbenzene/carboxen/polydimethylsiloxane. The vials were heated at controlled temperature (40 ± 0.5 °C) in order to reach equilibrium and 30 min exposure time. The GC-MS conditions were used as described by Corona (2010). Collected data were processed with the instrument data system. Salami volatile compounds were identified by comparison of the retention times with those of the reference compounds (NIST/EPA/MSDC Mass Spectral Database, T.G. House, Cambridge, UK). Semi-quantitative determination was carried out by the method of internal standard. The calibration curve was constructed with readings on five 2-pentanol-4-methyl methanol solutions with concentrations ranging from 1.5 μg mL^{-1} to 8 μg mL^{-1} (R^2 0.994). All analyses were performed in triplicate.

2.7. Sensory evaluation

The evaluation of the sensory profiles of full ripened salami was performed following ISO guidelines (2005).

The descriptive panel consisted of twelve judges (6 females and 6 males, 25–35 years old) which regularly performs sensory analysis and they have experience with evaluation of meat products (included fermented products), and with the methodology and technical aspects. Judges were trained in preliminary sessions to gain consensus on the sensory descriptors and the use of scale (Cenci-Goga et al., 2012). Each attribute was extensively described and explained to avoid any doubt about the relevant meaning as reported by Cenci-Goga et al. (2012). A total of 17 descriptors were included in the analysis for the external aspect (color uniformity, color intensity, fat/lean connection, fat/lean distribution), flavor (acid, rancid, mold, lactic, bitter, salty and intensity), rheology (elasticity, hardness, chewiness, juiciness and fattiness), as well as overall acceptability.

The overall acceptability is a subjective parameter useful for the evaluation of the palatability of experimental salami. The salami samples were randomly evaluated by assigning a score between 1.00 (the absence of sensation) and 9.00 (extremely intense) in individual booths under incandescent white light.

Each assessor evaluated three replicates of the six experimental salami produced. The individual scores for each assessor were then averaged to give a score for the taste panel as a whole. Each evaluation was carried out in different test sessions at the same time of day.

Assessors conducted no more than three tests per day lasting a maximum of 1 h (Cenci-Goga et al., 2012). The sensory analysis was carried out for both independent productions, during two different sensory sessions at the end of ripening of salami.

2.8. Statistical and explorative multivariate analysis

Results from physico-chemical and microbiological investigation, as well as VOCs and sensory data were analyzed using a generalized linear model (GLM) based on ANOVA model that included effects of week (1st and 2nd week), commercial categories of bovine breed (cow on retirement, beef and young bull) and microbial starter (inoculated and uninoculated), as well as the interaction between commercial categories of bovine breed and microbial starter. The post-hoc Tukey's method was applied for pairwise comparison. Statistical significance was attributed to P values of <0.05.

In addition, explorative multivariate analysis was employed to investigate relationship among data obtained from the different experimentations. A hierarchical cluster analysis (HCA) (joining, tree clustering) was carried out for grouping the trials according to their similarity, measured by Euclidean distances, whereas cluster aggregation was based on the single linkage method. Furthermore, the principal component analysis (PCAn) was employed to investigate relationships among samples. The input matrix used for HCA and PCAn consisted of the total area under growth/decline curves based on data from analysis of pH, a_w, weight loss and microbial populations (Blana, Grounta, Tassou, Nychas, & Panagou, 2014; Martorana et al., 2015a). Areas were calculated by integration using the OriginPro 7.5 software (OriginLab Corporation, Northampton, USA). In addition, other relevant indexes were taken into account as follows: maximum and minimum values of pH, maximum and minimum values of microbial populations (Bautista-Gallego et al., 2011; Martorana et al., 2015b).

Results of VOCs determination on salami samples at 45 days were also considered for both HCA and PCAn. The latter analysis was also employed to investigate relationship among salami samples based on sensory analysis (Martorana et al., 2015a). The number of principal factors was selected according to the Kaiser criterion (Jolliffe, 1986) and only factors with Eigen-values higher than 1.00 were retained.

All data were preliminary evaluated by using the Barlett's sphericity test (Dillon & Goldstein, 1984; Mazzei, Francesca, Moschetti, & Piccolo, 2010) in order to check the statistically significant difference among samples within each data set.

Statistical data processing and graphic construction were achieved by using SPSS software package (SPSS Inc. Chicago, IL, USA) and STATISTICA software version 10 (StatSoft Inc., Tulsa, OK, USA) and XLStat software version 7.5.2 (Addinsoft, New York, USA) for excel.

3. Results

3.1. Determination of physical and chemical parameters

The physical parameters registered throughout salami fermentation and ripening are reported in Table 1. No statistical differences according to Tukey's test were found between the formulations with or without addition of starters in terms of pH and a_w. However, minced meat and salami just after stuffing, showed pH of about 5.6 that decreased to about 5.3 in all trials at day 45. Mean values of a_w just after stuffing, were above 0.94 and decreased to about 0.87 in all trials.

The weight loss values, in the range 30–40%, were statistically different for the six formulations at the end of ripening for all trials. The highest values were found in samples of trial ST2 and SP2 that reached weight loss of 45.43 and 46.43%, respectively.

Salami color, expressed as luminosity (L*), red (a*) and yellow (b*) index, showed statistical differences among trials. The highest values of luminosity, red and yellow were detected for trials ST1 and SP3, respectively. The analysis of texture by Instron machine showed no significant statistically differences among trials for maximum resistance to compressive stress at 40% and at the end of the run.

Table 1
Results of physico-chemical parameters at the end of ripening of dry-cured bovine salami.

Physicochemical characteristics	Storage time (days)	Formulations[a]						Statistical significance[a]
		ST-1	ST-2	ST-3	SP-1	SP-2	SP-3	
pH	0	5.59 ± 0.06^{A}	5.55 ± 0.06^{A}	5.57 ± 0.05^{A}	5.6 ± 0.07^{A}	5.54 ± 0.05^{A}	5.53 ± 0.08^{A}	N.S.
	10	5.36 ± 0.02^{A}	5.38 ± 0.06^{A}	5.33 ± 0.04^{A}	5.32 ± 0.02^{A}	5.4 ± 0.03^{A}	5.31 ± 0.01^{A}	N.S.
	25	5.37 ± 0.02^{A}	5.34 ± 0.02^{A}	5.37 ± 0.01^{A}	5.21 ± 0.03^{A}	5.3 ± 0.02^{A}	5.29 ± 0.05^{A}	N.S.
	45	5.3 ± 0.01^{A}	5.25 ± 0.01^{A}	5.23 ± 0.01^{A}	5.26 ± 0.02^{A}	5.3 ± 0.04^{A}	5.29 ± 0.02^{A}	N.S.
a_w	0	0.94 ± 0.01^{A}	0.94 ± 0.01^{A}	0.94 ± 0.01^{A}	0.95 ± 0.05^{A}	0.94 ± 0.01^{A}	0.94 ± 0.06^{A}	N.S.
	10	0.91 ± 0.02^{A}	0.91 ± 0.05^{A}	0.92 ± 0.03^{A}	0.93 ± 0.01^{A}	0.93 ± 0.05^{A}	0.93 ± 0.03^{A}	N.S.
	25	0.89 ± 0.02^{A}	0.9 ± 0.03^{A}	0.91 ± 0.02^{A}	0.9 ± 0.04^{A}	0.91 ± 0.01^{A}	0.9 ± 0.04^{A}	N.S.
	45	0.86 ± 0.01^{A}	0.88 ± 0.02^{A}	0.88 ± 0.01^{A}	0.87 ± 0.01^{A}	0.88 ± 0.01^{A}	0.88 ± 0.01^{A}	N.S.
Weight loss	10	26.17 ± 0.54^{AB}	26.94 ± 0.42^{B}	26.06 ± 0.9^{AB}	25 ± 0.42^{A}	27.36 ± 0.43^{B}	24.86 ± 0.64^{A}	*
	25	37.48 ± 0.32^{A}	39.84 ± 0.52^{AB}	38.06 ± 0.52^{A}	36.74 ± 0.72^{A}	40.61 ± 0.56^{B}	38.01 ± 0.65^{B}	*
	45	41.91 ± 0.39^{A}	45.43 ± 0.65^{B}	42.98 ± 0.66^{A}	42.16 ± 0.78^{A}	46.43 ± 0.68^{B}	44.22 ± 0.3^{AB}	**
L[b]. Lightness	0	44.06 ± 0.63^{A}	47.11 ± 0.79^{B}	46 ± 1.58^{AB}	46.38 ± 1.35^{AB}	45.49 ± 1.19^{AB}	46.47 ± 1.5^{AB}	*
	10	49.16 ± 0.59^{C}	35.15 ± 1.12^{A}	59.99 ± 1.05^{D}	39.87 ± 0.91^{B}	48.04 ± 0.42^{C}	39.69 ± 1.58^{B}	***
	25	47.46 ± 0.89^{C}	33.04 ± 0.79^{A}	53.92 ± 0.87^{D}	40.6 ± 1.13^{B}	42.3 ± 0.72^{B}	36.01 ± 1.14^{A}	***
	45	47.02 ± 1.03^{C}	40.85 ± 1.1^{B}	37.66 ± 0.95^{AB}	35.45 ± 1.07^{A}	33.36 ± 1.45^{A}	36.44 ± 1.04^{A}	***
a[c]. Red index	0	11.43 ± 0.62^{BC}	9.51 ± 0.27^{A}	18.34 ± 0.32^{D}	10.28 ± 0.59^{AB}	13.67 ± 1.02^{C}	10.36 ± 0.47^{AB}	**
	10	13.72 ± 1.59^{AB}	17.56 ± 0.7^{BC}	11.47 ± 1.27^{A}	18.86 ± 1.41^{C}	13.66 ± 1.15^{A}	17.96 ± 1.33^{BC}	**
	25	11.48 ± 1.32^{A}	16.55 ± 0.69^{B}	10.76 ± 1.36^{A}	16.17 ± 1.41^{AB}	15.72 ± 1.2^{AB}	15.9 ± 1.39^{AB}	**
	45	10.44 ± 1.37^{A}	15.47 ± 0.93^{BC}	15.39 ± 0.69^{BC}	15.9 ± 1.01^{BC}	11.83 ± 1.14^{AB}	18.58 ± 1.18^{C}	**
b[d]. Yellow index	0	15.51 ± 1.24^{A}	12.48 ± 0.65^{A}	13.32 ± 0.15^{A}	14.13 ± 0.47^{A}	14.01 ± 1.15^{A}	12.45 ± 1.23^{A}	N.S.
	10	6.8 ± 1.38^{A}	7.1 ± 1.09^{A}	6.99 ± 0.77^{A}	9.11 ± 1.12^{C}	6.69 ± 0.87^{A}	7.83 ± 0.49^{B}	***
	25	8.32 ± 0.56^{E}	5.43 ± 0.51^{B}	4.12 ± 0.87^{A}	6.41 ± 0.43^{C}	7.72 ± 0.94^{D}	8.59 ± 0.77^{E}	***
	45	3.25 ± 0.6^{A}	6.65 ± 0.89^{C}	8.24 ± 1.03^{D}	6.65 ± 0.7^{C}	5.44 ± 0.8^{B}	8.76 ± 0.8^{D}	***
CS[e] 40%	45	0.06 ± 0.01^{A}	0.06 ± 0.01^{A}	0.06 ± 0.01^{A}	0.09 ± 0.01^{A}	0.07 ± 0.01^{A}	0.05 ± 0.01^{A}	N.S.
CS maximum load	45	0.49 ± 0.01^{A}	0.77 ± 0.01^{A}	0.71 ± 0.01^{A}	0.79 ± 0.01^{A}	0.62 ± 0.01^{A}	0.57 ± 0.01^{A}	N.S.

Results indicate mean values ± standard error of three measurements (carried out in triplicate for two independent productions).
[a] Data within a line followed by the same letter are not significantly different according to Tukey's test. P value: $^{*}P \le 0.05$; $^{**}P \le 0.01$; $^{***}P \le 0.001$; NS, not significant.
[b] L, ranges from 0 (black) to 100 (white).
[c] a, ranges from red (+a) to green (−a).
[d] b, ranges from yellow (+b) to blue (−b).
[e] CS, compressive stress at 40% and at the end of the run.

Chemical composition of full ripened salami is reported in Table 2. Mean values of proteins were slightly higher (about 56.35 g 100 g^{-1} total solids) in trials ST2 and SP2. At the same time, those trials showed the lowest values (about 32.50 g 100 g^{-1} total solids) of fat. No significant statistically differences were found among trials in terms of dry extract, ashes and non-protein nitrogen.

3.2. Microbial counts

The viable counts of microbial groups investigated during salami production are reported in Tables 3 and 4. Minced meats and swine fats showed high concentrations of coccus LAB, pseudomonads, TMC and TPC. Enterococci were not detected into ingredients.

In day 0, all inoculated trails showed a concentration of coccus LAB (on M17 agar) at least 2 log cycles higher than uninoculated samples. Rod LAB was at undetectable levels in all trials, and no staphylococci were found for uninoculated experimentations. During salami fermentation, TPC and TMC values increased in all trials and remained almost constant at approximately 7 log CFU g^{-1} until the end of the ripening period. Rod and coccus LAB showed a similar trend of development and the concentrations were comparable in all trails for the entire period of observation. The highest coccus LAB concentrations were registered for the trials ST1 (7.86 log CFU g^{-1}) and SP1 (7.82 log CFU g^{-1}) at days 10 and 25, respectively. On the other hand, the lowest counts of rod LAB were found at the end of process only for trial ST1 (5.72 log CFU g^{-1}). Significant difference of enterococci counts was found among trials inoculated with starter at day 10; from day 25 onwards, enterococci developed at levels similar to those registered for the trials spontaneously fermented and they remained between 5 and 6 log CFU g^{-1} until the end of production. Yeast concentration significantly differed among samples of both groups of trials during the entire period of observation. This microbial group increased slightly during fermentation until 7.06 log CFU g^{-1}, and the final count was found to be one log cycle lower than that observed for LAB.

CNC level noted for trials inoculated with starter were about 4.5 log CFU g^{-1} at the beginning of experimentation. The highest counts were found for trial ST1 at day 25. After that, CNC decreased to about 3.5 log CFU g^{-1}. High variability in concentration was found among CNC population for the uninoculated trials. Undesired staphylococci (CPS) were at undetectable levels in both groups of trials during the entire period of monitoring.

Pseudomonads were at about 5 log CFU g^{-1} in all trials at the beginning of their investigation and increased at maximum 1 log cycle at the end of the process. In contrast, lower counts were found for the uninoculated trials. *Enterobacteriaceae* decreased to undetectable concentration within day 25 of ripening in all trials.

The presence of pathogens was investigated at the end of the ripening period in all samples, but neither *L. monocytogenes* nor *Salmonella* spp. was detected.

3.3. Isolation, grouping and genetic differentiation of LAB and staphylococci

Seven thousand two hundred and forty-three bacterial cultures were picked up from MRS, M17 and BP agar plates, at highest dilutions, on the basis of appearance (color, morphology, edge, surface and elevation). All isolates were propagated in the corresponding broth media, with the exception of CNC cultures which were cultivated in nutrient broth (NB) applying the same incubation conditions.

All isolates were grouped for the isolation source, sampling time and agar medium. Three-thousands and seventy-five isolates representative of the LAB group were purified and, after microscopic inspection, separated in 2583 cocci and 492 rods. After Gram characterization and catalase testing, 1921 cocci and 342 rods were still considered presumptive LAB cultures, as being Gram-positive and catalase-negative. Nine hundreds and sixty-three presumptive staphylococci were also purified, differentiated and identified.

After the phenotypic characterization, LAB and staphylococcus cultures were separated into five and one groups, respectively (data not shown).

About 30% of the isolates from each phenotypic group were further investigated by RAPD analysis using primer M13 (data no shown). One strain representative per each RAPD profiles was identified at species level by 16S rRNA gene sequencing (Table S1). The sequences were compared by a BLAST search in GenBank/EMBL/DDBJ database, and the 15 strains were clearly identified as *Carnobacterium maltaromaticum*, *Enterococcus devriesei*, *Lactobacillus curvatus*, *Lactobacillus sakei*, *P. pentosaceus* and *S. xylosus*. The BLAST searches produced percentages of identity with sequences available in the NCBI database of at least 97%, which is considered the minimum level of similarity for 16S rRNA genes of two strains belonging to the same species (Scatassa et al., 2015).

The distribution of LAB species among bovine salami during the fermentation and ripening processes is reported in the Table S2. Both starters (*P. pentosaceus* and *S. xylosus*) represented the majority of isolates from the inoculated experimental trials during the entire period of monitoring. Among these samples, *L. curvatus*, *L. sakei* and *E. devriesei* were also detected during the ripening (days 25 and 45) of salami. On the other hand, *L. curvatus* and *L. sakei* were mainly isolated during the fermentation phase (days 25 and 45) of samples not added with LAB starter. The species *C. maltaromaticum* was detected only in trial ST2 at the beginning (day 0) of salami manufacturing.

3.4. Analysis of VOCs and sensory evaluation

The results of VOC analyses carried out on salami samples at day 45 are reported in Table 5. Acids, esters and ketones were detected at the highest mainly for salami samples that were spontaneously fermented. In contrast, samples inoculated with starters showed high concentration of aldehydes. In detail, the acids were mainly represented by acetic, isobutyric and 2-ethyl-hexanoic acids that

Table 2
Chemical parameters of bovine salami at the end of ripening (day 45).

Chemical parameters	Trials						Statistical significance[b]
	ST1	ST2	ST3	SP1	SP2	SP3	
Dry extract[a]	62.99 ± 0.32[D]	58.88 ± 0.57[AB]	58.23 ± 0.40[A]	62.86 ± 0.57[D]	61.79 ± 0.32[CD]	60.34 ± 0.57[BC]	*
Ashes[a]	9.34 ± 0.06[A]	11.21 ± 0.10[D]	10.93 ± 0.10[CD]	9.62 ± 0.10[A]	10.49 ± 0.09[B]	10.79 ± 0.10[BC]	*
Total nitrogen[a]	8.13 ± 0.12[AB]	9.54 ± 0.24[D]	8.87 ± 0.16[BC]	7.67 ± 0.17[A]	9.04 ± 0.05[C]	8.41 ± 0.09[B]	*
Proteins[a]	49.07 ± 0.17[A]	56.25 ± 0.54[C]	53.27 ± 0.38[B]	49.42 ± 0.24[A]	56.49 ± 0.10[C]	51.53 ± 0.51[B]	**
Fat[a]	41.66 ± 0.51[D]	32.07 ± 0.44[A]	35.92 ± 0.54[B]	41.27 ± 0.44[D]	33.17 ± 0.40[A]	38.03 ± 0.10[C]	***
Not protein nitrogen[a]	1.05 ± 0.03[A]	1.17 ± 0.16[A]	1.42 ± 0.16[A]	1.05 ± 0.02[A]	1.14 ± 0.05[A]	1.01 ± 0.05[A]	NS
Not-protein nitrogen/total nitrogen	0.13 ± 0.02[A]	0.12 ± 0.01[A]	0.16 ± 0.02[A]	0.14 ± 0.02[A]	0.12 ± 0.03[A]	0.12 ± 0.01[A]	NS

Results indicate mean values ± standard error of three measurements (carried out in triplicate for two independent productions).
[a] 100 g^{-1} total solids.
[b] Data within a line followed by the same letter are not significantly different according to Tukey's test. *P* value: *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$; NS, not significant.

Table 3
Microbial loads of meat and ingredients used to produce experimental bovine salame[a].

Ingredients	Media[b]							
	PCA-7 °C	PCA-30 °C	VRBGA	PAB	BP	M17	MRS	YGC
Minced meat from cow-120 m	5.38 ± 0.09	5.00 ± 0.09	2.00 ± 0.07	5.71 ± 0.09	n.d.	7.56 ± 0.07	n.d.	5.42 ± 0.09
Minced meat from calf-18 m-g	n.d.	6.36 ± 0.06	4.00 ± 0.06	6.48 ± 0.08	n.d.	6.65 ± 0.06	3.23 ± 0.07	5.01 ± 0.06
Minced meat from calf-18 m-b	6.10 ± 0.06	6.11 ± 0.08	3.15 ± 0.09	6.44 ± 0.06	n.d.	5.78 ± 0.09	3.26 ± 0.07	4.46 ± 0.07
LAB starter	4.85 ± 0.13	8.70 ± 0.07	n.d.	3.48 ± 0.23	n.d.	7.70 ± 0.10	8.34 ± 0.07	n.d.
Swine fat	7.03 ± 0.08	7.04 ± 0.06	n.d.	6.35 ± 0.06	2.48 ± 0.10	5.89 ± 0.09	2.00 ± 0.06	4.18 ± 0.13
Pepper	n.d.	1.87 ± 0.17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Salt mixture	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Casing	4.74 ± 0.13	5.70 ± 0.08	n.d.	4.60 ± 0.13	n.d.	2.99 ± 0.07	1.90 ± 0.06	4.54 ± 0.07

Abbreviations: PCA-7 °C, plate count agar incubated at 7 °C for total psychrotrophic counts; PCA-30 °C, plate count agar incubated at 30 °C for total mesophilic counts; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; PAB, pseudomonas agar base for pseudomonads; BP, Baird Parker only for negative coagulase cocci; M17 agar for mesophilic coccus LAB; MRS, de Man Rogosa Sharpe agar for mesophilic rod LAB; YGC. yeast glucose chloramphenicol agar for yeasts; n.d. not determined (value < detectable limit of method).

[a] Units are log (CFU g^{-1}) for all ingredients. Results indicate mean values ± standard error of three plate counts (carried out in triplicate for two independent productions).

[b] Results of microbial counts on KAA kanamycin aesculin azide agar for enterococci agar have been not reported because they were at level lower than detectable limit of method in all samples.

reached the highest concentration in trial SP1. At the same time the trial SP1 showed also the highest concentrations of ethyl octanoate and ethyl decanoate. High concentration of isoamyl acetate and methyl hexanoate was found in salami of trial SP3. The main compounds within the class of aldehyde were 1-octanal and nonanal that were mainly found in trial ST2. Alcohols were slightly higher

Table 4
Microbial loads[a] of bovine salami during fermentation and ripening.

Media	Trials						Statistical significance[b]
	ST1	ST2	ST3	SP1	SP2	SP3	
Day 0							
PCA-7 °C	5.04 ± 0.06^{B}	4.34 ± 0.07^{A}	4.32 ± 0.06^{A}	5.13 ± 0.06^{B}	4.34 ± 0.05^{A}	4.33 ± 0.07^{A}	*
PCA-30 °C	5.07 ± 0.06^{B}	4.10 ± 0.06^{A}	5.86 ± 0.06^{D}	5.11 ± 0.17^{BC}	4.22 ± 0.08^{A}	5.56 ± 0.08^{CD}	***
KAA	4.14 ± 0.46^{B}	4.31 ± 0.06^{BC}	4.06 ± 0.29^{AB}	4.14 ± 0.52^{B}	4.67 ± 0.01^{C}	3.60 ± 0.08^{A}	**
PAB	4.70 ± 0.40^{AB}	5.49 ± 0.10^{C}	4.98 ± 0.14^{B}	4.26 ± 0.07^{A}	5.51 ± 0.08^{C}	4.60 ± 0.07^{AB}	**
BP	4.36 ± 0.06^{A}	4.40 ± 0.17^{A}	4.29 ± 0.40^{A}	n.d.	n.d.	n.d.	NS
VRBGA	1.73 ± 0.06^{AB}	2.83 ± 0.12^{C}	2.01 ± 0.30^{B}	1.50 ± 0.13^{A}	3.21 ± 0.07^{C}	2.20 ± 0.36^{B}	***
M17	7.27 ± 0.06^{B}	6.96 ± 0.07^{B}	7.11 ± 0.29^{B}	4.23 ± 0.08^{A}	4.13 ± 0.07^{A}	4.56 ± 0.46^{A}	***
MRS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
YGC	4.08 ± 0.06^{A}	4.31 ± 0.06^{A}	5.14 ± 0.07^{B}	4.32 ± 0.08^{A}	3.90 ± 0.08^{A}	5.04 ± 0.07^{B}	**
Day 10							
PCA-7 °C	7.23 ± 0.06^{AB}	6.84 ± 0.29^{A}	7.44 ± 0.12^{B}	7.97 ± 0.17^{C}	7.92 ± 0.17^{C}	8.28 ± 0.46^{D}	***
PCA-30 °C	7.92 ± 0.29^{B}	7.46 ± 0.29^{A}	7.27 ± 0.29^{A}	8.64 ± 0.40^{D}	8.40 ± 0.40^{CD}	8.63 ± 0.06^{D}	**
KAA	6.43 ± 0.12^{C}	5.63 ± 0.40^{B}	6.53 ± 0.29^{C}	5.08 ± 0.40^{A}	5.23 ± 0.07^{AB}	5.22 ± 0.40^{AB}	***
PAB	5.09 ± 0.06^{C}	4.73 ± 0.29^{BC}	4.88 ± 0.40^{C}	3.66 ± 0.46^{A}	4.39 ± 0.07^{B}	4.35 ± 0.29^{B}	***
BP	5.09 ± 0.29^{A}	4.73 ± 0.40^{A}	4.88 ± 0.14^{A}	4.65 ± 0.23^{A}	4.77 ± 0.17^{A}	n.d.	NS
VRBGA	1.19 ± 0.14^{A}	1.42 ± 0.22^{A}	1.31 ± 0.24^{A}	1.28 ± 0.10^{A}	2.00 ± 0.21^{B}	1.24 ± 0.17^{A}	*
M17	7.86 ± 0.06^{C}	7.72 ± 0.29^{BC}	7.77 ± 0.06^{BC}	7.19 ± 0.40^{A}	7.13 ± 0.06^{A}	7.31 ± 0.23^{AB}	*
MRS	7.58 ± 0.23^{AB}	7.17 ± 0.40^{A}	7.53 ± 0.07^{AB}	7.68 ± 0.06^{B}	7.69 ± 0.17^{B}	7.68 ± 0.35^{B}	*
YGC	4.51 ± 0.06^{A}	5.74 ± 0.29^{BC}	6.84 ± 0.40^{D}	5.38 ± 0.46^{B}	4.86 ± 0.17^{A}	6.02 ± 0.17^{C}	***
Day 25							
PCA-7 °C	7.65 ± 0.17^{C}	7.43 ± 0.06^{BC}	8.78 ± 0.29^{D}	7.04 ± 0.23^{AB}	6.77 ± 0.40^{A}	6.84 ± 0.06^{A}	***
PCA-30 °C	7.82 ± 0.29^{B}	7.55 ± 0.29^{AB}	7.76 ± 0.10^{B}	7.49 ± 0.02^{AB}	7.15 ± 0.06^{A}	7.36 ± 0.07^{AB}	*
KAA	5.70 ± 0.12^{B}	5.13 ± 0.18^{A}	5.80 ± 0.07^{B}	5.14 ± 0.07^{A}	5.37 ± 0.23^{AB}	5.56 ± 0.06^{AB}	*
PAB	5.40 ± 0.23^{D}	4.89 ± 0.13^{C}	4.73 ± 0.17^{C}	3.39 ± 0.01^{A}	3.92 ± 0.23^{B}	4.16 ± 0.40^{B}	***
BP	5.40 ± 0.17^{C}	4.89 ± 0.29^{B}	4.73 ± 0.29^{B}	5.18 ± 0.06^{BC}	3.26 ± 0.06^{A}	5.04 ± 0.17^{BC}	***
VRBGA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
M17	7.61 ± 0.06^{A}	7.35 ± 0.18^{A}	7.46 ± 0.07^{A}	7.81 ± 0.46^{A}	7.57 ± 0.52^{A}	7.49 ± 0.10^{A}	NS
MRS	7.26 ± 0.29^{BC}	7.46 ± 0.06^{CD}	7.76 ± 0.40^{D}	6.55 ± 0.07^{A}	6.99 ± 0.06^{AB}	7.45 ± 0.07^{CD}	**
YGC	6.07 ± 0.06^{A}	5.86 ± 0.14^{A}	6.08 ± 0.06^{A}	6.88 ± 0.05^{B}	6.87 ± 0.52^{B}	7.06 ± 0.40^{B}	**
Day 45							
PCA-7 °C	7.19 ± 0.06^{B}	7.21 ± 0.10^{B}	7.19 ± 0.40^{B}	6.49 ± 0.12^{A}	6.03 ± 0.12^{A}	6.40 ± 0.52^{A}	**
PCA-30 °C	7.16 ± 0.17^{AB}	7.24 ± 0.18^{B}	7.19 ± 0.40^{B}	7.09 ± 0.23^{AB}	6.72 ± 0.29^{A}	6.93 ± 0.17^{AB}	*
KAA	5.39 ± 0.06^{A}	5.46 ± 0.06^{A}	5.36 ± 0.06^{A}	5.64 ± 0.06^{A}	5.58 ± 0.23^{A}	5.67 ± 0.29^{A}	NS
PAB	6.21 ± 0.17^{E}	5.55 ± 0.18^{D}	4.55 ± 0.06^{C}	2.94 ± 0.40^{A}	3.05 ± 0.17^{A}	3.73 ± 0.40^{B}	***
BP	3.40 ± 0.17^{A}	3.54 ± 0.06^{A}	3.82 ± 0.12^{A}	n.d.	n.d.	n.d.	NS
VRBGA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
M17	7.22 ± 0.12^{B}	6.34 ± 0.06^{A}	6.59 ± 0.23^{A}	6.80 ± 0.12^{B}	6.67 ± 0.17^{A}	6.36 ± 0.17^{A}	*
MRS	5.72 ± 0.17^{A}	6.32 ± 0.17^{B}	6.51 ± 0.12^{B}	6.80 ± 0.23^{BC}	7.01 ± 0.17^{C}	7.03 ± 0.06^{C}	**
YGC	6.48 ± 0.06^{A}	6.60 ± 0.18^{A}	6.74 ± 0.17^{A}	6.67 ± 0.06^{A}	6.69 ± 0.52^{A}	6.35 ± 0.52^{A}	NS

Abbreviations: PCA-7 °C, plate count agar incubated at 7 °C for total psychrotrophic counts; PCA-30 °C, plate count agar incubated at 30 °C for total mesophilic counts; KAA, kanamycin aesculin azide agar for enterococci; PAB, Pseudomonas agar base for pseudomonads; BP, Baird Parker only for negative coagulase cocci; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; M17 agar for mesophilic coccus LAB; MRS, de Man Rogosa Sharpe agar for mesophilic rod LAB; YGC, yeast glucose chloramphenicol agar for yeasts; n.d., not determined (value < detectable limit of method).

[a] Units are log (CFU g^{-1}) for all samples. Results indicate mean values ± standard error of three plate counts (carried out in triplicate for two independent productions).

[b] Data within a line followed by the same letter are not significantly different according to Tukey's test. *P* value: *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$; NS, not significant.

Table 5
Analysis of volatile organic compounds (VOCs) at the end of ripening (45 day) of bovine salami.

Chemical compounds[a]	Trials					
	ST1	ST2	ST3	SP1	SP2	SP3
Isoamyl acetate	26.27 ± 1.22	21.81 ± 1.87	20.89 ± 1.56	40.52 ± 3.42	28.60 ± 2.65	45.02 ± 3.15
Methyl hexanoate	8.78 ± 0.32	70.31 ± 5.95	76.08 ± 6.17	53.50 ± 4.42	85.71 ± 6.95	118.65 ± 10.08
Isoamyl alcohol	262.42 ± 9.10	215.36 ± 16.91	218.94 ± 14.44	234.62 ± 19.87	130.24 ± 10.21	154.16 ± 11.54
Ethyl hexanoate	10.99 ± 0.47	16.44 ± 1.27	18.55 ± 1.61	16.44 ± 1.49	10.75 ± 0.93	29.09 ± 2.11
1-Octanal	52.35 ± 1.92	166.13 ± 12.41	n.d.	n.d.	n.d.	n.d.
Ethyl lactate	24.51 ± 1.74	n.d.	n.d.	32.60 ± 2.46	n.d.	n.d.
Methyl octanoate	n.d.	21.18 ± 1.94	24.84 ± 2.13	19.01 ± 1.81	19.62 ± 1.79	18.40 ± 1.61
Nonanal	53.22 ± 2.42	138.09 ± 15.63	49.63 ± 3.97	40.79 ± 4.31	34.71 ± 3.27	90.04 ± 8.37
Ethyl octanoate	27.66 ± 1.68	21.14 ± 1.91	18.00 ± 1.61	49.59 ± 3.19	21.29 ± 1.49	29.96 ± 3.09
Acetic acid	241.53 ± 11.82	240.86 ± 24.17	308.38 ± 27.26	695.12 ± 7.19	224.03 ± 21.51	257.10 ± 26.27
1-Octen-3-ol	n.d.	14.18 ± 1.17	15.81 ± 1.67	23.30 ± 2.31	12.28 ± 1.11	215.11 ± 23.31
Decanal	n.d.	72.13 ± 6.55	16.78 ± 2.03	61.95 ± 5.97	n.d.	n.d.
Benzaldehyde	15.92 ± 1.07	20.53 ± 2.43	17.20 ± 2.01	27.95 ± 3.15	23.34 ± 2.19	31.09 ± 2.95
Propionic acid	n.d.	n.d.	n.d.	10.85 ± 0.91	4.14 ± 0.55	5.07 ± 0.39
2,3-Butanediol	n.d.	25.57 ± 2.95	24.62 ± 2.09	n.d.	n.d.	n.d.
1-Octanol	11.53 ± 0.43	21.48 ± 2.61	9.86 ± 0.81	8.73 ± 0.63	10.65 ± 1.17	24.35 ± 3.03
Isobutyric acid	n.d.	12.93 ± 1.11	n.d.	51.02 ± 2.28	19.86 ± 1.27	n.d.
Butanoic acid	9.14 ± 0.48	9.20 ± 0.78	12.24 ± 0.99	24.47 ± 3.13	9.24 ± 0.69	14.80 ± 1.47
Acetophenon	8.12 ± 0.33	8.38 ± 0.41	8.73 ± 0.61	9.60 ± 0.99	8.95 ± 0.27	10.83 ± 1.41
Ethyl decanoate	60.45 ± 3.70	37.14 ± 3.99	38.65 ± 2.84	83.30 ± 9.19	32.47 ± 4.11	47.60 ± 5.49
Ethyl dodecanoate	5.77 ± 0.21	7.58 ± 0.73	9.93 ± 0.63	14.33 ± 1.25	14.04 ± 1.65	14.39 ± 1.55
Hexanoic acid	14.09 ± 1.15	7.78 ± 1.19	6.56 ± 0.27	11.83 ± 2.03	8.42 ± 1.11	8.65 ± 0.93
Phenylethyl alcohol	43.65 ± 2.05	61.51 ± 7.15	61.15 ± 6.61	78.47 ± 8.15	62.50 ± 7.41	59.99 ± 7.19
2-Ethyl-hexanoic acid	27.38 ± 1.26	20.19 ± 1.81	27.58 ± 2.43	31.97 ± 2.97	16.80 ± 1.27	16.06 ± 2.15
Phenol	17.84 ± 0.69	17.28 ± 1.55	15.86 ± 1.95	19.26 ± 2.19	16.77 ± 1.91	15.64 ± 1.77
Octanoic acid	2.54 ± 0.16	5.84 ± 0.61	3.55 ± 0.47	6.88 ± 0.97	2.56 ± 0.19	4.65 ± 0.31
1-(3-Ethylphenyl) ethanone	17.87 ± 1.14	21.57 ± 2.05	17.83 ± 1.81	31.16 ± 2.94	28.07 ± 2.41	31.08 ± 3.11
Methyl tridecanoate	8.61 ± 0.44	11.48 ± 1.09	8.66 ± 0.71	10.95 ± 0.91	13.64 ± 1.27	24.90 ± 2.44

Results indicate mean values ± standard error of three measurements (carried out in triplicate for two independent productions) and are expressed (in mg kg^{-1}) as 4-methyl-2-pentanone.
n.d., not detected.
[a] The chemicals are shown following their retention time.

in trials spontaneously fermented such as SP3 that showed the highest concentration of 1-octen-3-ol and 1-octanol.

The results of the sensory analysis are reported in Table 6. Significant ($P < 0.05$) differences were mainly found among the trials performed with different meats. The main differences were estimated in terms of color uniformity and intensity, flavor intensity and overall acceptability. Those scores were registered at high values mainly for trials SP1 and ST1. In details, trial SP1 showed the highest values both for flavor intensity and overall acceptability, as well as for chewiness. In contrast, trials ST2 and SP2 showed high scores for off-flavors (rancid and mold) as well as the lowest for elasticity descriptors.

Table 6
Sensory characteristics of bovine salami at the end of ripening (day 45).

Attributes	Salami samples						Statistical significance[a]
	ST1	ST2	ST3	SP1	SP2	SP3	
Color uniformity	6.72 ± 0.08^{B}	5.78 ± 0.06^{A}	5.66 ± 0.05^{A}	6.88 ± 0.05^{B}	6.02 ± 0.07^{A}	5.98 ± 0.10^{A}	**
Color intensity	6.10 ± 0.01^{CD}	5.54 ± 0.08^{AB}	5.48 ± 0.13^{AB}	6.46 ± 0.02^{D}	5.37 ± 0.02^{A}	5.90 ± 0.09^{BC}	**
Fat/lean connection	5.76 ± 0.05^{B}	5.11 ± 0.12^{A}	5.04 ± 0.06^{A}	5.92 ± 0.07^{B}	5.00 ± 0.03^{A}	5.24 ± 0.04^{A}	**
Fat/lean distribution	4.09 ± 0.02^{A}	4.04 ± 0.06^{A}	3.98 ± 0.01^{A}	3.74 ± 0.02^{A}	3.79 ± 0.08^{A}	3.90 ± 0.01^{A}	NS
Acid flavor	1.00 ± 0.01^{A}	1.16 ± 0.06^{A}	1.08 ± 0.13^{A}	1.02 ± 0.13^{A}	1.19 ± 0.08^{A}	1.11 ± 0.04^{A}	NS
Rancid flavor	0.27 ± 0.01^{A}	1.10 ± 0.06^{B}	0.20 ± 0.12^{A}	0.24 ± 0.12^{A}	0.22 ± 0.10^{A}	0.23 ± 0.01^{A}	*
Mold flavor	0.86 ± 0.08^{A}	1.12 ± 0.10^{A}	1.45 ± 0.06^{B}	1.00 ± 0.05^{A}	1.10 ± 0.04^{A}	1.31 ± 0.03^{A}	*
Lactic flavor	3.84 ± 0.01^{B}	3.83 ± 0.04^{B}	4.33 ± 0.18^{C}	3.34 ± 0.08^{A}	3.13 ± 0.02^{A}	3.20 ± 0.10^{A}	**
Bitter	0.86 ± 0.04^{A}	0.94 ± 0.08^{A}	0.99 ± 0.12^{A}	0.66 ± 0.14^{A}	0.96 ± 0.02^{A}	1.12 ± 0.01^{A}	NS
Salty	2.97 ± 0.01^{AB}	3.00 ± 0.10^{AB}	2.62 ± 0.06^{A}	2.86 ± 0.04^{AB}	3.04 ± 0.06^{AB}	3.11 ± 0.04^{B}	*
Flavor intensity	7.26 ± 0.01^{B}	6.51 ± 0.16^{A}	6.42 ± 0.08^{A}	7.83 ± 0.09^{C}	7.25 ± 0.04^{B}	7.32 ± 0.07^{B}	**
Elasticity	4.36 ± 0.10^{B}	4.06 ± 0.02^{B}	3.52 ± 0.08^{A}	4.24 ± 0.02^{B}	4.02 ± 0.02^{B}	3.52 ± 0.09^{A}	**
Hardness	3.72 ± 0.05^{A}	3.99 ± 0.10^{A}	3.88 ± 0.05^{A}	3.90 ± 0.09^{A}	4.02 ± 0.12^{A}	3.70 ± 0.09^{A}	NS
Chewiness	4.82 ± 0.01^{C}	4.64 ± 0.16^{BC}	4.33 ± 0.08^{B}	5.62 ± 0.01^{D}	4.52 ± 0.02^{BC}	3.62 ± 0.06^{A}	***
Juiciness	4.00 ± 0.04^{A}	4.14 ± 0.12^{A}	4.13 ± 0.12^{A}	4.32 ± 0.07^{A}	4.10 ± 0.02^{A}	3.97 ± 0.18^{A}	NS
Fattiness	3.72 ± 0.06^{A}	4.00 ± 0.13^{AB}	3.88 ± 0.05^{AB}	4.11 ± 0.06^{AB}	4.25 ± 0.13^{B}	3.93 ± 0.10^{AB}	*
Overall acceptability	7.12 ± 0.04^{CD}	6.31 ± 0.01^{AB}	6.05 ± 0.08^{A}	7.52 ± 0.06^{D}	6.82 ± 0.09^{C}	6.71 ± 0.01^{BC}	***

Results indicate mean values ± standard error of three replicate analyses (carried out in triplicate for two independent productions).
NS, not significant.
[a] Data within a line followed by the same letter are not significantly different according to Tukey's test.
* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

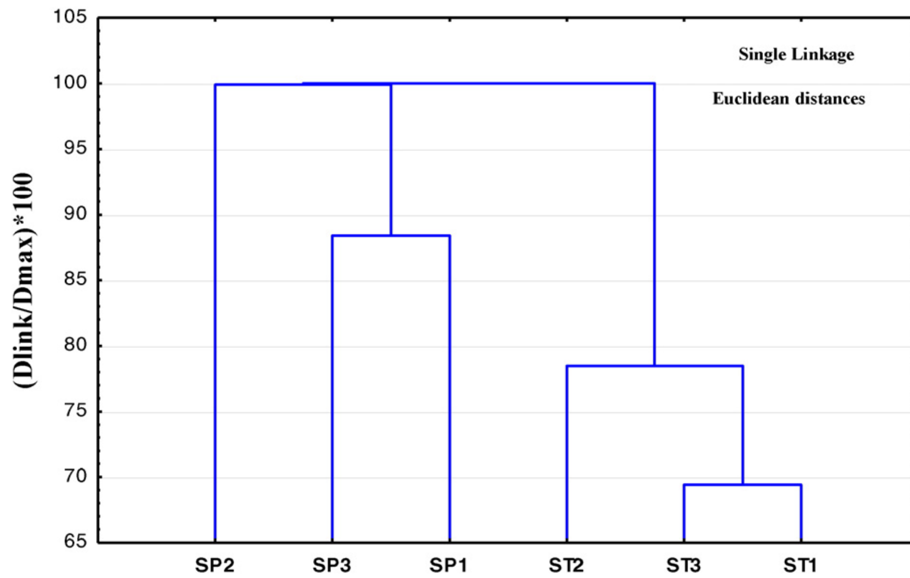

Fig. 2. Dendrogram of bovine salami samples resulting from HCA analysis based on values of physico-chemical (pH, a_w, color and texture) microbiological and VOCs changes. The dissimilarity among samples was measured by Euclidean distance, whereas cluster aggregation was achieved by single linkage. Abbreviations: SP and ST from 1 to 3, correspond to codes used for the experimental trials (see Fig. 1 for details). Results indicate mean values of three replicate per each trials (carried out in triplicate for two independent productions).

3.5. Statistical and explorative multivariate analysis

The analysis of variance over the two productions performed during two consecutive weeks showed no significant effect between weeks and the dependent variables corresponding to physico-chemical, microbiological data, VOCs and sensory scores.

HCA differentiated trials in accordance with their mutual dissimilarity and relationship by using 21 variables selected on the basis of results from physico-chemical, microbiological data and VOCs (Fig. 2). In detail, all trials were classified in two mega clusters on the basis of method of fermentation. Furthermore, within each mega cluster all trials were separated on the basis of type of raw meat used to produce experimental salami. The dissimilarity found among trials spontaneously fermented was significantly higher than that detected for the inoculated trials. In particular, trial SP2 showed the highest values of mutual dissimilarity; in contrast the lowest was found for trials ST1 and ST3 both inoculated with starter.

The results obtained by monitoring physico-chemical, microbiological data and VOCs data were also subjected to PCAn (Fig. 3). The correlation among variables and component of PCAn are shown in Fig. 3A, and the corresponding values of factor coordinates of the variables are reported in Table S3. The discrimination of trials can be visualized in

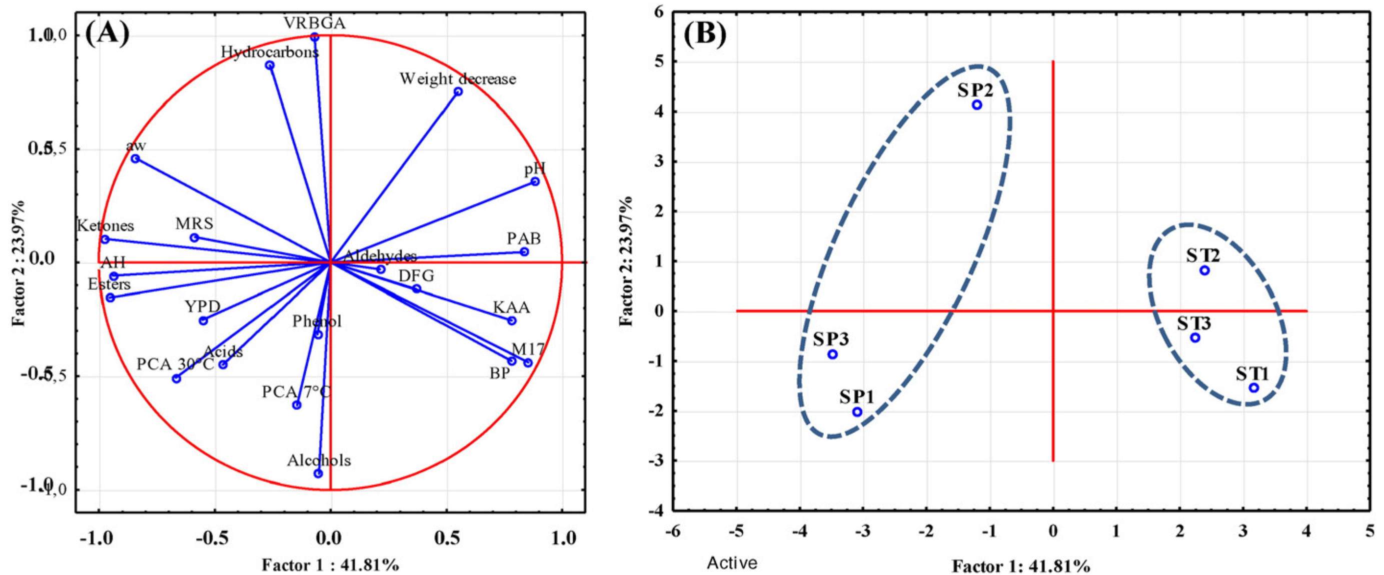

Fig. 3. PCA analysis [A (loading plot); B (score plot)] based on the values of physico-chemical (pH, a_w, color and texture) microbiological and VOCs changes estimated during the experimental salami productions. Abbreviations in Figure A: PCA-7 °C, plate count agar incubated at 7 °C for total psychrotrophic counts; PCA-30 °C, plate count agar incubated at 30 °C for total mesophilic counts; KAA, kanamycin aesculin azide agar for enterococci; PAB, Pseudomonas agar base for pseudomonads; BP, Baird Parker only for negative coagulase cocci; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; M17 agar for mesophilic coccus LAB; MRS, de Man Rogosa Sharpe agar for mesophilic rod LAB; YGC, yeast glucose chloramphenicol agar for yeasts; DFG, diverse functional groups; AH, aromatic hydrocarbons. Abbreviations in Figure B: SP and ST from 1 to 3, correspond to codes used for the experimental trials (see Fig. 1 for details). Results indicate mean values of three replicate per each trials (carried out in triplicate for two independent productions). Circles have been only reported to display the distribution of trials onto graphic representation of loading plot of Fig. 3B.

the plot of the scores (Fig. 3B). The trials were significantly separated along Factor 1 on the basis of fermentation method, and also by this type of analysis, the trial SP2 was significantly separated from the other trials. These results confirmed those obtained by HCA.

The PCAn was used to analyze also sensory profiles of full ripened salami (day 45). To this purpose three biplot graphs were constructed as illustrated in Fig. 4. The six trials were separated mainly along the F1 component. Trials SP1 and ST1 were closely and positively related to appearance attributes, as well as to flavor intensity and overall acceptability.

The Barlett's sphericity test was applied to all data matrix inputs and differences statistically significant ($P < 0.001$) were found among trials.

4. Discussion

Several productions based on the use of different microbiological and physical parameters have been proposed to produce salami, but, up to now, none of them has been carried out by using meat from bovine breed. The intrinsic properties of meat could affect the growth and survival of several (pro-technological and/or spoilage and/or pathogenic) microbial groups and, mainly, the chemical organic compositions, as well as the sensory properties of final product.

The evolution of physico-chemical parameters in all trials followed trends commonly reported for fermented meat products (Cenci-Goga, Ranucci, Miraglia, & Cioffi, 2008; Cenci-Goga et al., 2012; Francesca et al., 2013). Both groups (inoculated and uninoculated) of salami reached similar values of pH endpoints. Generally, the inoculum of LAB starter provides a rapid decrease of pH thanks to the production of lactic acid inhibiting the growth of several unwanted (spoilage and/or pathogenic) microorganisms. The pH decrease is crucial to assure the hygienic safety of fermented meat products (Lücke, 1998; Työppönen, Petäjä, & Mattila-Sandholm, 2003). A pH lower than 5.2 is mandatory to carry out "shelf-stable" meat products such as sausages and salami (Ambrosiadis, Soultos, Abrahim, & Bloukas, 2003). On the other hand, several work performed on other meat foods reported pH higher than 5.2 (Aymerich, Martín, Garriga, & Hugas, 2003; Barbut, 2006; Urso, Comi, & Cocolin, 2006). Furthermore, other studies clearly showed that non-acid salami productions, characterized by pH endpoints higher than 6.0 are quite common in some Italian regions (D'Ascenzi, Nuvoloni, Pedonese, & Rindi, 2005; Forzale, Nuvoloni, Pedonese, D'Ascenzi, & Giorgi, 2011; Severini et al., 1999) because of intense proteolytic activities of microorganisms might increase final values of pH. At the same time, it should be stressed that microbial hygienic safety of salami could be assured by inhibitory compounds other than acids (Francesca et al., 2013; Moschetti, Blaiotta, Villani, & Coppola, 2001; Villani et al., 1994).

The final values of weight loss were statistically different for the six formulations. These results demonstrate the large variations in the processing of fermented salami, which may be due to formulations, raw materials and processing, among other factors. However, the weight

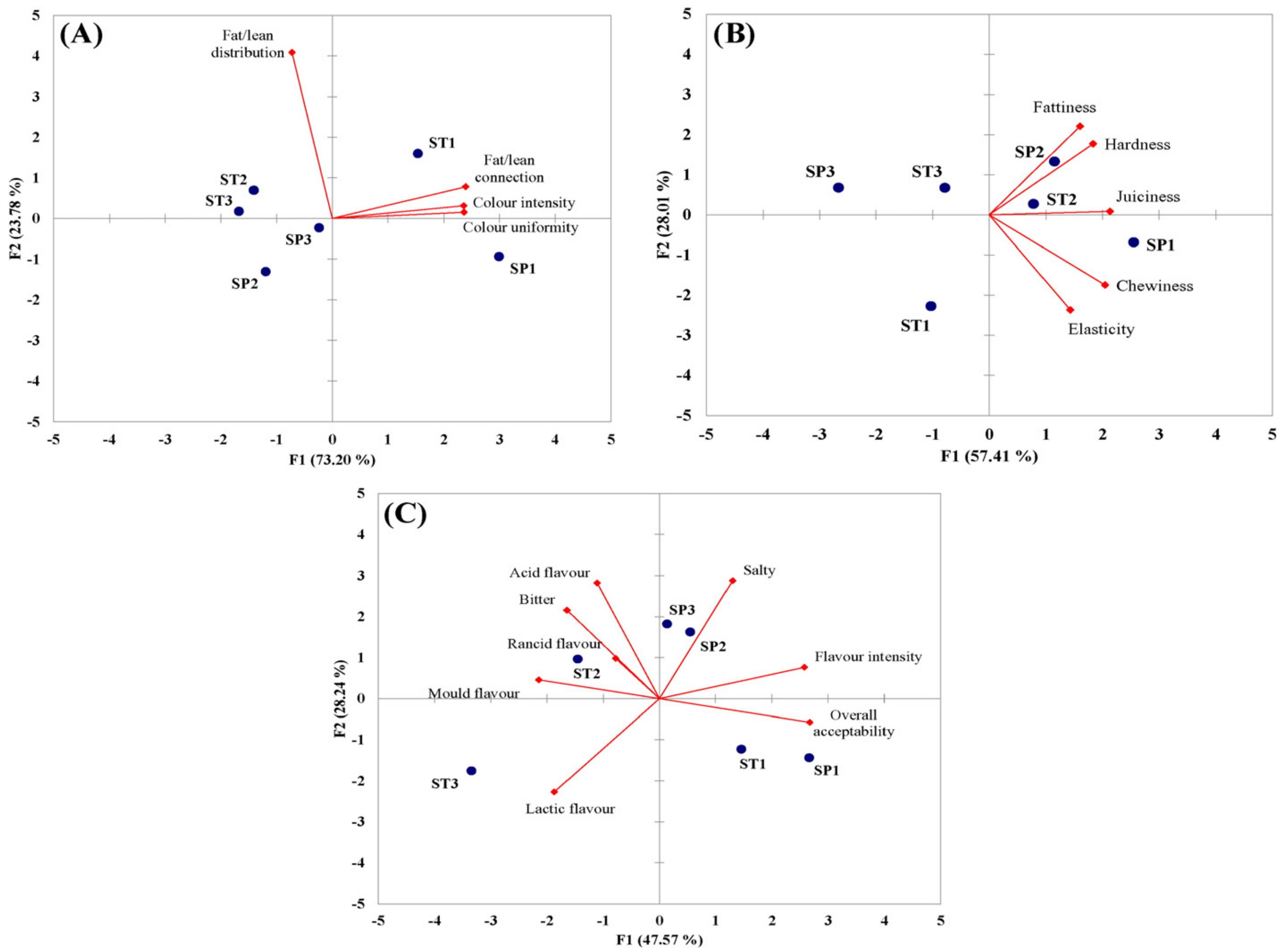

Fig. 4. PCA for sensory data of salami at the end of process (45 day). Biplot graphs show relationships among factors, variables and trials. (A, appearance attributes; B, texture attributes; C, basic tastes, odor, aroma and overall acceptability). Results indicate mean values of three replicate per each trials (carried out in triplicate for two independent productions). Abbreviations: SP and ST from 1 to 3, correspond to codes used for the experimental trials (see Fig. 1 for details).

loss values reached at the end of the drying period were (40 to 46%) slightly higher than those reported by Rust (1994), that are considered ideal.

Microbiological results evidenced a substantial concentration of LAB populations during the entire salami manufacturing. Although, one group of trials was spontaneously fermented, LAB evolution during the entire process was superimposable to that registered for inoculated trials. In accordance to other works (Bonomo, Ricciardi, & Salzano, 2011; Giraffa, 2002), our raw meat was contaminated by LAB detected at high concentration, probably as consequence of microbial contamination due to intestinal gutting of animals.

At the beginning of process, not inoculated thesis showed CNC counts significantly lower than that registered for the inoculated trials; probably, too rapid increase of LAB population, thus an intense acidification of samples, inhibited and/or slowed the CNC growth (Francesca et al., 2013; Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003). Contradictory results have been reported on the role and concentration of *Enterococcus* populations during salami production. However, several studies showed the ability of both enterococci and CNC to influence the flavor of final product by proteolysis and lipolysis (Talon, Walter, Chartier, Barriere, & Montel, 1999), as well as in the case of CNC to participate in the stability of salami red color (Toldrà, 2008).

As noted in both groups of our trials, the rapid increase of LAB, enterococci and CNC populations, in combination to nitrite and nitrate salt effect, significantly inhibited the growth both of spoilage and pathogenic microorganisms. Interestingly, *Enterobacteriaceae* were found at very low concentration during salami ripening, and they decreased rapidly up to uncountable level after day 10 both for inoculated and uninoculated trials. Although, one group of experimental trials was spontaneously fermented, it is worth noting that *Listeria* spp. and *Salmonella* spp. were never detected during the entire meat process.

The microbial investigation on experimental bovine salami were also performed by species identification of coccus and rod LAB, as well as CNC, isolated at the highest concentration during the entire process. All species identified in the present study are commonly detected in raw meat before to be processed and during productions of fermented meats (Francesca et al., 2013). Both *L. curvatus* and *L. sakei* are the most frequently isolated LAB from fermented meat products and, in particular, *L. sakei*, is considered to be one of the best choices for further use as a starter culture in fermented meats (Aymerich et al., 2005). Interestingly, strains of *L. curvatus* and *L. sakei* are reported to be protective culture in the biopreservation of meat products because they were found to be bacteriocinogenic by generating small peptides and amino acids from hydrolysis of muscle sarcoplasmic and myofibrillar proteins (Fadda, Vignolo, Ruiz Holgado, & Oliver, 1998; Fadda et al., 1999).

Explorative multivariate analysis has been widely applied in food processes (Berrueta, Alonso-Salces, & Héberger, 2007; Martorana et al., 2015a). Results from HCA and PCAn clearly showed a significant effect of type of meat on salami composition. Although salami produced with the same type of fermentation were closely in terms of microbiological and chemical data, the type of meat mainly effect the sensory profile of full ripened salami. Interestingly, independently on the starter inoculum, the use of meat from cow 120 months positively affected flavor and overall acceptability of full ripened salami.

In conclusion, our study provided, for the first time, an extended overview on microbial ecology and chemical parameters of salami manufactured with meat of cow on retirement, beef and young bull representing three different commercial categories of bovine breed. The results showed the possibility of using cattle meat for salami production, in particular using cow on retirement meat, which represents a valuable style of products with high sensorial characteristics. Experimental salami produced with meat of cow on retirement, beef and young bull were hygienically safe, since no pathogenic bacteria were found in any samples.

The goal of the present study was to show that cattle meat might be a commercial alternative for the meat of autochthonous breed appreciated on the market. The formulation of salami with indigenous breed meat (Cinisara and Suino Nero dei Nebrodi) can be used for the production of a traditional food. This production strategy could be particularly appreciated by consumers of "traditional/typical and niche" foods (Settanni & Moschetti, 2014) and, at the same time, it might be an interesting alternative for the enhancement of the Cinisara meat. In recent years, typical products of high quality, linked to place of origin, show, indeed, a growing appreciation by consumers (Ciotola et al., 2009).

Further investigations will be carried out for a more thorough validation of this manufacturing method for salami considering also the addition of other local ingredients, to a greater characterization of the products, tying them to the origin territory.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meatsci.2015.12.014.

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