

Research Article

Chemical and Microbiological Characterization for PDO Labelling of Typical East Piedmont (Italy) Salami

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This study is focused on the characterisation of typical salami produced in Alessandria province (North West of Italy). Seventeen small or medium salami producers from this area were involved in the study and provided the samples investigated. The aim is double and consists in obtaining a screening of the characteristics of different products and following their evolution along ripening. The study involved five types of typical salami that were characterised for aroma components and nutritional features. This approach could provide a basis for a possible PDO or PGI label request. Principal Component Analysis and cluster analysis were used as multivariate statistical tools for data treatment. The overall results obtained point out that the products investigated do not deviate from analogous European products and show the possibility of characterising by specific parameters three main groups of samples: *Salamini di Mandrogne*, *Muletta*, and *Nobile Giarolo*; moreover some considerations can also be drawn with respect to the nutritional characterization considering the biogenic amines profile.

1. Introduction

The general term “salami” indicates stuffed meat products, very diffused and largely consumed because of their textural, sensorial, and nutritional properties. Different kinds of salami can be distinguished as a function of several factors, that is, fineness of the meat, formulation, consistency, and storage conditions [1]. The different appearance and taste also depend on the production strategies, the addition of spices, the use of microbial starters, and the environmental parameters experienced during fermentation and ripening processes. To protect the peculiarity of a typical product, it is first necessary to identify and quantify those variables that better describe its characteristics. These features permit promoting the product through the development of a certificate of origin that also reports the production process and the geographical origin. To this purpose a series of chemical and microbiological analyses are generally performed. Volatile organic compounds (VOCs) characterisation is useful to investigate the aroma properties of meat products [2–7].

The typical aroma of the products depends on a large number of volatile species, whose nature and amount can be related to the raw matter composition and the different ingredients as well as to the processing conditions including fermentation and ripening. The aroma can arise from a complex pattern of chemical reactions that take place among components, as, for example, oxidation of unsaturated fatty acids or microbiological metabolisms of lipids, proteins, and carbohydrates. The analysis of the volatile fraction has been associated with the compositional, biochemical, and microbiological characterisation to compare three Italian PDO (Protected Denomination of Origin) fermented sausages (namely, Varzi, Brianza, and Piacentino) [8]. Only few data are present regarding the characterisation of long ripened salami and, to our knowledge, no study simultaneously treats the chemical and microbiological data with methods of multivariate data analysis [7, 9]. Characterisation analyses concerning the distinctive properties of typical products are often promoted by authorities with the aim to support the possible request of PDO and PGI (Protected Geographical

Indication) labelling. The present paper presents a wide characterisation study of typical homemade salami produced in the Alessandria province (North West of Italy). The scope of this work regards screening the characteristics of five different products (*Muletta Monferrina*, *Salame Nobile del Giarolo*, *Filetto Baciato*, *Tipico Tortonese*, and *Salamini di Mandrogne*) and following their evolution along ripening: microbiological and chemical analyses were carried out regarding both nonvolatile and volatile fractions. The analysis of the nonvolatile fraction and the microbiological determinations gives information about taste, as well as about ripening time and hygienic conditions of production. In particular, the iodine value (index of the unsaturation degree of fat) [10, 11] and the saponification number (measure of the average molecular weight of all fat present in the sample) give useful information about the nutritional characteristics; metal content instead, in particular the rare earth elemental composition, can be very useful to provide information about the geographical provenience [12, 13]. All data collected were treated by multivariate statistical analysis techniques as Principal Component Analysis (PCA) and Cluster Analysis. PCA was firstly applied to the overall set of data collected at all the ripening stages considered, to provide a general description of the relationships existing between samples and variables. Then, the analysis was focussed on the samples collected at the selling stage only, to provide a description of the products as they reach the consumer table.

2. Materials and Methods

2.1. Salami Samples. Five different local salami products were involved in the study, namely, *Muletta Monferrina*, *Salame Nobile del Giarolo*, *Filetto Baciato*, *Tipico Tortonese*, *Filzetta*, and *Salamini di Mandrogne*. The samples were provided by the 17 producers cooperating to the study, in particular, nine different producers regarding *Salamini di Mandrogne* and two different salami factories for the other salami products. Samples were provided both at their production time (T_0) and at different time points during ripening, comprising the selling time. Different typical products show different monitoring schedule along time, as they show different optimal ripening periods until selling: 2, 3, 4, 5, and 6 months (T_2 – T_6) for *Muletta Monferrina*; 1, 2, 3, and 4 months (T_1 – T_4) for *Nobile del Giarolo*; 1 and 2 months (T_1 – T_2) for *Filetto Baciato* and *Tipico Tortonese*. *Salamini di Mandrogne* were analysed only at the production time (T_0) as they are sold fresh. The salami analysed was identified by a four-character label: the first two letters indicate the type of sample (MU = *Muletta*, FI = *Filetto*, GI = *Giarolo*, SM = *Salamini di Mandrogne*, and SA = *Tipico Tortonese*); the third letter indicates the manufacturer (A or B); the fourth character is a number indicating the months of ripening and ranges from 0 (production time) to 6 (number of months of ripening). Samples were provided in triplicate by each producer for each ripening time; results for each determination were provided for each sample and the three replicates for each producer were then averaged.

2.2. Chemicals. KOH \geq 85.5%, $\text{CCl}_4 \geq$ 99.8%, $\text{CH}_3\text{COONa} \geq$ 99%, $\text{Na}_2\text{SO}_4 \geq$ 99.5%, $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O} \geq$ 99%, and

$\text{Na}_2\text{C}_2\text{O}_4 \geq$ 98% were purchased from Carlo Erba (Milan, Italy). $\text{CuSO}_4 \geq$ 99%, $\text{K}_2\text{SO}_4 \geq$ 99%, Se \geq 99%, $\text{H}_2\text{SO}_4 \geq$ 95–98%, NaOH \geq 98%, KI \geq 99%, HCOOH \geq 96% ACS, $\text{KNO}_2 \geq$ 97%, $\text{CH}_3\text{COONH}_4 \geq$ 99.9%, $\text{Na}_2\text{S}_2\text{O}_3 \geq$ 99%, 1% (w/V) water solution of starch indicator, L-lysine \geq 98%, tryptamine hydrochloride 99%, methyl red crystals ACS reagent, phenolphthalein RPE-ACS, and the C6–C22 series of *n*-alkanes were purchased from Sigma-Aldrich (Schnellendorf, Germany). Acetonitrile \geq 99.9% HPLC grade, $\text{HCOONH}_4 \geq$ 99.9%, and HCl \geq 37% were purchased from VWR International (Darmstadt, Germany). Ethyl alcohol \geq 99.8%, cadaverine dihydrochloride $>$ 99%, histamine dihydrochloride $>$ 99%, dansyl chloride \geq 99%, petroleum ether puriss. p.a. ACS reagent bp 40–60°C, L-histidine \geq 99.5%, L-tyrosine \geq 99%, octylamine \geq 99%, $\text{NaHCO}_3 \geq$ 99%, and $\text{HNO}_3 \geq$ 69.5% were purchased from Fluka (Buchs, Switzerland). $\text{H}_3\text{PO}_4 \geq$ 85%, $\text{KNO}_3 \geq$ 99%, KCl \geq 99.5%, and Symphony potentiometric buffer solutions (pH 4.00, 7.00, and 10.00) were purchased from Merck (Darmstadt, Germany). Hydromatrix was purchased from Varian (Palo Alto, CA, USA) and Wijs solution 0.1 M in acetic acid from Riedel de Haen (Seelze, Germany). MRS agar was purchased from LAB M (Bury, UK), Tryptone Soya Agar and Mannitol Salt Agar were purchased from Oxoid (Rodano Milan, Italy). Ultrapure water was produced by a Millipore Milli-Q system (Milford, MA, USA).

2.3. Equipment. The following apparatus were used for the preparation of the samples: Stomacher Circulator (PBI International, Milan, Italy), oven EWTQ905 (Falc Instruments, Treviglio, Bergamo, Italy), muffle Pyro High Temperature Microwave (Milestone, Shelton, CT, USA), accelerated solvent extractor ASE 100 (Dionex, Sunnyvale, CA), centrifuge IC CL31R Multispeed (Thermo Electron Corporation, Waltham, MA, USA), and Sartorius balance CP225D-0CE (0.00001 g) (Goettingen, Germany). pH measurements were performed by a Symphony SB70P pH meter (VWR, Darmstadt, Germany), equipped with a combined glass Ag/AgCl electrode. Conductivity was measured by a conductometer ATC HI 9033 (Hanna Instruments, Woonsocket, RI, USA). HPLC analyses of nitrite and nitrate ions were carried out by a Merck-Hitachi HPLC system (Tokyo, Japan) equipped with L-6200 intelligent pump interfaced to an L-4250 UV-Vis detector and to D-2500 Chromato-integrator. Biogenic amines and their precursor amino acids were determined by HPLC Spectra System (Providence, RI, USA), equipped with a Spectra System pump P4000, a Spectra System SCM 1000 degasser, an autosampler Spectra System AS 3000, UV 6000 LP detector, and the software ChromQuest. Metals determination was performed by a Thermo Fisher XSeries 2 ICP-MS (Winsford, UK), equipped with an Apex-Q fully-integrated inlet system (Elemental Scientific Inc. Omaha, USA) and the software PlasmaLab V2.5.4.289. The analysis of volatile compounds was carried out on a TraceGC Ultra gas chromatograph (Thermo Finnigan, Milan, Italy) equipped with a split/splitless (SSL) injector, a CombiPal (CTC analytics, Zwingen, Switzerland) autosampler and coupled with a TRACE DSQ (Finnigan, Milan, Italy) mass spectrometer.

2.4. Sample Pretreatment. The salami samples delivered to our laboratory were immediately sliced and minced. In a portion of the fresh sample, treated as successively described, pH, moisture, conductivity, nitrite, and nitrate content were immediately determined, while all other analyses were performed on the fraction stored in freezer at -25°C . To measure pH, conductivity, nitrite, and nitrate, 10.0 g of fresh sample was kept in contact with 90.0 mL of ultrapure water in a stomacher bag and digested for 5.0 min at 270 rpm; then the extract was filtered on filter paper. For the determination of fat and iodine value, a sample of 10.0 g of thawed salami was put in an ASE (Accelerated Solvent Extraction) cell with 5.0 g of hydromatrix and extracted by petroleum ether performing 3 cycles of extraction of 10.0 min each, at 160.0°C .

For ash percentage and metal ion determinations, 5.0 g of sample was treated in muffle under a linear temperature gradient reaching 820.0°C in 1 h 20'. The analysis of volatile compounds was performed on 10.0 g of sample cut in very thin slices and weighted into a 20.0 mL headspace vial, sealed with polytetrafluoroethylene- (PTFE-) coated silicone rubber septum (20 mm diameter), where they were left for 60 min at 25°C . After each analysis the fibre was kept for 15 min at 280°C .

2.5. Methods

2.5.1. HPLC Determination of Nitrite and Nitrate. Nitrite and nitrate determination was performed on the stomacher aqueous extract filtered on a $0.22\ \mu\text{m}$ PTFE filter (VWR International, Darmstadt, Germany) and diluted 1/10 v/v.

The mobile phase was an aqueous solution of octylamine 5.0 mM brought to pH 6.4 by o-phosphoric acid; the stationary phase was a Lichrospher C18e ($250 \times 4\ \text{mm}$, $5\ \mu\text{m}$) column with a Lichrospher RP-18 ($5\ \mu\text{m}$) precolumn (Merck, Darmstadt, Germany). The mobile phase flow rate was $1.0\ \text{mL min}^{-1}$, the injection volume was $100.0\ \mu\text{L}$, and wavelength of the UV detector was set at 205 nm.

2.5.2. HPLC Determination of Amino Acids and Biogenic Amines. The dansylation reaction of aminoacids and biogenic amines was performed on a solution obtained putting 10.0 g of sample in contact with 100.0 mL HCl 0.1 M in a stomacher bag and digested for 5.0 min at 270 rpm; the extract was centrifuged for 10.0 min at 8000 rpm ($25000 \times g$). $1200\ \mu\text{L}$ of extract was put in contact with $1200\ \mu\text{L}$ of NaHCO_3 and $1200\ \mu\text{L}$ of a dansyl chloride solution 0.02 M in acetone. The mixture was kept at dark for 40.0 min at 65°C and then centrifuged for 2.0 min at 10000 rpm and undergone to solid phase extraction (SPE). After conditioning the C18 SPE cartridge (50 mg of sorbent) (Phenomenex, Bologna, Italy) with 2.00 mL of methanol and 2.00 mL of a water/acetone 50/50 (v/v) mixture, 2.00 mL of the derivatized sample was loaded and a washing step was performed with 3.00 mL of Milli Q water. The cartridge was dried under nitrogen and the sample recovered in 2.00 mL of methanol. For the HPLC determination of biogenic amines and precursor aminoacids, a Lichrospher C18e ($250 \times 4\ \text{mm}$, $5\ \mu\text{m}$) column with a Lichrospher RP-18 ($5\ \mu\text{m}$) precolumn (Merck, Darmstadt, Germany) was employed, while the mobile phase was

a mixture of $\text{CH}_3\text{COONH}_4$ 9.0 mM (at pH 3.40 for HCOOH) (41%) and acetonitrile (59%). The mobile phase flow rate was $1.0\ \text{mL min}^{-1}$ and the UV detector set at 254 nm.

2.5.3. Water Content. Water content was determined by the comparison of the solid sample weights before (5.0 g) and after the oven treatment performed at 105.0°C for 2.5 h [15].

2.5.4. Saponification Value. The saponification value (S) was determined directly on 5.0 g of salami put in contact with 25.00 mL of 0.5 N ethanol solution of KOH in a flask equipped with a reflux condenser. The system was heated for 20 min and then the mixture was centrifuged for 5 min at 5000 rpm; the supernatant was titrated with HCl 0.5 N, phenolphthalein being the indicator.

The saponification value was calculated through the following equation:

$$S = \frac{(V_b - V_s) \cdot 28.05}{w}, \quad (1)$$

where V_b is the volume (mL) of titrant used in the blank titration, V_s is the volume (mL) obtained in the titration of the sample, and w is the weight (5.0 g) of the sample.

2.5.5. Fat Content. The fat content was determined by weighting the residual of the ASE extract after complete solvent evaporation [16].

2.5.6. Iodine Value. The iodine value (I) was determined by adding 20.00 mL of CCl_4 to the ASE extract. After agitation for 5 min, 25.00 mL of Wijs solution was added and the resulting mixture was kept at dark for 1 h; after addition of 20.00 mL of KI solution 10% w/v, the solution was titrated with $\text{Na}_2\text{S}_2\text{O}_3$ 0.1 N with starch solution as indicator. The iodine value is calculated as follows:

$$I = \frac{(V_b - V_s) \cdot 12.69}{w}, \quad (2)$$

where V_b is the volume (mL) employed in the titration of the blank, V_s is the volume (mL) employed in the titration of the sample, and w is the weight (g) of sample [17].

2.5.7. Ash Content. The ash content was determined by weighting the muffle mineralised sample after 20 min of cooling at room temperature.

2.5.8. Protein Determination according to the Kjeldahl Method. 1.0 g of the sample was placed in Kjeldahl flask and added with 20.0 g of K_2SO_4 , 0.55 g of CuSO_4 , 0.75 g of Se, and 35.0 mL of H_2SO_4 18 M. The flask was heated in a mantle for one hour at the solution fuming temperature. Then 100.0 mL of ultrapure water and 250.0 mL of NaOH 20% (w/v) were added to the cooled solution and the resulting mixture was heated. The first 50 mL of the distilled fraction was recovered in a flask, containing 50.0 mL of HCl 0.1 N. The excess of HCl was

titrated with NaOH 0.1 N, methyl red as the indicator. The % of nitrogen (%N) was calculated as

$$\%N = \frac{eq_{H^+} \cdot 14.008}{w} \cdot 100, \quad (3)$$

where eq_{H^+} are the equivalents of H^+ consumed by the distilled basic fraction and w is the weight (g) of the sample. Protein content was estimated by multiplying the *Kjeldahl* nitrogen content by 6.25 [18].

2.5.9. Determination of Volatile Compounds. The analysis of volatile compounds was performed on 10.00 g of sample cut in very thin slices and weighted into a 20.00 mL headspace vial, sealed with polytetrafluoroethylene- (PTFE-) coated silicone rubber septum (20 mm diameter), added with 1.00 mL of the internal standard 4-methyl-2-pentanone aqueous solution at the concentration of $2.00 \mu\text{g mL}^{-1}$. They were left for 60 min at 25°C . Headspace was extracted by SPME technique using a CAR/PDMS fibre, $75 \mu\text{m}$ film thickness (Supelco, Bellefonte, PA, USA). Fibres exposition time of 90 min at 25°C was adopted [19]. The fibre was then introduced into the injector of a gas chromatograph at 220°C and the sample injected by splitless mode for 8 minutes. The source and transfer line temperatures were set at 250°C and 230°C , respectively. After each analysis the fibre was kept for 15 min at 280°C . The GC system was equipped with a fused-silica capillary column (Rtx-WAX, $30 \text{ m} \times 0.25 \text{ mm i.d.}$, film thickness $0.25 \mu\text{m}$). Helium was used as carrier gas at 1 mL min^{-1} flow rate. The column temperature was held at 35°C for 8 min, increased from 35°C to 60°C at 4°C min^{-1} , from 60°C to 160°C at 6°C min^{-1} , and from 160°C to 200°C at $20^\circ\text{C min}^{-1}$. The mass spectra were obtained by electron impact at 70 eV with the detector operating in scan mode (total ion current) from m/z 35 to 350 a.m.u., with scanning velocity of 2.48 scan s^{-1} . The identification of volatile compounds was carried out by comparing GC retention time and MS spectra with those of standard compounds. Nist 98 and Wiley 275 mass spectral libraries were used when standard compounds were unavailable. A series of *n*-alkanes (C6–C22) was also determined under the same conditions to obtain linear retention index (LRI) values for the aroma components. Quantitative analyses of samples were carried out by using the internal standard procedure and expressed as ng IS equivalents.

2.5.10. Microbiological Analysis. The microbiological analyses were performed on 10.0 g of sample. Lactic acid bacteria (*Lactobacillus*) counts were determined by the overlay technique using MRS agar and colonies counted after incubation in anaerobic conditions after 72 h at 30°C ; total count was performed on Tryptone Soya Agar after 72 h at 30°C ; *Micrococcaceae* were determined on Mannitol Salt Agar after 24 h at 42°C . All bacteria counts were expressed as colony forming per gram of sample (CFU g^{-1}).

2.6. Statistical Analysis. All statistical treatments, Principal Component Analysis (PCA), and graphical representations

were carried out by Statistica version 7.1 (StatSoft Inc, USA) and Microsoft Excel (Microsoft Corporation, USA).

3. Results and Discussion

3.1. Physical-Chemical and Nonvolatile Fraction Analyses. While PDO protocols are already available for other Italian Salami, as *Varzi*, *Brianza*, and *Piacentino* salami [8] and UNI standard values are reported for *Felino* or *Milano* [6, 15, 16], this is not yet the case for the salami here considered. Taking into account that the samples investigated are produced at homemade level, in order to identify parameters suitable for the definition of PDO and PGI, a high number of variables have been evaluated. As reported above, the products considered are characterised by different ripening procedures: *Salamini di Mandrogne* are sold without ripening, *Muletta* after a six-month ripening period, and *Filetto Baciato*, *Nobile del Giarolo*, and *Tipico Tortonese* after ripening times that vary between two and three months. The analyses were performed at regular time intervals (one month) during the ripening period, ranging from the production time to the selling time for all products except for *Salamini di Mandrogne*, for which the analyses were performed only at the production-commercialisation time. Some considerations and comparison can be made among the data reported in Tables 1 and 2.

Water percentage (Table 1) varies from about 56% at the production time for *Salamini di Mandrogne* to about 20% at the end of ripening of *Nobile del Giarolo* samples. Weight loss during ripening varies from about 29% of MUA samples to about 7% of SAA samples: different variations can be ascribed to the ripening conditions, performed in a traditional cellar for *Muletta* and *Nobile del Giarolo* samples and in industrial climatic chambers for the other products. pH values at the production time are all lower than 6.0 and, in agreement with data found for other meat products [8] trend to increase during ripening. Likely due to an intense deaminase oxidative activity, induced in particular by moulds [8], pH values increase during ripening for MUA and GIB and in particular for GIA sample, probably due to the traditional room in which this product is ripened [9]. Table 1 also shows that the content of fat and proteins is similar, at the end of ripening, for nearly all samples, in agreement with literature data for typical north Italian salami [8, 9, 13], with the only exception of *Filetto Baciato* and *Salamini di Mandrogne*. The lower fat content for *Filetto* can be explained taking into account that this product is constituted by a central lean fillet of pork surrounded by a salami mixture. The iodine value largely varies within the different samples. The values at the selling time are generally lower than literature data for European salami [20] that, in turn, are lower than values obtained for products from other countries. Saponification number, providing a measure of chain length of fatty acids, ranges from 78 to 179 mg KOH g^{-1} and is consistent with average values for meat products [11]. Nitrite and nitrate contents (Table 1) must be compared with Italian law threshold concentration levels, reported in the D.M. 27/02/1996 n. 209, that considers two different levels. One indicates the maximum amount that can be added (150 mg Kg^{-1} for sodium nitrite and 300 mg Kg^{-1} for sodium nitrate) while the other gives

TABLE 1: Results of physical-chemical analysis at the production and selling times.

	Proteins ^a %	pH	Cond mS	H ₂ O %	Saponification number Mg KOH/g DM	Fat %	Iodine number gI ₂ /100 g DM	Ash %	Nitrite mg NaNO ₂ Kg ⁻¹	Nitrate mg NaNO ₃ Kg ⁻¹
MUA0	25.4 ± 0.6	5.5 ± 0.2	5.9 ± 0.3	57.4 ± 0.9	180 ± 2	11.7 ± 0.7	92 ± 3	5.0 ± 0.1	61 ± 1	139 ± 3
MUA6	13.6 ± 0.3	6.4 ± 0.2	7.7 ± 0.4	28.2 ± 0.4	128 ± 3	19.1 ± 0.8	41 ± 2	7.2 ± 0.2	29 ± 1	24 ± 1
MUB0	23.9 ± 0.6	5.1 ± 0.2	5.8 ± 0.3	52.5 ± 0.8	142 ± 2	15.2 ± 0.9	96 ± 3	4.8 ± 0.1	77 ± 1	248 ± 5
MUB6	34.6 ± 0.9	5.6 ± 0.2	8.5 ± 0.5	28.3 ± 0.4	143 ± 3	21.5 ± 0.3	35 ± 1	6.8 ± 0.2	114 ± 2	57 ± 1
GIA0	29.9 ± 0.7	5.5 ± 0.2	6.9 ± 0.4	40.2 ± 0.6	150 ± 2	14.9 ± 0.9	52 ± 2	4.6 ± 0.1	76 ± 1	49 ± 1
GIA4	13.0 ± 0.3	6.7 ± 0.2	8.5 ± 0.5	20.1 ± 0.3	113 ± 3	27 ± 2	25 ± 1	5.6 ± 0.1	53 ± 1	72 ± 1
GIB0	28.6 ± 0.7	5.5 ± 0.2	6.7 ± 0.4	43.1 ± 0.6	141 ± 2	13.7 ± 0.8	73 ± 3	4.7 ± 0.1	106 ± 2	56 ± 1
GIB3	132 ± 3	6.2 ± 0.2	7.4 ± 0.4	26.2 ± 0.4	135 ± 3	25 ± 2	30 ± 1	5.7 ± 0.1	52 ± 1	47 ± 1
SAA0	22.6 ± 0.6	5.9 ± 0.2	4.7 ± 0.3	44.3 ± 0.7	79 ± 1	10.0 ± 0.6	101 ± 4	3.4 ± 0.1	37 ± 1	163 ± 3
SAA2	3.9 ± 0.1	5.3 ± 0.2	7.7 ± 0.4	29.9 ± 0.4	150 ± 3	18.1 ± 0.8	40 ± 2	4.9 ± 0.1	110 ± 2	49 ± 1
SAB0	9.4 ± 0.2	5.3 ± 0.2	6.8 ± 0.4	36.1 ± 0.5	92 ± 2	21.5 ± 0.8	42 ± 2	4.2 ± 0.1	76 ± 1	110 ± 2
SAB2	33.6 ± 0.8	5.8 ± 0.2	8.9 ± 0.5	29.4 ± 0.4	102 ± 2	17.4 ± 0.9	48 ± 2	6.9 ± 0.2	95 ± 2	42 ± 1
FIA0	9.5 ± 0.2	6.1 ± 0.2	4.7 ± 0.3	49.0 ± 0.7	142 ± 2	6.3 ± 0.4	163 ± 6	3.6 ± 0.1	42 ± 1	87 ± 2
FIA2	25.4 ± 0.6	5.7 ± 0.2	8.5 ± 0.5	40.5 ± 0.6	107 ± 2	8.6 ± 0.5	103 ± 4	6.5 ± 0.2	108 ± 2	33 ± 1
FIB0	9.7 ± 0.2	5.8 ± 0.2	6.9 ± 0.4	51.9 ± 0.8	130 ± 2	6.0 ± 0.4	176 ± 6	3.6 ± 0.1	44 ± 1	52 ± 1
FIB2	171 ± 0.4	6.1 ± 0.2	10.1 ± 0.6	37.1 ± 0.6	136 ± 2	12.8 ± 0.8	103 ± 4	7.2 ± 0.2	81 ± 2	62 ± 1
SMA0	18.7 ± 0.5	5.9 ± 0.2	4.1 ± 0.2	52.1 ± 0.8	148 ± 2	4.4 ± 0.3	105 ± 3	3.0 ± 0.1	49 ± 1	<LOD
SMB0	27.9 ± 0.7	5.8 ± 0.2	3.5 ± 0.2	54.5 ± 0.8	150 ± 2	11.8 ± 0.7	88 ± 3	2.5 ± 0.1	30 ± 1	<LOD
SMC0	4.4 ± 0.1	5.6 ± 0.2	3.5 ± 0.2	56.4 ± 0.9	121 ± 1	9.4 ± 0.6	130 ± 4	2.4 ± 0.1	35 ± 1	<LOD
SMD0	7.3 ± 0.2	5.7 ± 0.2	3.6 ± 0.2	56.8 ± 0.9	126 ± 1	5.1 ± 0.3	104 ± 3	2.6 ± 0.1	39 ± 1	<LOD
SME0	15.5 ± 0.4	5.8 ± 0.2	3.5 ± 0.2	53.8 ± 0.8	126 ± 2	14.9 ± 0.9	81 ± 3	2.7 ± 0.1	41 ± 1	19 ± 1
SMF0	31.9 ± 0.8	5.7 ± 0.2	4.1 ± 0.2	46.2 ± 0.7	137 ± 2	15.6 ± 0.9	66 ± 2	2.6 ± 0.1	43 ± 1	7 ± 1
SMG0	171 ± 0.4	5.6 ± 0.2	3.7 ± 0.2	45.8 ± 0.7	138 ± 2	9.2 ± 0.6	104 ± 4	2.4 ± 0.1	36 ± 1	3 ± 1
SMH0	19.6 ± 0.5	5.6 ± 0.2	3.5 ± 0.2	52.4 ± 0.8	141 ± 2	7.7 ± 0.5	118 ± 4	2.7 ± 0.1	38 ± 1	<LOD
SMI0	19.8 ± 0.5	5.8 ± 0.2	3.7 ± 0.2	56.4 ± 0.9	133 ± 1	10.4 ± 0.6	104 ± 3	2.7 ± 0.1	40 ± 1	<LOD

^adetermined by Kjeldahl method. DM = dry matter; LOD NaNO₃ = 0.10 mg L⁻¹.

TABLE 2: Results of biogenic amines and precursor amino acids determination for the production and selling times.

	LYS mg Kg ⁻¹ DM	HISTID mg Kg ⁻¹ DM	TRYPT mg Kg ⁻¹ DM	TYROS mg Kg ⁻¹ DM	CAD mg Kg ⁻¹ DM	HIS mg Kg ⁻¹ DM	TYR mg Kg ⁻¹ DM	Total ABS mg Kg ⁻¹ DM
MUA0	<LOD	460 ± 14	62 ± 2	<LOD	94 ± 3	375 ± 12	304 ± 9	835
MUA6	269 ± 8	112 ± 3	68 ± 2	<LOD	204 ± 6	406 ± 13	192 ± 6	870
MUB0	506 ± 15	351 ± 10	<LOD	<LOD	<LOD	<LOD	146 ± 4	146
MUB6	1103 ± 34	888 ± 26	<LOD	<LOD	30 ± 1	208 ± 7	280 ± 9	519
GIA0	485 ± 15	207 ± 6	<LOD	<LOD	59 ± 2	236 ± 7	128 ± 4	423
GIA4	649 ± 20	213 ± 6	35 ± 1	<LOD	72 ± 2	244 ± 8	113 ± 3	465
GIB0	511 ± 15	437 ± 13	45 ± 1	198 ± 4	<LOD	<LOD	119 ± 4	410
GIB3	<LOD	418 ± 12	38 ± 1	<LOD	<LOD	246 ± 8	95 ± 3	133
SAA0	129 ± 3	127 ± 4	<LOD	<LOD	<LOD	252 ± 8	<LOD	252
SAA2	1602 ± 49	397 ± 12	51 ± 1	<LOD	<LOD	257 ± 8	134 ± 4	443
SAB0	431 ± 13	313 ± 9	<LOD	179 ± 4	<LOD	<LOD	<LOD	—
SAB2	<LOD	662 ± 20	44 ± 1	273 ± 6	<LOD	216 ± 7	159 ± 5	—
FIA0	130 ± 4	147 ± 4	33 ± 1	<LOD	<LOD	<LOQ	43 ± 1	273
FIA2	595 ± 18	322 ± 10	43 ± 1	<LOD	<LOD	197 ± 6	118 ± 4	160
FIB0	139 ± 4	158 ± 5	33 ± 1	<LOD	<LOD	290 ± 9	<LOD	323
FIB2	341 ± 10	131 ± 4	43 ± 1	<LOD	35 ± 1	214 ± 7	112 ± 3	405
SMA0	138 ± 4	149 ± 4	<LOD	<LOD	<LOD	<LOD	<LOD	—
SMB0	<LOD	173 ± 5	<LOD	<LOD	<LOD	<LOD	<LOD	—
SMC0	139 ± 4	229 ± 7	<LOD	<LOD	<LOD	311 ± 10	<LOD	311
SMD0	175 ± 5	178 ± 5	<LOD	<LOD	<LOD	328 ± 11	<LOD	328
SME0	180 ± 5	181 ± 5	<LOD	<LOD	<LOD	<LOD	<LOD	—
SMF0	109 ± 4	141 ± 4	<LOD	<LOD	<LOD	257 ± 8	<LOD	258
SMG0	61 ± 2	126 ± 3	<LOD	<LOD	<LOD	<LOD	<LOD	—
SMH0	90 ± 2	146 ± 4	<LOD	<LOD	<LOD	<LOD	<LOD	—
SMI0	124 ± 4	210 ± 6	<LOD	<LOD	<LOD	313 ± 10	<LOD	313

DM = dry matter; LOD lysine = 40 µg L⁻¹, cadaverine = 52 µg L⁻¹, histamine = 104 µg L⁻¹, histidine = 159 µg L⁻¹, tyramine = 62 µg L⁻¹, and tryptamine = 45 µg L⁻¹.

the maximum residual content that can be present at the selling time and corresponds to 50 mg Kg⁻¹ for sodium nitrite and to 250 mg Kg⁻¹ for sodium nitrate. Regarding nitrite, its amount is always larger than 29 mg Kg⁻¹ and for five samples (namely, *MUB6*, *SAA2*, *SAB2*, *FIA2*, and *FIB2*) they are above the law limit at the selling time: this is a quite common situation since nitrite slowly transforms into nitrate during ripening and these products are not supposed to be consumed fresh. Table 2 reports the amounts, corrected for moisture, obtained for cadaverine (CAD), histamine (HIS), histidine (HISTID), tyramine (TYR), tryptamine (TRYPT), tyrosine (TYROS), and lysine (LYS). Both the total amount of BAs and the HIS/HISTID concentration ratio increase during ripening. At the selling time, tyramine is present in all samples at concentrations ranging from 95 to 280 mg Kg⁻¹. Anyway the total BAs amount is always lower than the level (1000 mg Kg⁻¹) reported as dangerous for human health [21], where 870 mg Kg⁻¹ is the maximum amount obtained at the selling time. However, a univocal toxic level is difficult to define since individual sensitivity can be very different and can also be related to the specific biogenic amine considered.

Regarding the content of BAs in fresh products (production time), the samples generally show content unexpectedly high of histamine and tyramine [1, 22].

Salamini di Mandrogne do not contain tyramine, cadaverine, and tryptamine but the samples *SMC*, *SMD*, *SMF*, and *SMI* show concentrations of histamine of about 300 mg Kg⁻¹.

3.2. Microbiological Analyses. The results of microbiological analyses are presented in Figures 1 and 2, in which lactic acid bacteria and *Micrococcaceae* are reported as a function of ripening time. The results showed that the raw mixtures (T_0) were characterised by good hygienic conditions and suitable presence of lactic acid bacteria and *Micrococcaceae* that assists a correct fermentation process.

The microbiological trend is similar for all products. The counts of acid lactic bacteria and *Micrococcaceae* generally showed a maximum at one month of ripening (T_1) for all the samples and then decreased until the end of ripening. The only exception is represented by *Muletta* that showed the highest counts of lactic acid bacteria and *Micrococcaceae* at T_2 , probably due to its large size, that can affect

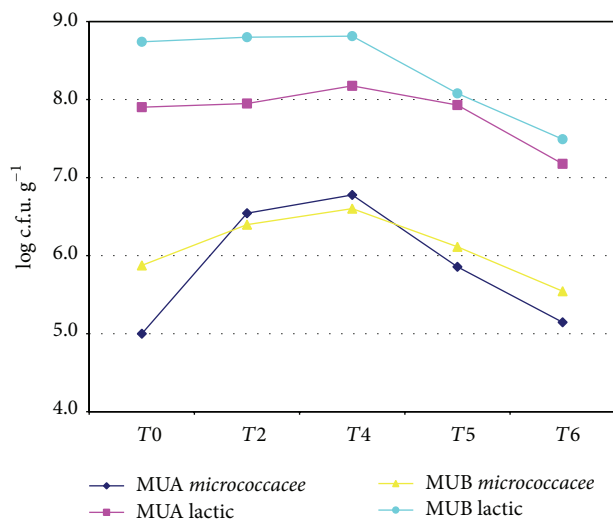


FIGURE 1: Evolution of *Micrococcaceae* and lactic acid bacteria during ripening of *Muletta* salami (log c.f.u. g⁻¹).

the growth of lactobacilli and *Micrococcaceae*. Also the amount of volatile compounds showed a similar trend, likely due to the formation of metabolites (e.g., esters, alcohols, and ketones) produced during the fermentation process. Salamini of Mandrogne (fresh sausages) showed a mean value of 8×10^6 u.f.c. g⁻¹ for acid lactic bacteria.

3.3. Volatile Compounds Analysis. About 70 volatile substances including terpenes, esters, ketones, alcohols, aldehydes, and sulphur compounds were searched and determined in all samples (Tables 3, 4, 5, and 6).

The compounds were identified using both chromatographic (Kovats indices) and spectrometric (mass spectra, EI, 70 eV) criteria. Kovats indices were calculated for each chromatographic peak and compared with those stored in a proprietary database including about 250 volatile compounds usually found in food matrices [9, 14, 23, 24]. Determination of the volatile constituents was carried out by spiking the salami, before the extraction, with 4-methyl-2-pentanone ($2.0 \mu\text{g mL}^{-1}$), used as the internal standard since preliminary results indicated its absence in all the samples. The lowest content of volatile species was found in *Filetto Baciato*: the result is likely due to its composition, constituted by a central lean fillet of pork inside the salami texture. In all products the largest group of volatiles was represented by terpenes, where α -pinene, β -pinene, sabinene, limonene, and β -caryophyllene are the most abundant. Terpenes can derive from animal feedstuffs and mainly from the spices as black pepper, nutmeg, and clove added during production. In particular nutmeg contains α -pinene, β -pinene, sabinene, and limonene and clove β -caryophyllene [7, 25]. The maximum terpene compounds concentration was observed at the end of the ripening period in *Nobile del Giarolo* and *Filetto Baciato*, while in *Muletta* it was reached at about 4 months of ripening (T_4) [26, 27]. Four sulphur containing compounds were identified and quantified in *Filetto Baciato*

and *Nobile del Giarolo* and six compounds in *Muletta*, the most abundant being allyl methyl sulphide. Sulphur compounds mainly derive from garlic and represent important aroma compounds, since they are characterised by very low sensory thresholds [28]. The amounts of sulphur containing species increased during the ripening, except for *Muletta* that showed a decreasing trend after the T_4 of ripening. Many ketones and alcohols were found to be present. The most abundant ketones were 2-butanone, 3-hydroxy-2-butanone (acetoin), and 2-propanone; their concentrations increased reaching a maximum at the end of ripening except in *Muletta* where the amount increases until T_5 and then decreases.

The most abundant alcohols isolated were ethanol, 2-butanol, 3-methyl-1-butanol, 1-propanol, and 1-octen-3-ol, which in particular is produced during lipid oxidation and is recognised for a characteristic mushroom note and a very low sensory threshold [4].

High amounts of ethanol were found in all products, likely arising from the wine added during preparation. Also 3-methyl-1-butanol was found in all the products, likely formed through the reduction reaction of the corresponding aldehyde [3, 19, 29].

Several ethyl esters were isolated in particular in *Muletta*. Since esters can be formed in a complex chain of reactions such as alcohol-aldehyde-acid-ester, ethyl esters are usually present in fermented meat products and contribute to the fruity note of the flavour [6, 20, 30, 31]. The long ripening time undergone by *Muletta* likely favoured therefore their formation. Aldehydes were identified and quantified in *Filetto Baciato*, *Muletta*, and *Nobile del Giarolo*. The total aldehyde content increased during ripening, especially in *Filetto Baciato* and *Nobile del Giarolo*, while showing a maximum at T_4 for *Muletta*. Many aldehydes are products of lipid oxidation. In particular hexanal, which is produced during the oxidation of n-6 unsaturated fatty acids, imparts a green odour and is considered a good indicator of oxidation [30]. The low amount of hexanal found in all the salami could likely be attributed to the antioxidative activity of terpenes of spices found at higher concentration levels in all the products. All the products contain benzenacetaldehyde that is considered one of the substances giving a specific flavour note to pork meat and can form from phenylalanine. Among free fatty acids the most abundant compounds identified in all the salami were acetic acid, butanoic acid, 2-methyl propanoic acid, and 3-methyl butanoic acid. The total amount of fatty acids increased during the ripening in *Nobile del Giarolo* and *Filetto Baciato*, while reaching a maximum at T_4 in *Muletta*. As regards such salami therefore we can conclude that a decrease of several volatile compounds occurred in the last ripening period, probably due to the natural loss from the matrix surface.

44 volatile compounds were identified and quantified in *Salamini di Mandrogne*. As mentioned before, a different behaviour characterizes *Salamini di Mandrogne*, which are sold just after production. The most abundant compounds were ketones, alcohols, terpenes, sulphur compounds, free fatty acids, and lactones. High concentrations of volatile species formed in carbohydrate fermentation were found,

TABLE 3: Volatile compounds detected in "Muletta" salami during ripening (ng g⁻¹).

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₂		T ₄		T ₅		T ₆		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	Min	Max	Min	Max	
<i>Terpenes (19)</i>														
5.55	755	α -Pinene	S	111.03	507.39	345.67	1143.06	38.40	6145.03	140.64	26771	31.99	309.08	MS + LRI
5.84	766	α -Thujene	S	0.00	221.22	0.00	46.92	0.00	0.00	0.00	0.00	0.00	4.48	MS + LRI
8.81	884	β -Pinene	S	263.65	710.08	453.30	1855.70	30.60	11530.05	281.01	365.64	48.46	536.68	MS + LRI
9.79	923	Sabinene	S	0.00	744.65	0.00	419.57	0.00	0.00	0.00	94.55	177.45	MS + LRI	
11.20	979	3-Carene	S	205.30	867.22	331.35	2051.75	195.87	18524.57	454.80	837.22	34.51	374.29	MS + LRI
12.05	1013	α -phellandrene	S	4.18	26.48	0.00	0.00	0.00	0.00	0.00	0.00	51.54	345.89	MS + LRI
12.11	1015	Terpen	S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ms
12.51	1031	β -Mircene	S	3.62	41.42	8.36	35.85	9.42	550.28	24.55	31.85	29.40	257.49	MS + LRI
12.79	1042	α -Terpinene	S	0.00	16.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
13.69	1078	Limonene	S	53.44	305.81	90.11	366.58	60.46	3769.76	75.89	163.02	131.85	1321.38	MS + LRI
14.87	1125	β -Phellandrene	S	0.00	79.65	0.00	42.94	831.08	669.13	0.00	0.00	0.00	0.00	MS + LRI
15.17	1137	Terpen	S	0.00	0.00	0.00	8.15	0.00	0.00	0.00	0.00	0.00	0.00	ms
16.51	1190	γ -Terpinene	S	0.00	26.58	0.00	0.00	0.00	0.00	0.00	0.00	1.99	0.06	MS + LRI
16.61	1194	Cymene	S	0.00	46.71	0.00	12.28	338.55	243.82	0.00	41.06	14.02	0.25	MS + LRI
22.82	1441	Terpen	S	0.00	6.12	0.00	0.00	0.00	0.00	0.00	0.00	2.48	0.21	ms
24.66	1514	Terpen	S	0.00	1.69	0.00	2.4	0.00	0.00	0.00	0.00	0.00	0.00	ms
25.24	1537	Terpen	S	6.08	6.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ms
25.13	1533	trans- β -Caryophyllene	S	24.02	129.96	22.12	307.58	13.94	1413.19	53.32	278.67	41.62	1507.61	MS + LRI
27.18	1614	Humulene	S	0.00	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.08	MS + LRI
Tot.				276.06	1408.28	1250.91	6290.38	1518.32	42845.83	1030.21	2079.72	582.42	4835.95	
<i>Aldehydes (6)</i>														
1.77	604	Acetaldehyde		0.00	69.07	0.00	84.10	0.00	806.23	0.00	0.00	0.00	0.00	MS + LRI
8.18	859	Hexanal	LO	33.00	50.30	0.00	157.22	0.00	20.31	0.00	49.16	0.00	0.00	MS + LRI
18.69	1277	2-Heptenal		0.00	1.48	0.00	0.00	0.00	1.77	0.00	0.00	0.00	0.00	MS + LRI
20.50	1349	Nonanal	LO	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00	1.79	44.88	MS + LRI
23.95	1486	Benzaldehyde	AC	0.00	5.76	0.00	8.95	1044.19	0.00	0.00	123.44	0.00	17.42	MS + LRI
25.86	1562	Benzenacetaldehyde	AC	0.00	9.29	0.00	8.41	785.02	0.00	5.56	423.71	104.00	145.16	MS + LRI
Tot.				33.00	135.90	0.00	258.68	1829.21	828.31	5.56	596.31	105.79	207.46	

TABLE 3: Continued.

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₂		T ₄		T ₅		T ₆		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	Min	Max	Min	Max	
<i>Ketones (12)</i>														
2.12	618	2-Propanone	MI	0.00	406.93	33.62	91.69	0.00	505.84	0.00	51.34	0.00	11.97	MS + LRI
2.91	650	2-Butanone	F	0.00	1623.42	0.00	0.00	0.00	0.00	0.00	3521.42	0.00	166.12	MS + LRI
5.25	743	2,3-Butanedione	F	0.00	142.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
13.97	1089	2-Hexanone	MI	0.00	0.00	0.00	5.12	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
14.01	1091	2-Heptanone	LO	0.00	3.68	0.00	0.00	0.00	0.00	0.00	21.19	0.00	4.41	MS + LRI
16.27	1181	3-Octanone	MI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.59	MS + LRI
16.97	1208	Ketone	MI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.67	Ms
16.53	1191	Ketone	MI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.60	Ms
16.98	1209	3-Hydroxy-2-butanone	F	59.37	1966.78	827.33	1172.70	6.85	2038.92	7.29	223.42	0.00	15.51	MS + LRI
18.91	1286	6-Methyl-5-hepten-2-one	MI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	MS + LRI
20.92	1365	2-Nonanone	LO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.18	0.00	0.00	MS + LRI
24.5	1508	4-Hydroxy-2-butanone	MI	0.00	4.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Ms
Tot.				59.37	4239.47	860.95	1187.51	6.85	2544.76	7.29	3804.55	0.00	202.12	
<i>Alcohols (22)</i>														
3.49	673	Ethanol	F	7522.25	6370.77	11973.63	47046.29	648.81	101837.44	2241.97	3420.26	320.23	11467.61	MS + LRI
5.97	771	2-Butanol	F	0.00	2987.55	248.58	2729.31	0.00	234404.24	0.00	3594.77	0.00	72.96	MS + LRI
7.22	821	1-Propanol	LO	0.00	205.73	211.15	441.08	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
10.9	967	2-Methyl-1-propanol	AC	10710	442.94	56.05	897.74	19.46	2616.34	53.74	72.37	0.00	97.35	MS + LRI
11.65	997	2-Pentanol		0.00	34.63	22.17	205.84	0.00	19.92	0.00	33.60	0.00	176.61	MS + LRI
12.69	1038	1-Butanol		0.00	20.65	0.00	13.53	0.00	654.85	0.00	40.62	0.00	0.00	MS + LRI
14.75	1120	3-Methyl-1-butanol	AC	599.71	3086.67	384.91	4016.50	59.35	20241.68	211.95	328.12	28.17	1468.75	MS + LRI
16.87	1204	3-Methyl-3-buten-1-ol		0.00	6.04	6.75	40.25	0.00	3.94	0.00	0.00	0.00	0.52	MS + LRI
16.96	1208	1-Pentanol		0.00	33.84	15.22	180.02	0.00	0.00	0.00	0.00	0.00	1.19	MS + LRI
18.53	1270	3-Methyl-2-buten-1-ol		0.00	5.96	0.00	7.14	0.00	0.00	0.00	0.00	0.00	0.48	MS + LRI
19.24	1299	2-Heptanol		0.00	1.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.92	MS + LRI
19.53	1310	1-Hexanol		0.00	77.84	0.00	406.92	3.13	449.85	0.00	2.29	2.88	60.97	MS + LRI
20.31	1341	Alcohol		0.00	0.00	0.00	212.00	0.00	0.00	0.00	0.00	0.00	0.00	Ms
22.04	1410	1-Octen-3-ol	LO	0.00	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.69	MS + LRI
23.12	1453	Alcohol		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Ms
24.74	1517	Alcohol		0.00	6.36	0.00	1.31	0.00	168.84	0.00	3.75	0.00	4.10	Ms
30.61	1751	Alcohol		0.00	0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00	Ms
31.05	1768	Benzenethanol	MI	0.00	19.63	24.62	45.64	0.00	0.46	0.00	64.40	0.00	26.74	MS + LRI
Tot.				8229.06	13302.15	12943.08	56244.27	730.75	360397.56	2507.66	7560.18	351.28	13421.89	

TABLE 3: Continued.

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₂		T ₄		T ₅		T ₆		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	Min	Max	Min	Max	
<i>Free fatty acids (9)</i>														
21.61	1393	Acetic acid		2550.58	2928.98	2788.50	24333.05	562.28	52236.16	952.94	2049.15	100.55	9039.94	MS + LRI
23.52	1469	Formic acid		0.00	2.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
23.71	1476	Propanoic acid		0.00	133.16	143.09	1486.92	38.26	2954.71	54.40	141.12	5.99	716.58	MS + LRI
24.99	1527	2-Methyl-propanoic acid		0.00	19.34	30.73	475.17	7.06	817.86	11.22	21.07	0.00	21.63	MS + LRI
26.22	1576	Butanoic acid		74.75	122.94	68.00	774.76	37.87	2360.26	27.97	79.15	1.81	232.99	MS + LRI
27.09	1611	3-Methyl butanoic acid		36.16	400.93	51.91	893.17	19.40	1413.97	15.82	32.27	1.59	57.79	MS + LRI
27.38	1622	2-Methyl-2-propenoic acid		0.00	0.00	0.00	0.00	0.00	157.74	0.00	0.00	0.00	0.00	MS + LRI
28.38	1662	Pentanoic acid		0.00	2.67	2.65	11.27	1.98	75.10	0.96	2.58	0.00	47.65	MS + LRI
29.80	1718	Hexanoic acid		0.00	2.19	4.96	32.28	12.01	162.80	2.95	10.01	0.69	9.63	MS + LRI
Tot.				2661.49	3612.31	3089.84	28007.15	678.86	60178.60	1066.26	2335.35	110.63	10126.21	
<i>Hydrocarbons (3)</i>														
1.51	500	Pentane	LO	0.00	280.40	75.81	2368.70	85.92	553.22	80.65	295.70	2.40	161.09	MS + LRI
6.08	776	Toluene	MI	0.00	0.00	0.00	57.66	0.00	0.00	0.00	0.00	77.60	1487.15	MS + LRI
23.42	1465	Hydrocarbon	MI	0.00	2.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Ms
Tot.				0.00	282.70	75.81	2426.36	85.92	553.22	80.65	295.70	80.00	1648.24	
<i>Esters (9)</i>														
3.08	656	Acetic acid ethyl ester	ME	1261.40	7371.93	926.44	9092.92	586.21	16420.94	566.78	1836.03	0.00	6755.62	MS + LRI
7.56	834	Butanoic acid ethyl ester	ME	3.59	32.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
19.7	1317	Propanoic acid ethyl ester	ME	0.00	76.40	0.00	167.25	0.00	15.52	0.00	0	0.00	0.00	MS + LRI
20.04	1330	2-Hydroxy propanoic acid ethyl ester	ME	0.00	38.26	53.30	164.14	0.00	492.76	3.80	17.86	0.00	28.04	Ms
30.39	1742	Hexanoic acid ethyl ester	ME	0.00	3.68	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00	MS + LRI
Tot.				1345.07	7522.94	979.74	9424.31	586.21	16929.22	570.58	1853.89	0.00	6783.66	

TABLE 3: Continued.

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₂		T ₄		T ₅		T ₆		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	Min	Max	Min	Max	
<i>Sulfur compounds (6)</i>														
2.86	648	Sulfur oxide	S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	8.76	MS + LRI
3.80	685	Allyl methyl sulfide	S	482.66	6499.78	296.74	1764.14	115.64	11593.25	835.35	836.38	133.51	2404.39	MS + LRI
23.12	1453	Sulfur compound	S	0.00	2.11	0.00	0.00	0.00	2.59	0.00	0.00	0.00	0.00	Ms
27.98	1646	Sulfur compound	S	0.56	5.75	2.12	10.12	0.00	0.00	0.00	1.19	2.92	64.32	Ms
28.3	1659	Diallyl sulfone	S	0.00	13.52	0.00	18.18	0.00	744.68	0.00	7.02	0.00	0.00	MS + LRI
40.76	2154	Decanethiol	S	0.00	11.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
Tot.				483.22	6532.34	298.86	1792.44	115.64	12340.52	842.37	844.59	136.43	2477.47	Ms
<i>Lactones (2)</i>														
26.04	1569	2(3H)-Furanone, dihydro	LO	7.12	10.92	9.68	48.62	2.49	139.58	2.73	4.79	0.46	55.14	MS + LRI
27.57	1630	Lactone	MI	0.00	1.65	0.00	0.00	0.00	32.91	0.00	0.00	0.00	0.00	Ms
Tot.				8.77	12.57	9.68	48.62	2.49	172.49	2.73	4.79	0.46	55.14	

^aRetention time of volatile compounds. ^bKovats index calculated for RTX-WAX capillary column (Castello, 1999) [14]. ^cOrigin: F (carbohydrate fermentation); AC (amino acid catabolism); LO (lipid oxidation); ME (microbial esterification); S (spices and condiments); MI (miscellaneous; contaminants, unknown). ^dRipening time according to experimental plan. ^eMinimum extracted quantities (ng 4-methyl-2-pentanone equivalents g salami⁻¹). Value 0 means that trace amounts were detected (<0.1 ng g⁻¹). ^fMaximum extracted quantities (ng 4-methyl-2-pentanone equivalents g salami⁻¹). Value 0 means that trace amounts were detected (<0.1 ng g⁻¹). ^gMS + LRI, mass spectrum, and LRI agree with those of authentic compounds; ms + lri, mass spectrum, and LRI in agreement with the literature; mass spectrum agrees with spectrum in the NIST library Mass Spectral Database.

TABLE 4: Supplementary material V. Volatile compounds detected in "Filetto Baciato" salami during ripening (ng g⁻¹).

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₁		T ₂		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	
<i>Terpenes (17)</i>										
5.55	755	α -Pinene	S	20.65	25.69	36.63	791.65	0.00	95.20	MS + LRI
8.81	884	β -Pinene	S	25.99	72.45	0.00	50.48	138.03	1384.00	MS + LRI
9.79	923	Sabinene	S	0.00	51.02	7.84	735.94	19.92	1316.24	MS + LRI
11.20	979	3-Carene	S	0.00	98.43	292.32	2246.35	771.73	3978.16	MS + LRI
12.05	1013	α -Phellandrene	S	0.00	1.89	20.21	177.46	54.41	316.97	MS + LRI
12.51	1031	β -Mircene	S	0.00	3.75	14.98	146.02	39.23	258.11	MS + LRI
13.69	1078	Limonene	S	20.10	22.28	108.44	1178.92	282.31	2082.59	MS + LRI
16.51	1190	γ -Terpinene	S	0.00	0.00	0.00	39.94	0.00	70.46	MS + LRI
16.74	1199	Cymene	S	0.00	0.00	11.68	182.24	30.73	318.32	MS + LRI
20.84	1362	Fenchone	S	0.00	2.26	0.00	2.29	0.00	5.19	MS + LRI
22.26	1419	Copaene	S	0.00	0.00	0.67	5.72	1.50	9.99	MS + LRI
22.46	1427	Terpen	S	0.00	0.00	0.00	15.73	0.00	27.48	ms
22.98	1447	Terpen	S	0.00	0.00	0.00	17.02	0.00	29.32	ms
23.31	1460	Canphor	S	0.00	0.00	0.00	32.78	0.00	56.49	MS + LRI
24.37	1503	Terpen	S	1.20	13.01	1.99	423.55	4.80	733.92	ms
25.13	1533	trans- β -Caryophyllene	S	0.00	10.37	49.71	826.57	118.47	1391.62	MS + LRI
26.75	1597	Humulene	S	0.00	0.00	1.28	11.08	3.00	19.82	MS + LRI
Tot.				67.94	301.15	545.75	6883.74	1464.13	12093.88	
<i>Aldehydes (2)</i>										
20.50	1349	Nonanal	LO	0.00	0.00	4.56	26.24	10.84	45.64	MS + LRI
25.86	1562	Benzenacetaldehyde	AC	0.00	0.00	13.29	68.57	32.60	122.71	MS + LRI
Tot.				0.00	0.00	5.90	94.81	43.44	168.35	
<i>Ketones (4)</i>										
2.12	618	2-Propanone	MI	0.00	0.00	97.39	493.34	256.34	835.77	MS + LRI
2.91	650	2-Butanone	F	0.00	0.00	106.31	1387.93	267.35	2484.82	MS + LRI
16.98	1209	3-Hydroxy-2-butanone	F	0.00	19.53	0.00	1365.82	0.00	2418.43	MS + LRI
20.92	1365	2-Nonanone	LO	0.00	0.00	0.00	10.24	0.00	17.85	MS + LRI
Tot.				0.00	0.00	203.70	3257.33	523.69	5756.87	

TABLE 4: Continued.

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₁		T ₂		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	
<i>Alcohols (15)</i>										
3.49	673	Ethanol	F	0.00	1048.40	122.71	6118.70	306.49	10849.91	MS + LRI
5.97	771	2-Butanol	F	0.00	0.00	25.67	459.59	74.57	831.53	MS + LRI
10.9	967	2-Methyl-1-propanol	AC	0.00	28.74	0.00	0.00	0.00	0.00	MS + LRI
13.40	1067	Alcohol	MI	0.00	10.47	0.00	1100.83	0.00	1752.47	ms
14.55	1112	Alcohol	MI	0.00	3.27	0.00	0.00	0.00	0.00	ms
14.75	1120	3-Methyl-1-butanol	AC	0.00	123.69	14.11	154.43	38.10	271.58	MS + LRI
16.87	1204	3-Methyl-3-buten-1-ol	AC	0.00	0.00	0.00	29.60	0.00	51.69	MS + LRI
16.36	1184	1-Pentanol	LO	0.00	0.00	1.03	1705	2.80	30.05	MS + LRI
18.68	1276	3-Methyl-2-buten-1-ol	AC	0.00	0.00	0.44	54.84	1.11	96.30	MS + LRI
18.85	1283	Alcohol	MI	0.00	0.00	0.00	17.40	0.00	30.50	MS + LRI
19.53	1310	1-Hexanol	LO	0.00	0.00	2.62	15.71	6.59	27.39	ms
22.04	1410	1-Octen-3-ol	LO	0.00	0.00	1.85	10.27	4.75	17.81	MS + LRI
23.09	1452	2-Ethyl-1-hexanol	MI	0.00	0.00	0.00	39.98	0.00	68.54	MS + LRI
23.99	1487	Alcohol	ME	0.00	0.00	0.00	80.67	0.00	144.95	MS + LRI
31.05	1768	Benzenethanol	MI	0.00	0.00	0.00	10.54	0.00	18.48	MS + LRI
Tot.				0.00	1214.57	168.43	8109.61	434.41	14191.20	
<i>Free fatty acids (7)</i>										
21.61	1393	Acetic acid		88.19	144.31	30.18	2017.36	67.93	3533.31	MS + LRI
23.71	1476	Propanoic acid		0.00	1.90	0.00	31.71	0.00	55.38	MS + LRI
24.99	1527	2-Methyl-propanoic acid		0.00	1.04	0.00	46.84	0.00	82.87	MS + LRI
26.22	1576	Butanoic acid		5.51	24.77	0.00	162.43	0.00	283.71	MS + LRI
27.09	1611	3-Methyl butanoic acid		1.40	1.68	0.00	81.99	0.00	144.55	MS + LRI
28.34	1660	Pentanoic acid		0.00	0.51	0.00	0.00	0.00	0.00	MS + LRI
29.80	1718	Hexanoic acid		0.71	2.86	0.00	16.99	0.00	30.28	MS + LRI
Tot.				95.81	177.07	30.18	2357.32	67.93	4130.10	

TABLE 4: Continued.

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₁		T ₂		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	
3.08	656	Acetic acid ethyl ester	ME	75.96	138.53	0.00	0.00	0.00	0.00	MS + LRI
Tot.				75.96	138.53	0.00	0.00	0.00	0.00	
3.80	685	<i>Sulfur compounds</i> (4)	S	0.00	80.77	24.34	1554.58	72.00	2742.01	MS + LRI
17.04	1211	Allyl methyl sulfide	S	0.00	0.00	5.05	62.31	13.03	107.33	MS + LRI
22.59	1432	Dithiopentane	S	0.00	0.00	0.00	61.81	0.00	106.95	ms
28.3	1659	Sulfur compound	S	0.00	0.72	0.00	35.92	0.00	62.22	MS + LRI
Tot.				0.00	81.49	29.39	1714.62	85.03	3018.51	
28.62	1671	<i>Nitrogen compounds</i> (1)	MI	0.00	0.00	0.00	90.89	0.00	156.80	ms
Tot.				0.00	0.00	0.00	90.89	0.00	156.80	
26.04	1569	<i>Lactones</i> (1)	LO	0.00	2.29	0.00	0.00	0.00	0.00	MS + LRI
Tot.				0.00	2.29	0.00	0.00	0.00	0.00	

^aRetention time of volatile compounds. ^bKovats index calculated for RTX-WAX capillary column (Castello, 1999) [14]. ^cOrigin: F (carbohydrate fermentation); AC (amino acid catabolism); LO (lipid oxidation); ME (microbial esterification); S (spices and condiments); MI (miscellaneous; contaminants, unknown). ^dRipening time according to experimental plan. ^eMinimum extracted quantities (ng 4-methyl-2-pentanone equivalents g salami⁻¹). Value 0 means that trace amounts were detected (<0.1 ng g⁻¹). ^fMaximum extracted quantities (ng 4-methyl-2-pentanone equivalents g salami⁻¹). Value 0 means that trace amounts were detected (<0.1 ng g⁻¹). ^gMS + LRI, mass spectrum, and LRI in agreement with those of authentic compounds; ms + lri, mass spectrum, and LRI in agreement with the literature; mass spectrum agrees with spectrum in the NIST library Mass Spectral Database.

TABLE 5: Volatile compounds detected in "Nobile del Giarolo" salami during ripening (ng g⁻¹).

RT ^a	LR ^b	Compounds	Origin ^c	T ₀ ^d		T ₁		T ₂		T ₃		T ₄		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	Min	Max	Min	Max	
<i>Terpenes (20)</i>														
5.55	755	α -Pinene	S	0.00	286.01	0.00	2367.21	210.31	1081.54	210.31	655.96	0.00	6752.46	MS + LRI
5.84	766	α -Thujene	S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	773.52	MS + LRI
8.81	884	β -Pinene	S	5.13	448.13	15.92	4000.17	200.93	3171.51	628.94	1020.19	0.00	2029.65	MS + LRI
9.79	923	Sabinene	S	0.00	0.00	0.00	0.00	0.00	21.68	0.00	68.34	0.00	3821.96	MS + LRI
10.62	956	Terpen	S	0.00	29.41	0.00	0.00	0.00	382.73	0.00	3248.16	0.00	0.00	ms
11.20	979	3-Carene	S	0.00	4062.38	1686.41	141.61	655.80	955.02	1035.26	2165.81	0.00	9440.59	MS + LRI
12.05	1013	α -Phellandrene	S	0.00	996.05	157.89	90.44	30.92	42.83	45.39	97.00	0.00	574.63	MS + LRI
12.51	1031	β -Mircene	S	0.00	760.74	85.42	4.00	27.73	36.28	39.51	86.71	0.00	587.80	MS + LRI
12.79	1042	α -Terpinene	S	0.00	24.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	66.61	MS + LRI
13.69	1078	Limonene	S	0.00	4667.83	6.57	897.05	200.33	629.05	511.25	636.40	0.00	5448.64	MS + LRI
13.99	1090	Terpen	S	0.00	33.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	637.33	ms
16.61	1194	p-Cymene	S	0.00	636.21	0.00	0.00	0.00	28.82	0.00	91.31	0.00	275.18	MS + LRI
17.06	1212	Terpen	S	56.01	56.01	45.73	3.59	0.00	12.32	0.00	27.40	0.00	34.47	ms
22.31	1421	Terpen	S	15.82	15.82	24.92	0.25	0.00	9.17	0.00	79.47	0.00	125.49	ms
24.24	1497	Terpen	S	21.44	21.44	22.80	3.75	0.00	17.03	0.00	4.45	0.00	141.82	ms
24.46	1506	Terpen	S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.23	ms
25.13	1533	trans- β -Caryophyllene	S	0.35	35.63	1.76	4.50	0.00	14.09	0.00	46.61	0.00	39.47	MS + LRI
25.26	1538	Terpineol	S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	88.89	MS + LRI
27.16	1613	Terpen	S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.30	ms
Tot.				98.75	12073.70	2047.42	7512.57	1326.02	6402.07	2470.66	8227.81	0.00	30864.04	
<i>Aldehydes (4)</i>														
8.18	859	Hexanal	LO	0.00	6.78	29.93	104.69	12.57	72.86	17.25	40.93	0.00	128.46	MS + LRI
20.50	1349	Nonanal	LO	0.00	0.00	0.00	0.00	0.00	9.08	0.00	29.57	0.00	84.54	MS + LRI
23.27	1459	Benzaldehyde	AC	1.13	79.31	4.49	95.17	58.98	277.60	0.00	130.98	0.00	431.17	MS + LRI
25.86	1562	Benzenacetaldehyde	AC	0.00	677.86	0.00	117.94	0.00	1103.61	192.62	197.08	0.00	990.64	MS + LRI
Tot.				1.13	763.95	34.42	317.80	71.55	1463.15	209.87	398.56	0.00	1634.81	

TABLE 5: Continued.

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₁		T ₂		T ₃		T ₄		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	Min	Max	Min	Max	
<i>Ketones (9)</i>														
2.12	618	2-Propanone	MI	35.77	260.91	23.67	4254.54	31714	3168.90	181.12	1003.98	0.00	939.69	MS + LRI
2.91	650	2-Butanone	F	0.00	1035.38	0.00	24868.69	0.00	43993.89	0.00	3561.24	0.00	6257.35	MS + LRI
4.77	724	2-Pentanone	LO	0.00	0.00	0.00	63.08	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
14.01	1091	2-Heptanone	LO	0.00	63.11	0.00	13.65	0.00	10.02	0.00	33.22	0.00	0.00	MS + LRI
16.27	1181	3-Octanone	LO	0.00	28.28	0.00	2.95	3.22	15.18	3.77	10.96	0.00	33.42	MS + LRI
16.98	1209	3-Hydroxy-2-butanone	F	4.24	571.19	0.00	653.99	11.87	1428.91	37.81	51.11	0.00	180.04	MS + LRI
18.91	1286	6-Methyl-5-hepten-2-one	MI	0.00	0.00	0.00	0.00	0.00	2.10	0.00	6.85	0.00	10.06	MS + LRI
20.92	1365	2-Nonanone	LO	0.00	177.82	0.00	3.39	0.00	32.67	0.00	0.00	0.00	0.00	MS + LRI
24.5	1508	4-Hydroxy-2-butanone	MI	0.00	0.00	0.00	76.68	0.00	0.00	0.00	17.58	0.00	0.00	MS + LRI
Tot.				40.01	2136.69	23.67	29936.97	332.23	48651.67	222.70	4684.94	0.00	7420.56	
<i>Alcohols (13)</i>														
3.49	673	Ethanol	F	176.04	2617.49	492.29	5961.66	1738.35	6176.15	988.74	5503.44	0.00	3687.51	MS + LRI
5.97	771	2-Butanol	F	0.00	0.00	0.00	279.15	0.00	956.62	0.00	5015.19	0.00	723.36	MS + LRI
7.22	821	1-Propanol	LO	0.00	0.00	0.00	202.90	0.00	381.67	0.00	184.94	0.00	0.00	MS + LRI
10.9	967	2-Methyl-1-propanol	AC	0.00	11.08	28.84	49.33	0.00	11.23	0.00	35.75	0.00	0.0	MS + LRI
14.75	1120	3-Methyl-1-butanol	AC	0.00	0.00	0.00	329.06	36.95	269.77	159.35	861.51	0.00	325.12	MS + LRI
16.87	1204	3-Methyl-3-buten-1-ol	AC	0.00	0.00	20.49	85.84	0.00	2.62	0.00	8.45	0.00	0.00	MS + LRI
16.96	1208	1-Pentanol	LO	0.00	1.92	0.00	11.15	0.00	10.41	0.00	33.48	0.00	26.42	MS + LRI
18.39	1265	Alcohol	MI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.86	ms
18.53	1270	3-Methyl-2-buten-1-ol	AC	0.00	0.00	0.00	19.87	0.00	0.00	0.00	0.00	0.00	2.75	MS + LRI
19.42	1306	2,3-Butanediol	ME	0.00	0.00	0.00	3.01	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
19.53	1310	1-Hexanol	LO	0.00	75.08	0.00	6.32	0.00	35.96	0.00	116.95	0.00	17.63	MS + LRI
22.04	1410	1-Octen-3-ol	LO	1.19	190.82	5.27	7.07	0.00	28.42	0.00	92.39	0.00	62.06	MS + LRI
31.05	1768	Benzenethanol	MI	0.00	53.82	0.00	6.82	0.00	14.92	0.00	48.69	0.00	48.21	MS + LRI
Tot.				177.23	2950.21	546.89	6962.18	1175.30	7887.77	1148.09	11900.79	0.00	4901.92	
<i>Free fatty acids (8)</i>														
21.61	1393	Acetic acid		249.63	870.62	303.09	22463.67	1025.56	6761.99	765.92	3197.54	0.00	2707.34	MS + LRI
23.71	1476	Propanoic acid		2.76	11.21	2.27	72.15	13.54	119.99	37.23	44.71	0.00	31.52	MS + LRI
24.99	1527	2-Methyl-propanoic acid		0.00	1.89	1.19	35.26	2.74	26.57	4.86	8.53	0.00	0.00	MS + LRI
26.22	1576	Butanoic acid		12.56	58.34	17.23	181.33	33.22	111.91	19.09	103.77	0.00	0.00	MS + LRI
27.09	1611	3-Methyl butanoic acid		2.54	30.87	2.47	111.75	7.42	79.42	11.97	23.15	0.00	0.00	MS + LRI
28.38	1662	Pentanoic acid		0.00	0.24	0.63	6.74	0.00	4.91	1.71	0.73	0.00	0.00	MS + LRI
29.80	1718	Hexanoic acid		1.13	40.16	2.56	19.92	5.11	20.51	6.58	16.21	0.00	12.55	MS + LRI
32.75	1836	Octanoic acid		0.00	0.00	0.00	0.00	0.00	1.47	0.00	4.79	0.00	6.19	MS + LRI
Tot.				268.62	1013.33	329.44	22890.82	1087.59	7126.77	847.36	3399.43	0.00	2757.60	

TABLE 5: Continued.

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		T ₁		T ₂		T ₃		T ₄		Method of identification ^g
				Min ^e	Max ^f	Min	Max	Min	Max	Min	Max	Min	Max	
<i>Hydrocarbons (5)</i>														
1.51	500	Pentane	LO	0.00	20.14	0.00	18.21	27.32	1177.39	0.00	85.37	0.00	0.00	MS + LRI
1.66	600	Hexane	LO	0.00	20151.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
6.08	776	Toluene	MI	0.00	4.26	0.00	0.00	312.75	763.96	0.00	2513.08	0.00	2127.98	MS + LRI
11.61	995	o-Xylene	MI	0.00	33.56	0.00	0.00	1.42	45.29	0.00	0.00	0.00	0.00	MS + LRI
12.04	1013	p-Xylene	MI	0.65	65.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
Tot.				0.65	20275.64	0.00	18.21	341.49	1986.64	0.00	2598.45	0.00	2127.98	
<i>Esters (2)</i>														
2.56	636	Acetic acid methyl ester	ME	0.00	604.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	MS + LRI
3.08	656	Acetic acid ethyl ester	ME	25.15	728.97	28.47	167.91	57.05	69.93	75.03	231.11	0.00	0.00	MS + LRI
Tot.				25.15	1333.37	28.47	167.91	57.05	69.93	75.03	231.11	0.00	0.00	
<i>Sulfur compounds (4)</i>														
1.95	612	Carbon disulfide	S	1.34	34.08	0.00	0.00	0.00	0.00	0.00	381.66	0.00	0.00	MS + LRI
3.80	685	Allyl methyl sulfide	S	20.81	590.28	0.00	26.37	0.00	290.23	0.00	942.49	0.00	1098.17	MS + LRI
5.34	746	Sulfur compound	S	0.00	0.00	0.00	1.50	0.00	66.23	20.72	217.64	0.00	110.10	ms
27.85	1641	Dimethyl disulfide	S	0.00	21.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.76	MS + LRI
Tot.				22.15	645.92	0.00	27.87	0.00	356.46	20.72	1541.79	0.00	1242.03	
<i>Nitrogen compounds (2)</i>														
18.45	1267	2,6-Dimethyl pirazin	MI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.82	MS + LRI
28.12	1652	Acetamide	MI	0.00	0.00	0.00	0.00	0.00	1.35	0.00	4.30	0.00	6.64	MS + LRI
Tot.				0.00	0.00	0.00	0.00	0.00	1.35	0.00	4.30	0.00	12.46	
<i>Lactones (1)</i>														
26.03	1569	2(3H)-Furanone, dihydro	LO	0.00	0.54	1.05	21.88	9.07	21.00	4.85	28.52	0.00	0.00	MS + LRI
Tot.				0.00	0.54	1.05	21.88	9.07	21.00	4.85	28.52	0.00	0.00	
<i>Ethers (1)</i>														
27.99	1646	Dimethoxy benzene	MI	0.00	54.88	0.00	0.00	1.77	7.39	0.00	5.81	0.00	9.05	MS + LRI
Tot.				0.00	54.88	0.00	0.00	1.77	7.39	0.00	5.81	0.00	9.05	

^aRetention time of volatile compounds. ^bKovats index calculated for RTX-WAX capillary column (Castello, 1999) [14]. ^cOrigin: F (carbohydrate fermentation); AC (amino acid catabolism); LO (lipid oxidation); ME (microbial esterification); S (spices and condiments); MI (miscellaneous; contaminants, unknown). ^dRipening time according to experimental plan. ^eMinimum extracted quantities (ng 4-methyl-2-pentanone equivalents g salami⁻¹). Value 0 means that trace amounts were detected (<0.1 ng g⁻¹). ^fMaximum extracted quantities (ng 4-methyl-2-pentanone equivalents g salami⁻¹). Value 0 means that trace amounts were detected (<0.1 ng g⁻¹). ^gMS + LRI, mass spectrum, and LRI agree with those of authentic compounds; ms + lri, mass spectrum, and LRI in agreement with the literature; mass spectrum agrees with spectrum in the NIST library Mass Spectral Database.

TABLE 6: Volatile compounds detected in “Salamini di Mandrogne” salami (ng g⁻¹).

RT ^a	LRI ^b	Compounds	Origin ^c	T ₀ ^d		Method of identification ^e
				Min ^e	Max ^f	
<i>Terpenes (14)</i>						
6.14	755	α -Pinene	S	0.00	675.10	MS + LRI
9.75	884	β -Pinene	S	31.35	1636.87	MS + LRI
12.17	979	3-Carene	S	0.00	204.03	MS + LRI
12.98	1013	α -Phellandrene	S	0.00	7.32	MS + LRI
13.45	1031	β -Myrcene	S	0.00	55.99	MS + LRI
14.56	1078	Limonene	S	35.00	722.61	MS + LRI
14.87	1125	Eucalyptol	S	0.00	78.98	MS + LRI
16.51	1190	γ -Terpinene	S	0.00	36.01	MS + LRI
23.73	1477	Canphor	S	0.00	5.45	MS + LRI
24.74	1517	Linalool	S	5.92	26.52	MS + LRI
25.70	1533	trans- β -Caryophyllene	S	14.58	219.65	MS + LRI
27.17	1614	Humulene	S	0.00	7.98	MS + LRI
27.70	1635	Terpen	S	0.00	1.16	MS + LRI
34.20	1893	Eugenol	S	0.00	4.09	MS + LRI
Tot.				86.85	3681.76	
<i>Ketones (3)</i>						
4.92	743	2,3-Butanedione	F	0.00	446.48	MS + LRI
14.04	1092	2-Octanone	LO	0.00	1.70	MS + LRI
17.78	1209	3-Hydroxy-2-butanone	F	579.38	52656.86	MS + LRI
Tot.				579.38	53105.04	
<i>Alcohols (11)</i>						
3.85	673	Ethanol	F	110.24	15032.00	MS + LRI
10.24	967	2-Methyl-1-propanol		0.00	325.00	MS + LRI
11.54	993	1-Methoxy-2-propanol		0.00	39.18	MS + LRI
12.68	1038	1-Butanol	F	0.00	35.03	MS + LRI
13.86	1085	Alcohol		0.00	2.17	Ms
15.49	1120	3-Methyl-1-butanol	AC	0.00	4116.36	MS + LRI
16.82	1202	Alcohol		0.00	2.62	Ms
17.00	1208	1-Pentanol	LO	0.00	140.41	MS + LRI
20.11	1310	1-Hexanol	LO	0.00	201.62	MS + LRI
22.58	1431	1-Octen-3-ol	LO	4.63	115.49	MS + LRI
Tot.				114.87	20009.88	
<i>Free fatty acids (8)</i>						
22.27	1393	Acetic acid		131.03	626.36	MS + LRI
23.53	1469	Formic acid		0.00	1.20	MS + LRI
24.31	1476	Propanoic acid		0.00	14.52	MS + LRI
25.00	1527	2-Methyl-propanoic acid		0.00	12.57	MS + LRI
26.22	1576	Butanoic acid		72.00	8.86	MS + LRI
27.08	1611	3-Methyl-butanoic acid		7.12	37.29	MS + LRI
28.39	1662	Pentanoic acid		0.00	11.77	MS + LRI
30.38	1718	Hexanoic acid		4.00	30.96	MS + LRI
Tot.				214.15	743.53	
<i>Sulfur compounds (7)</i>						
4.24	685	Allyl methyl sulfide	S	0.00	947.78	MS + LRI
7.37	827	Mercapto acetone	S	0.00	22.06	MS + LRI
16.18	1177	Sulfur compound	S	0.00	41.57	Ms
23.12	1453	Diallyl disulfide	S	7.78	25.93	MS + LRI
28.30	1659	Sulfur compound	S	0.00	7.04	Ms
29.43	1704	Sulfur compound	S	0.00	0.60	Ms
Tot.				7.78	1044.98	

TABLE 6: Continued.

RT ^a	LRI ^b	Compounds	Origin ^c	T_0^d		Method of identification ^g
				Min ^e	Max ^f	
<i>Lactones (1)</i>						
26.04	1569	2(3H)-Furanone, dihydro	LO	1.61	18.29	MS + LRI
Tot.				1.61	18.29	

^aRetention time of volatile compounds. ^bKovats index calculated for RTX-WAX capillary column (Castello, 1999) [14]. ^cOrigin: F (carbohydrate fermentation); AC (amino acid catabolism); LO (lipid oxidation); ME (microbial esterification); S (spices and condiments); MI (miscellaneous: contaminants, unknown). ^dRipening time according to experimental plan. ^eMinimum extracted quantities (ng 4-methyl-2-pentanone equivalents g salami⁻¹). Value 0 means that trace amounts were detected (<0.1 ng g⁻¹). ^fMaximum extracted quantities (ng 4-methyl-2-pentanone equivalents g salami⁻¹). Value 0 means that trace amounts were detected (<0.1 ng g⁻¹). ^gMS + LRI, mass spectrum, and LRI agree with those of authentic compounds; ms + lri, mass spectrum, and LRI in agreement with the literature; mass spectrum agrees with spectrum in the NIST library Mass Spectral Database.

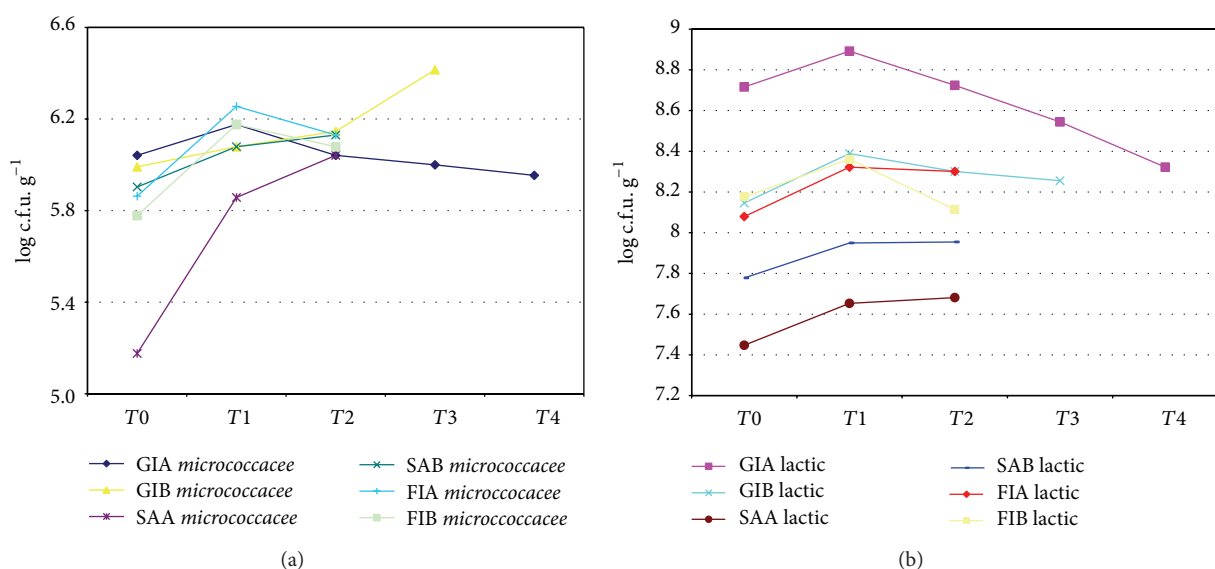


FIGURE 2: (a) Evolution of *Micrococcaceae* bacteria during ripening of *Giarolo*, *Tipico Tortonese*, and *Filetto Baciato* salami (log c.f.u. g⁻¹). (b) Evolution of lactic acid bacteria during ripening of *Giarolo*, *Tipico Tortonese*, and *Filetto Baciato* salami (log c.f.u. g⁻¹).

especially acetic acid, 2,3-butanedione (diacetyl), and 3-hydroxy-2-butanone (acetoin). The result can be related to the high content of *Lactobacillus*. 3-Methyl-1-butanol (likely formed by reduction of the corresponding aldehydes) and ethanol were the most abundant alcohols found in *Salamini di Mandrogne*. Some sulfur-containing compounds were also identified, the most abundant being allyl methyl sulphide, while the most abundant terpenes were δ -3-carene and β -caryophyllene, which probably derive from black pepper, cloves, and nutmeg used in the preparation [32–36].

3.4. Multivariate Analysis. Data were arranged in a 42 × 240 matrix (42 being the samples at different ripening times and 240 the variables). All variables expressed as concentrations and percentages were corrected for the amount of water present in each sample.

PCA on the Overall Dataset. PCA was performed on the overall dataset (42 × 248) after autoscaling and elimination of sample MUA4, resulting to be an outlier from a first analysis. The first two PCs explain about 22% of the overall variance, indicating a low correlated and redundant data structure.

Figure 3(a) represents the score plot of the first two PCs; three main groups of samples can be identified:

- group 1: constituted by almost all samples belonging to *Muletta* type;
- group 2: constituted by almost all samples belonging to *Salamini di Mandrogne* type;
- group 3: constituted by almost all other samples.

The samples appear separated according to the type of product. The two most different groups are those characterised by the most different maximum ripening times: *Salamini di Mandrogne* (sold fresh, group 2) and *Muletta* (six-month ripening, group 1). Moreover, *Salamini di Mandrogne* are produced with veal meat and are well separated from those produced with pork meat.

No trend as a function of ripening time can be observed.

PCA at the Selling Stage. A further PCA was then performed on the data collected at the selling stage, with the aim to further investigate the differences between the samples at the time when they are consumed. PCA was performed, after

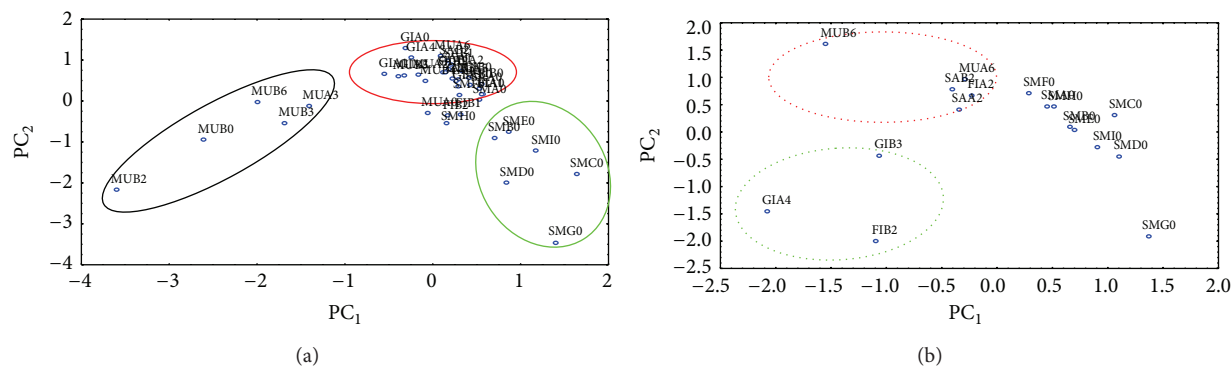


FIGURE 3: (a) Results of PCA applied to the overall dataset after autoscaling: score plot of PC_1 versus PC_2 ; (b) results of PCA applied to the samples analysed at the selling time after autoscaling: score plot of PC_1 versus PC_2 .

autoscaling, on the 17×214 matrix that contains all products, including *Salamini di Mandrogne*. Some variables showing a null variance for this subset of samples were eliminated from the dataset. The first two PCs (29% total variance) were considered significant and again indicate a low correlated data structure.

In the corresponding score plot (Figure 3(b)), *Salamini di Mandrogne* are grouped at positive scores along PC_1 , while the other samples lay at negative values. PC_1 seems mostly related to the ripening period that the samples undergo until selling, since the fresh products are at positive scores, while the most ripened ones are located at large negative scores along the first PC. This last group can be further divided in two groups according to the positive or negative score on PC_2 . PC_2 is therefore able to separate *Nobile del Giarolo* (negative scores on PC_2) from the other samples that came all from a zone of the Alessandria province around Tortona (positive scores on the same PC). The analysis of the corresponding loadings allowed the identification of the main differences between the groups identified. *Salamini di Mandrogne* are characterised, as expected, by large values of moisture. Moreover, they are characterised by a small aroma of pepper (α -phellandrene, δ limonene, α -pinene, and β -myrcene) and garlic (diallyl disulphide), a low content of spices (3-carene, sabinene), small amounts of biogenic amines, and a low oxidation of unsaturated fatty acids (hexanal). The other samples are characterised by an opposite behaviour, showing a larger contribution of variables related to aroma. These samples, however, are separated in two groups along PC_2 : *Nobile del Giarolo* is characterised by a larger amount of spices (β -myrcene, p-cymene) and pepper (α -pinene, α -phellandrene, sabinene) and a higher carbohydrate fermentation (2 butanol, 2-butanone) [7, 12]. The samples from the Tortona area instead (positive scores on PC_2) are characterised by a larger content of fats and tyramine. The analysis therefore points out the existence of three main groups of samples: their differences are mainly related to the ripening period they undergo (accounted for by PC_1) and to the ingredients used (different aromas and starting meat mixture). A further Cluster Analysis was applied on this dataset: Figure 4 represents the dendrogram obtained by the Ward method (Euclidean distances) applied

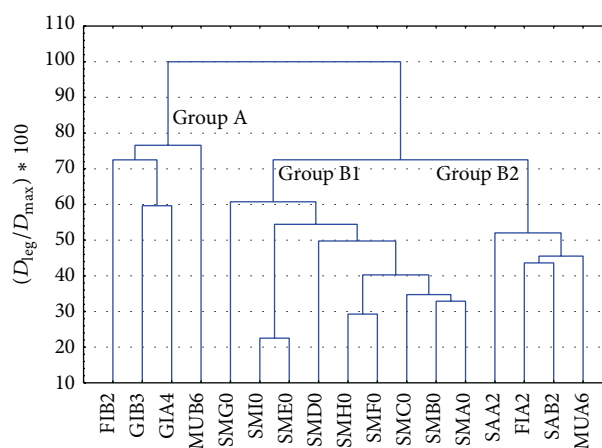


FIGURE 4: Dendrogram calculated by the Ward method with Euclidean distances on the dataset constituted by all the samples characterised at the selling time, after autoscaling. Samples are reported on the x-axis while distance between samples/groups is reported as percentage on the y-axis.

to the dataset after autoscaling. The dendrogram reports the samples on the x-axis; two main groups can be detected: the first one (group A) consists of the samples showing the largest negative scores along PC_1 . The other group can be divided in two subgroups (groups B1 and B2): group B1 contains all *Salamini di Mandrogne* samples that showed the largest positive scores on PC_1 ; group B2 instead is constituted by the other samples, showing intermediate scores along the first PC. Cluster Analysis therefore confirms the results obtained by PCA, showing that the most important information regards changes in the chemical composition that can be ascribed to ripening.

4. Conclusions

This study is focused on the characterisation of typical salami products of the Alessandria province territory (North West of Italy). Seventeen small or medium salami producers from this area were involved in the study and provided six types of typical salami. Samples were characterised for what regards

the aroma component and nutritional feature with a double aim: obtaining a screening of the characteristics of different products and following their evolution along with ripening.

The overall results obtained point out that the products investigated do not deviate from analogous European products. The attention was then focussed on the production and selling times to provide a characterisation of the samples at the moment when they are prepared and finally sold. The analysis was carried out with the help of multivariate statistical tools, as Principal Component Analysis and Cluster Analysis. The results show the existence of three main groups of samples: *Salamini di Mandrogne*, *Muletta*, and *Nobile del Giarolo*. Among them, *Salamini di Mandrogne* certainly appear as quite different products since they are sold fresh and present a particular recipe constituted mainly from veal meat: these features is reflected in a low content of biogenic amines, a low carbohydrate fermentation, and a low content of aroma components related to spices. The other two products are commercialised after a ripening period of four months for *Nobile del Giarolo* and of six months for *Muletta*. These two products can be differentiated mainly regarding carbohydrate fermentation and aroma component related to spices (larger in *Nobile del Giarolo*) and fats and content of tyramine (larger in *Muletta*). Some considerations can also be drawn with respect to the nutritional characterization of the samples observing BA content, as their profile can be related to a good or bad ripening working out, according to which BA is predominant. Tyramine is usually the dominant amine in salami and is considered an index of correct ripening working out: in the investigated samples, its values are in agreement with other traditional Italian [34] and European fermented sausages [1], even if it is not always the dominant amine: for many samples histamine is the most abundant one with concentration ranges larger than the law limit (100 mg Kg^{-1}). In conclusion results obtained by this study confirm that the determination of various typologies of parameters (volatile compounds, amino acids, chemical and microbiological parameters, and biogenic amine) may be important to assess the quality of raw and final products in terms of optimal condition of production and preservation of typical meat products during their shelf-life. The entire approach could provide a basis for a possible PDO or PGI label assignment.

Conflict of Interests

The authors declare that they have no conflict of interests.

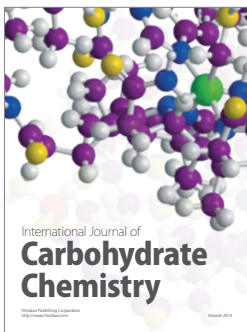
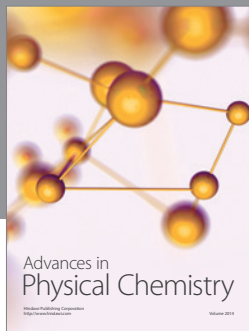
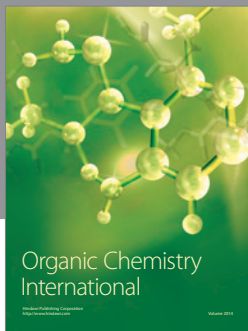
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