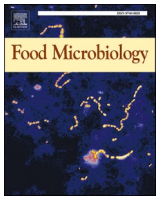
Food Microbiology 127 (2025) 104684

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm



Replacing preservative E 252 with powdered dried sumac (*Rhus coriaria* L.) fruits in "Suino Nero dei Nebrodi" salamis: Effects on microbiological, physicochemical, and antioxidant properties

Gabriele Busetta [a], Giuliana Garofalo [a], Marialetizia Ponte [a], Marcella Barbera [b], Antonio Alfonzo [a], Elena Franciosi [c], Nicola Francesca [a], Giuseppe Frusteri [d], Daniela Piazzese [b], Adriana Bonanno [a], Rosario Schicchi [a], Giancarlo Moschetti [a], Raimondo Gaglio [a,*], Luca Settanni [a]

[a] Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, Bldg. 5, 90128, Palermo, Italy
[b] Department of Earth and Marine Sciences (DiSTeM), University of Palermo, Via Archirafi, 90123, Palermo, Italy
[c] Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, 38098, San Michele all'Adige, Italy
[d] Organizzazione Prodotto Allevatori Nebrodi (OPAN), Via Provinciale 172, 98070, Rocca di Capri Leone, Italy

ARTICLE INFO

Keywords:
Rhus coriaria fruit powder
Fermented meat
Microbiological safety
Physicochemical properties
Volatile organic compounds
Sensory traits

ABSTRACT

The aim of this study was to develop health-oriented fermented salamis by replacing synthetic preservative (E 252) with dried Sumac (*Rhus coriaria*) fruit powder (DSFP). The salamis were produced at an industrial scale using meat from the "Suino Nero dei Nebrodi" breed, without adding starter cultures. The experimental design included four different salami productions: CTR, control production without nitrate salt and DSFP; CMC, commercial control production with nitrate salt but without DSFP; EXP1, experimental production without nitrate salt but with DSFP; and EXP2, experimental production with both nitrate salt and DSFP. Plate counts showed that DSFP did not inhibit the growth of lactic acid bacteria (LAB), coagulase-negative staphylococci, and yeasts, all of which reached approximately 7.0 log CFU/g in 45 d ripened salamis. Except for the CTR production, *Escherichia coli* levels decreased to undetectable amounts at 30 d of ripening. Culture-independent methods identified 16 taxonomic groups, with LAB being the predominant group across all trials, comprising 46.05–81.81 % of relative abundance (RA) in 45 d ripened salamis. Physicochemical analysis indicated that adding DSFP increased antioxidant activity by nearly 30 % and reduced primary lipid oxidation to levels comparable to those achieved with nitrate salt. The addition of DSFP in CMC, EXP1, and EXP2 salamis resulted in an approximate 11 % increase in total terpene aromatic profiles. Sensory evaluation indicated that the addition of DSFP did not impact overall acceptability ($p > 0.05$). Therefore, incorporating DSFP in fermented meat production offers a viable alternative to the use of synthetic preservatives.

1. Introduction

The art of producing cured meats is one of humanity's oldest food processes (Leroy et al., 2010). In fact, evidence of cured meat production dates back to the Roman Empire (Lücke, 1998; Wood, 2012). Nowadays, these products are widely consumed worldwide (Blaiotta et al., 2018). Italy, known for its culinary heritage, produces a diverse range of salami, each distinguished by unique ingredients added during processing. For instance, in Sicily's Nebrodi Park (province of Messina), Suino Nero dei Nebrodi salami is crafted. This salami type features the finest cuts of Sicilian Black pig including shoulder, ham, lard, belly, and neck, cut into small pieces "at knifepoint", carefully mixed with fat and seasoned with salt, pepper and spices (Moretti et al., 2004). Additionally, synthetic preservatives such as nitrates (sodium nitrate, E 251; potassium nitrate, E 252) and nitrites (potassium nitrite, E 249; sodium nitrite, E 250) are incorporated, in accordance with Commission Regulation (EC) no. 2108/2023, which allows their use in cured meat products (European Commission, 2023). These additives incorporated into salami formulations serve various purposes, including preventing lipid oxidation, preserving color, and ensuring microbiological safety by warding off

* Corresponding author.
E-mail address: raimondo.gaglio@unipa.it (R. Gaglio).

https://doi.org/10.1016/j.fm.2024.104684
Received 17 September 2024; Received in revised form 19 November 2024; Accepted 21 November 2024
Available online 27 November 2024

pathogenic agents (Majou and Christieans, 2018). On the final point, these nitrogen compounds are primarily used to inhibit the growth of enterobacteria, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium* toxins (European Food Safety Authority and European Centre for Disease Prevention and Control, 2019; Tack et al., 2020). However, despite their functional benefits, the addition of nitrate and nitrite addition in fermented meat productions has raised significant concerns due to their potential to form N-nitroso carcinogen compounds (nitrosamines) (Hammes, 2012). Numerous studies have shown that N-nitrosamines can contribute to the development of specific types of cancer (IARC, 2010; IARC, 2018). Consequently, there is growing interest in food technology to enhance nutritional and health-related qualities while minimizing harmful aspects.

Researchers and the meat industry face a significant challenge: creating innovative formulations that eliminate nitrate from food products without compromising quality and maintain microbiological safety (Alahakoon et al., 2015; Flores and Toldrá, 2021; Stoica et al., 2022). One promising approach involves replacing synthetic nitrites and nitrates with plant-derived alternatives in salami production.

To this purpose, plant-based ingredients such as beetroot, cabbage, celery, young radish, leek, olive leaves, parsley, spinach, and others have been used as alternatives to nitrates and nitrites in salami production (Ferysiuk and Wójciak, 2020). To our knowledge, no previous research has explored replacing synthetic preservatives with dried Sumac (*Rhus coriaria*) fruit powder (DSFP) in fermented salamis.

R. coriaria L., a shrub that grows naturally in the Mediterranean area, is gaining attention for its high antioxidant, antimicrobial, and health-promoting properties (Alsamri et al., 2021; Elagbar et al., 2020). Its fruits, in particular, are rich in organic acids, fibers, flavonoids, minerals, proteins, vitamins, and tannins compared to other parts of the plant (Zannou et al., 2024). Additionally, sumac fruits contain approximately 160 of nitrates and 36 mg/kg DM of nitrites (Özcan and Akbulut, 2008).

This study aims to develop a novel type of spontaneously fermented "Suino Nero dei Nebrodi" salami by substituting E 252 potassium nitrate salts with DSFP. The specific objectives include: (i) assessing microbial populations using both culture-dependent and independent methods throughout salami production; and (ii) characterizing the final salamis in terms of physicochemical properties, antioxidant capacity, volatile organic compounds, and sensory attributes.

2. Materials and methods

2.1. Production of sumac fruit powder

Fruits of *R. coriaria* were harvested in western Sicily, specifically in countryside of Gratteri and Collesano (Palermo province), between September 2023 and November 2023. The fruits were manually picked up (with hands covered by disposable gloves) from plants using dissecting scissors. The harvested fruits were then placed in plastic bags and transported at ambient temperature to the laboratory of Agricultural Microbiology, University of Palermo, within 6–8 h from harvest.

The entire sumac fruits (including both pulp and seeds) were spread out in a single layer on stainless steel trays and dried at 54 °C in a natural convection laboratory oven (mod. E34 WTB-Binder, Tuttlingen, Germany) until a constant weight was achieved. The dried fruits were subsequently milled into a powder (250 μm particles) using the semi-industrial Fritsch Pulverisette 14 mill (Fritsch GmbH, Idar-Oberstein, Germany). The resulting dried sumac fruit powder (DSFP) was transferred to sterile PolySilk® BagLight® 400 bags (Interscience, Saint Nom, France) and stored at room temperature in a dark environment.

2.2. Production of Suino Nero dei Nebrodi salamis

In this study, the meat and fat used exclusively came from Suino Nero dei Nebrodi pigs raised in an "en plein air" breeding system within the natural Nebrodi Park territory (Messina province, Sicily). These pigs were specifically reared in the area of "Bosco dei Caprioli" (38°01′29.78″ N/14°47′02.94″ E, Galati Mamertino, Italy). After slaughtering at the slaughterhouse Nebros Carni S.r.l. located in Mirto (Italy), the half carcasses were refrigerated and transported to the salami factory O.P.A.N. (Organizzazione Prodotto Allevatori Nebrodi) in Rocca di Capri Leone (Italy). There, they were kept for almost 24 h at 0 °C in a cooling chamber (Frigoveneta Spa, Villa Bartolomea, Italy). The deboning process involved manually removing bones from four half carcasses on a sanitized plastic table using Diverfoam SMS Chlor VF18 (GEMAGroup, Montecassiano, Italy) and washing them with red vinegar. The meat, cleansed of tendons and other connective tissues, was roughly chopped together with fat, maintaining a meat-to-fat ratio of 3:1. Finally, the meat/fat mixture was ground once using a grinder machine (COGEMAT S.r.l., Camporotondo Etneo, Italy) equipped with vertical and horizontal wires, resulting in cubes approximately 1 cm × 1 cm in size. Four 5-kg portions of ground meat/fat mixture were combined with a blend of spices, including black pepper (D&V S.r.l., San Floro, Italy) and fennel seeds (Royal Ingredients S.r.l., Castelnuovo Rangone, Italy). Additionally, kitchen salt (Raffineria Sale Scarpa, S. Agata di Militello, Italy), kitchen sugar (Suicrà S.r.l., Saviano, Italy), E 252 (potassium nitrate, Royal Ingredients S.r.l.), and DSFP were included in the recipes. These ingredients were used to obtain four distinct trials: CTR, control production without nitrate salt and DSFP; CMC, commercial control production with nitrate salt but without DSFP; EXP1, experimental production without nitrate salt but with DSFP; and EXP2, experimental production with both nitrate salt and DSFP. The four salami trials were carried out without adding any starter cultures. The meat/fat mixture was blended with the specific ingredients following their respective recipes using a mixer (COGEMAT S.r.l.) for 4 min at speed 1 (2 min of direct rotation and 2 min of reverse rotation). After blending, the mixer was cleaned with hot water (80 °C) to remove any residue from the meat/fat mixture. The resulting mixtures were collected separately in plastic boxes and stuffed into natural beef casings (approximately 4 cm in diameter) using a manual sausage filler mod. 5 Deluxe horizontal (FACEM S.p.A., Torino, Italy). Each salami weighed approximately 500 g, resulting in a total of 10 salamis per trial. All salamis were dried for 7 d and ripened for a total of 45 d following the protocol reported by Gaglio et al. (2016). Two rounds of salami production trials were carried out at the end of April and in mid-May 2024. Samples were collected before mixing (DSFP, casings, fat, and meat), immediately after stuffing (T_0), and during ripening at 15, 30, and 45 d. The analysis involved a total of 8 salamis per sampling time, with two batches of salamis from each of the four trials (32 salamis) for each round of production, resulting in 64 salamis overall. These salamis were transported under refrigeration to the Laboratories of Agricultural Microbiology at the University of Palermo for immediate analysis.

2.3. Microbiological analysis of the salami using culture-dependent approach

All samples collected during Suino Nero dei Nebrodi salami productions underwent microbiological analysis to evaluate the primary microbial groups associated with salami production. The analysis started with the transfer of 25 g of each sample into a sterile bag (BagFilter P, Interscience, Saint Nom, France), which was diluted with 225 mL of Ringer's solution (Sigma Aldrich, Milan, Italy) and homogenized using a stomacher BagMixer® 400 (Interscience) at the highest speed for 2 min. Serial decimal dilutions (1:10) in Ringer's solution were prepared, and the cell suspensions were plated on agar media. Total mesophilic microorganisms (TMM) on Plate Count Agar (PCA) incubated at 30 °C for 72 h (ISO 4833:2003). Mesophilic LAB in Man-Rogosa-Sharpe (MRS) incubated at 30 °C for 48 h (ISO 15214:1998). Yeasts and molds on Dicloran Rose-Bengal Chloramphenicol Agar (DRBC) incubated at 25 °C for 5 d (ISO 21527–1:2008). Members of the Enterobacteriaceae family were detected on Violet Red Bile Glucose Agar (VRBGA) after incubation

at 37 °C for 24 h (ISO 21528–2:2017). Staphylococci coagulase-positive (CPS) and coagulase-negative (CNS) were enumerated on Baird-Parker (BP) agar supplemented with rabbit plasma fibrinogen (RPF) incubated at 37 °C for 48 h (ISO 6888–2:1999). *Escherichia coli* on Hektoen Enteric Agar (HEA) incubated for 24 h at 37 °C. The detection of *Listeria monocytogenes* and *Salmonella* spp. was performed on 25 g of each sample following the ISO 11290-1, 2017 and ISO 6579–1:2017 guidelines, respectively. Aside from the mesophilic LAB and Enterobacteriaceae, which were inoculated using the pour plate technique, all other microbial populations were plated using the spread plate method (Sanders, 2012). All growth media and supplements were purchased from Oxoid (Hampshire, United Kingdom). All microbiological counts were carried out in duplicates for all samples at each collection time.

2.4. Culture-independent analysis of bacterial community

Total DNA extraction from DSFP and Suino Nero dei Nebrodi salami was performed using the DNeasy PowerFood Microbial Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA from four salamis per trial (two batches of salami for each of the two rounds of production) was pooledas individual salami's DNA per pool. DNA quality was assessed by gel electrophoresis and UV/Vis spectrophotometry. PCR amplification targeted 16S rRNA gene V3-V4 variable regions using bacterial primer set 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GACTACNVGGGTWTCTAATCC-3′). PCR amplification was performed in a 25 μL reaction volume, consisting of 12.5 μL of 2X KAPA Hifi HotStart Ready Mix (Kapa Biosystems Ltd., London, UK), 1 μM of each primer, 2 μL of DNA (10 ng/μL), and 9.5 μL of ddH_2O. All PCR reactions were carried out using a Verity™ 96-well Thermal Cycler with the following protocol: 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s, with a final elongation step at 72 °C for 5 min. PCR products were checked by gel electrophoresis and cleaned using an Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA), following the manufacturer's instructions. After seven PCR cycles (16S metataxonomic Sequencing Library Preparation, Illumina), Illumina adaptors were attached using the Illumina Nextera XT Index Primer. Libraries were purified using the Agencourt AMPure XP (Beckman Coulter, Brea, CA, USA) and then sequenced on an Illumina® MiSeq platform (Run Chemistry: 2 × 300 PE) using MiSeq Control Software 2.0.5 and Real-Time Analysis software 1.16.18 (Illumina, San Diego, CA, USA).

2.5. Illumina data analysis and sequence identification

The raw paired-end FASTQ files underwent demultiplexing using the idemp (available at https://github.com/yhwu/idemp/blob/master/idemp.cpp). Subsequently, they were imported into Quantitative Insights Into Microbial Ecology (Qiime2, version 2018.2). The sequences underwent quality filtering, trimming, de-noising, and merging using DADA2 (Callahan et al., 2016). Briefly, forward and reverse reads with more than two expected errors were discarded. Subsequently, the DADA2 workflow was used to trim quality-controlled reads to base pairs, accurately identify sequences with single-nucleotide resolution, and filter out *de novo* chimeras. Chimeric sequences were identified and removed using the consensus method within DADA2. Representative bacterial sequences were aligned using MAFFT and used for phylogenetic reconstruction in FastTree, leveraging the alignment and phylogeny plugins (Katoh and Standley, 2013; Price et al., 2009). Taxonomic and compositional analyses for bacteria were conducted using the feature-classifier plugin (available at https://github.com/qiime2/q2-feature-classifier). A pre-trained Naive Bayes classifier, based on the Greengenes 13_8 99% Operational Taxonomic Units (OTUs) database (previously trimmed to the V3-V4 region of 16S rDNA and bound by the 341F/805R primer pair), was used to generate taxonomy tables from paired-end sequence reads. As recommended by Logares et al. (2014), taxa with an individual relative abundance (RA) of 0.1 % or higher were considered abundant. The MiSeq Illumina sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) and are accessible under Accession Number PRJNA1161483.

2.6. Physicochemical analyses of salamis

Salami samples collected at 45 d of aging were assessed for their cross-sectional area using a caliper and were subjected to determination of colorimetric parameters using a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) with the illuminant C. The results are reported in terms of lightness (L*), redness (a*), and yellowness (b*), based on the International Commission on Illumination (CIE) L*a*b* color space system (Menegas et al., 2013). At 0 and 45 d of ripening, salami samples were assessed for pH using a HI 9025 pH meter (Hanna Instrument, Ann Arbor, MI, USA) and for water activity, measured at room temperature (approximately 23 °C) with an activity-meter (Rotronic Int., USA).

The tenderness of the salami at 15 and 45 d was assessed by measuring the compressive strength (N/mm^2) with an Instron 5564 tester (Instron, Trezzano sul Naviglio, Milan, Italy) at the point of maximum deformation. The measurements were done in duplicate on samples with both diameter and height of 2.5 cm, without casings, maintained at room temperature.

Initially, DSFP, pork meat, and all salami samples were frozen at −20 °C and then subjected to lyophilization for the analysis of their centesimal chemical composition. All analyses were carried out in duplicate. The content of dry matter (DM), protein, fat, and ash was determined following AOAC methods (AOAC, 2023).

2.7. Determination of antioxidant properties of salami

Extracts from the lyophilized salami samples were prepared using the method of Rashidinejad et al. (2013) with minor adjustments (Garofalo et al., 2024), and analysed to determine polyphenol content, antioxidant capacity, and oxidation status.

The total polyphenol content was measured using the Folin-Ciocalteau colorimetric method (ISO 14502–1:2005) with gallic acid as standard, as described by Ponte et al. (2022). Results were reported as gallic acid equivalent (g GAE/kg DM).

The salami extracts were analysed in duplicate to assess the Trolox equivalent antioxidant capacity (TEAC), using Trolox as standard, following the procedure outlined by Ponte et al. (2022). Results were expressed in Trolox equivalent (mmol/kg DM).

The oxidative stability of salami fat was assessed by measuring peroxide values (POV, meq O_2 kg/fat), which serves as a primary lipid oxidation index (IDF, 1991), and thiobarbituric acid reactive substances (TBARS, mg malonaldehyde (MDA)/kg DM) as a secondary lipid oxidation index. The TBARS measurement followed the method proposed by Tarladgis et al. (1960) and modified by Mele et al. (2011), as detailed by Ponte et al. (2022).

2.8. Volatile organic compounds emitted from salamis

Volatile organic compounds (VOCs) were extracted using headspace solid-phase microextraction (SPME) and identified via gas chromatography-mass spectrometry (GC/MS). Five grams of samples were exposed to SPME fiber (DVB/CAR/PDMS, 50 mm, Supelco) while stirring at 60 °C for 15 min, Desorption was performed for 1 min at 250 °C via a GC splitless injector. Chromatographic separation was achieved with a DB-624 capillary column (Agilent Technologies, 60 m, 0.25 mm, 1.40 μm). Helium was used as the carrier gas, with an ionization voltage of 70 eV and a flow rate of 1 mL/min. The oven temperature program started with a 5-min isothermal period at 40 °C, followed by a linear increase of 5 °C/min up to 200 °C, and held for 2 min. Mass spectra were acquired in full-scan mode over a range of *m/z* 40–400 amu, with the interface temperature set to 230 °C. Individual

VOCs were identified by comparing MS spectra with the commercial NIST05 library. The analysis was repeated three times, and results are reported as percentages relative to significant peaks. Coated fiber, not subjected to any extraction procedure, was run as blanks between each set of three analyses.

2.9. Sensory evaluation

Following salami production, a sensory analysis was conducted 45 d later. Slices of the different salamis were presented to a panel of 15 consumers ranging in age from 25 to 65 years. These judges were recruited from PhD students, researchers, and professors of the Department of Agricultural, Food and Forest Sciences (University of Palermo, Italy). The judges evaluated 17 sensory attributes, categorized into five aspects: odour, appearance, taste, rheology, and overall acceptability (Gaglio et al., 2016). Each sensory descriptor received a score on a continuous linear scale from 1 (low perception) to 9 (highest perception). The evaluations were facilitated using iPads with Smart Sensory Box software (Smart Sensory Solutions S.r.l., Sassari, Italy), and panelists had access to water and unsalted crackers during the assessments.

2.10. Statistical analysis

The microbiological and physicochemical data were analysed using One-Way Analysis of Variance (ANOVA). Pairwise comparisons were performed with Tukey's multiple comparison test at a significance level of $p < 0.05$. The concentrations of VOCs emitted from the salamis were graphically represented using Agglomerative Hierarchical Clustering (AHC). All statistical analyses were performed using XLStat software version 2020.3.1 for Excel (Addinsoft, New York, NY, USA).

3. Results and discussion

3.1. Microbial count evolution

The levels of viable microbial groups investigated from raw materials (including DSFP) to CTR, CMC, EXP1, and EXP2 after 45 d of ripening are summarized in Table 1. In all salami trials, initial levels of LAB, CNS, yeasts, and molds were around 3.0 log CFU/g and increased to just under 7.0 log CFU/g at 45 d of ripening. This trend is consistent with findings on traditional Thai fermented pork salamis where sodium nitrite was replaced with Karanda (*Carissa carandas* Linn.) extracts (Sueprasarn et al., 2017). CPS, *L. monocytogenes*, and *Salmonella* spp. were not detected in any salami production throughout the entire sampling period. After 30 d of ripening, members of the Enterobacteriaceae family, specifically *E. coli*, were found only in CTR salamis. The absence of these bacteria in CMC, EXP1 and EXP2 productions is attributed to the inhibitory effects of E 252 and DSFP on various microorganisms (Nikolova and Belichovska, 2022; Viola et al., 2024).

The microbiological analysis of DSFP did not detect pathogenic bacteria, including aerobic spore-forming bacteria, coagulase-positive staphylococci, *E. coli*, *L. monocytogenes*, and *Salmonella* spp. These findings are consistent with the results reported by Viola et al. (2024)

Table 1
Microbial loads of samples collected during experimental salami productions.

Samples	Bacterial counts						
	TMM	LAB	CNS	Yeasts	Molds	Enterobacteriaceae	*E. coli*
Raw materials							
DSFP	5.54	<1	<2	<2	<2	<1	<2
Casings	4.23	<1	<2	3.70	2.05	<1	<2
Fat	4.24	3.85	3.57	3.47	3.35	3.49	2.99
Pork meat	4.53	3.24	3.09	3.09	3.60	4.13	3.05
Salamis at t_0							
CTR	5.17	3.63	3.87	3.64	3.51	3.31	2.89
CMC	5.25	3.24	3.91	3.49	3.24	3.27	2.73
EXP1	5.01	3.53	3.99	3.34	3.52	3.39	2.80
EXP2	5.19	3.29	3.72	3.47	3.24	3.17	2.96
SEM	0.031	0.052	0.034	0.037	0.042	0.029	0.034
p value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Salamis at 15 d							
CTR	7.59	7.11	6.85	6.44	5.19	2.92 a	2.67 a
CMC	7.40	7.16	6.84	6.34	5.35	2.33 b	2.14 b
EXP1	7.63	7.20	6.58	6.59	5.54	2.46 b	2.23 b
EXP2	7.56	7.24	6.73	6.48	5.45	2.24 b	2.19 b
SEM	0.057	0.025	0.040	0.031	0.054	0.077	0.070
p value	n.s.	n.s.	n.s.	n.s.	n.s.	0.005	0.015
Salamis at 30 d							
CTR	7.39	7.08	6.26	6.35	6.22	2.54 a	2.31 a
CMC	7.15	6.98	6.42	6.17	6.18	<1 b	<2 b
EXP1	7.05	6.86	6.43	6.25	6.06	<1 b	<2 b
EXP2	7.21	7.03	6.18	6.26	6.29	<1 b	<2 b
SEM	0.059	0.029	0.038	0.027	0.073	0.288	0.262
p value	n.s.	n.s.	n.s.	n.s.	n.s.	<0.0001	<0.0001
Salamis at 45 d							
CTR	7.19	6.63	6.77	6.90	6.98	<1	<2
CMC	7.10	6.74	6.87	7.01	6.88	<1	<2
EXP1	7.00	6.79	6.65	6.81	6.85	<1	<2
EXP2	7.07	6.88	6.89	7.00	6.91	<1	<2
SEM	0.037	0.029	0.061	0.034	0.022	n.e.	n.e.
p value	n.s.	n.s.	n.s.	n.s.	n.s.	n.e.	n.e.

Results are expressed as log CFU/g and indicate mean values. Abbreviations: TMM, total mesophilic microorganisms; LAB, lactic acid bacteria, CNS, coagulase negative staphylococci; *E.*, *Escherichia*; DSFP, dried sumac fruit powder; CTR, control production without nitrate salt and DSFP; CMC, commercial control production with nitrate salt but without DSFP; EXP1, experimental production without nitrate salt but with DSFP; EXP2, experimental production with both nitrate salt and DSFP; SEM, standard error of the mean; n.s., not significant; n.e., not evaluated. Within each column for each trial (four salamis for typology), means followed by different letters are significantly different ($p < 0.05$).

from Sicilian sumac powder, affirming the hygienic suitability of this product for use in fermented foods. The natural casings had a TMM count at 30 °C of approximately 5.0 log CFU/g, which is similar to the findings by Comi et al. (2005) for natural casings used in northern Italian salami production. Pork fat and meat counts of TMM, LAB, and CNS were at levels ranging from 3.0 to 4.0 log CFU/g. These microbial populations are essential for the proper fermentation process (Sukumaran et al., 2018).

3.2. Characterization of salami microbiota by culture-independent approaches

In this study, Illumina sequencing was used to thoroughly examine the bacterial communities in the samples. This sequencing analysis allowed the classification of sequences into OTUs. The V3-V4 hypervariable region of the bacterial 16S ribosomal RNA gene was sequenced from nine samples, yielding a total of 1,021,802 raw reads. After processing the libraries with the DADA2 script in the QIIME2 pipeline, 1268 OTUs were identified with a minimum abundance of 10 reads across all samples (Table 2). The RA % of OTUs in DSFP and salamis was examined from independent productions at 0 and 45 d of ripening. The results of the classification, shown in Fig. 1 and Table S1, revealed 16 taxonomic groups, primarily at the genus level. *Staphylococcus* was detected in all salami samples. This bacterial genus, known for its technological importance during the fermentation and ripening of fermented meat products (Aro et al., 2010; Tabanelli et al., 2012), was initially found at high levels in all samples (accounting for 62.58 % of RA in the CTR samples). However, after 45 d of ripening, its abundance consistently decreased. This reduction in all salamis may be attributed to pH decrease caused by LAB (Metaxopoulos et al., 1981; Tabanelli et al., 2012). Additionally, the presence of DSFP, which has strong antibacterial activity against Gram-positive strains (Mahdavi et al., 2018; Viola et al., 2024), could contribute to this decline.

The genus *Lactobacillus* was also detected in all salami samples at day 0, but its presence significantly diminished at 45 d of fermentation. A similar trend was observed by Połka et al. (2015) in the production of typical Italian salami. LAB communities dominated all salami productions at 45 d of ripening, with high RA % observed in the EXP1 and CMC samples (81.81 % and 75.25 %, respectively). These results align with previous findings reported by Settanni et al. (2020).

The phyla Actinobacteria (Actinomycetota), Firmicutes (Bacillota), and Bacteroidetes (Bacteroidota); the classes Alphaproteobacteria and Betaproteobacteria; the family of Micrococcaceae; and the genera *Acinetobacter, Pseudoalteromonas*, and *Vibrio* were detected in DSFP. However, their presence was reduced or undetectable in ripened salami.

Alpha diversity, which measures the diversity within a specific region, sample, or ecosystem, is typically assessed by the number of species present. To calculate alpha diversity, the Shannon, Caho1, Simpson, Good Coverage Diversity Indexes, and the number of observed OTUs were used (Table 2). This allowed us to construct OTUs rarefaction curves for each of the sampled salami (Fig. 2).

3.3. Physicochemical characterization of salamis

Table 3 shows the physicochemical parameters of DSFP, pork meat and salami. The chemical composition of DSFP is comparable to the findings of Zannou et al., 2024, who reported 4.69 % protein and 18.74 % fat levels in dehydrated sumac fruits with 2.30 % moisture. In the salamis, the addition of DSFP increased protein levels and decreased fat content. A reduction in ash was observed only in the absence of nitrate salt (EXP1). These variations in experimental salamis (EXP1 and EXP2) derives from the chemical composition of sumac, which was lower in ash and fat than the pork meat. Consequently, the increase of protein in the experimental salamis has to be mostly related to the decrease of fat due to the addition of sumac powder to pork meat and fat mixture. Similar results were observed in salami formulations where pork fat was substituted with avocado pulp (Garofalo et al., 2024). However, the absence of studies providing fat and protein data on spontaneously fermented salamis made with plant-derived preservatives, makes direct comparison with existing literature challenging.

The inclusion of sumac did not significantly impact the pH levels measured immediately after stuffing (T0) and after 45 d of ripening. As anticipated, the higher water activity observed in CTR and EXP2 salamis at both 0 and 45 d was related to their lower dry matter content. Additionally, the combination of nitrate salt and sumac (EXP2) unexpectedly reduced the hardness after 15 d of ripening. However, after 45 d, the salami formulation did not affect tenderness or colour.

3.4. Oxidation level in salamis

The impact of sumac inclusion in salami was significant for the content of polyphenols, which have antioxidant properties. Indeed, it is known that *R. coriaria* plants are rich in tannins, phenolic acids, anthocyanins, gallic acid derivatives, flavonoid glycosides, and organic acids (Abu-Reidah et al., 2015). Several studies have shown that these substances have antioxidant activity (Zannou et al., 2024). In Table 4, it can be observed the higher polyphenols content resulted in experimental salami production (3.37 and 3.35 g GAE/kg DM in EXP1 and EXP2, respectively), and how the same corresponded to the improvement of their antioxidant activity, measured as TEAC.

The antioxidant activity of polyphenols is associated with the lowest levels of primary lipid oxidation (POV) in salami EXP1 and EXP2, which were fortified with sumac powder, regardless of nitrite salt presence. A reduction in hydroperoxides, compared to the control, was also

Table 2
Sequencing results for Salamis samples bacterial communities and alpha-diversity indices (Shannon, Simpson, Caho1 and Goods Coverage[a]).

Samples	Number of sequences (Raw reads)	Filtered Sequences	Observed OTUs	Shannon	Simpson	Caho1
DSFP	35,252	22,580	57	3.75	0.867	57
CTR T0	88,190	20,850	140	5.55	0.952	142
CTR T45	107,620	26,657	144	5.69	0.968	146
CMC T0	80,060	29,705	166	5.51	0.951	182
CMC T45	152,380	30,558	123	5.25	0.944	124
EXP1 T0	64,420	25,377	129	5.47	0.959	133
EXP1 T45	241,790	51,162	155	5.09	0.927	164
EXP2 T0	80,600	35,967	157	5.41	0.945	167
EXP2 T45	171,490	49,784	197	5.66	0.965	203
TOT	1,021,802	292,640	1268			

[a] Good coverage index was always over 0.99. Abbreviations: OTUs, Operational Taxonomic Units; DSFP, dried sumac fruit powder; CTR 0 d, control production without nitrate salt and DSFP immediately after stuffing; CTR 45 d, control production without nitrate salt and DSFP after 45 d of ripening; CMC 0 d, commercial control production with nitrate salt but without DSFP immediately after stuffing; CMC 45 d, commercial control production with nitrate salt but without DSFP after 45 d of ripening; EXP1 0 d, experimental production without nitrate salt but with DSFP immediately after stuffing; EXP1 45 d, experimental production without nitrate salt but with DSFP after 45 d of ripening; EXP2 0 d, experimental production with both nitrate salt and DSFP immediately after stuffing; EXP2 45d, experimental production with both nitrate salt and DSFP after 45 d of ripening.

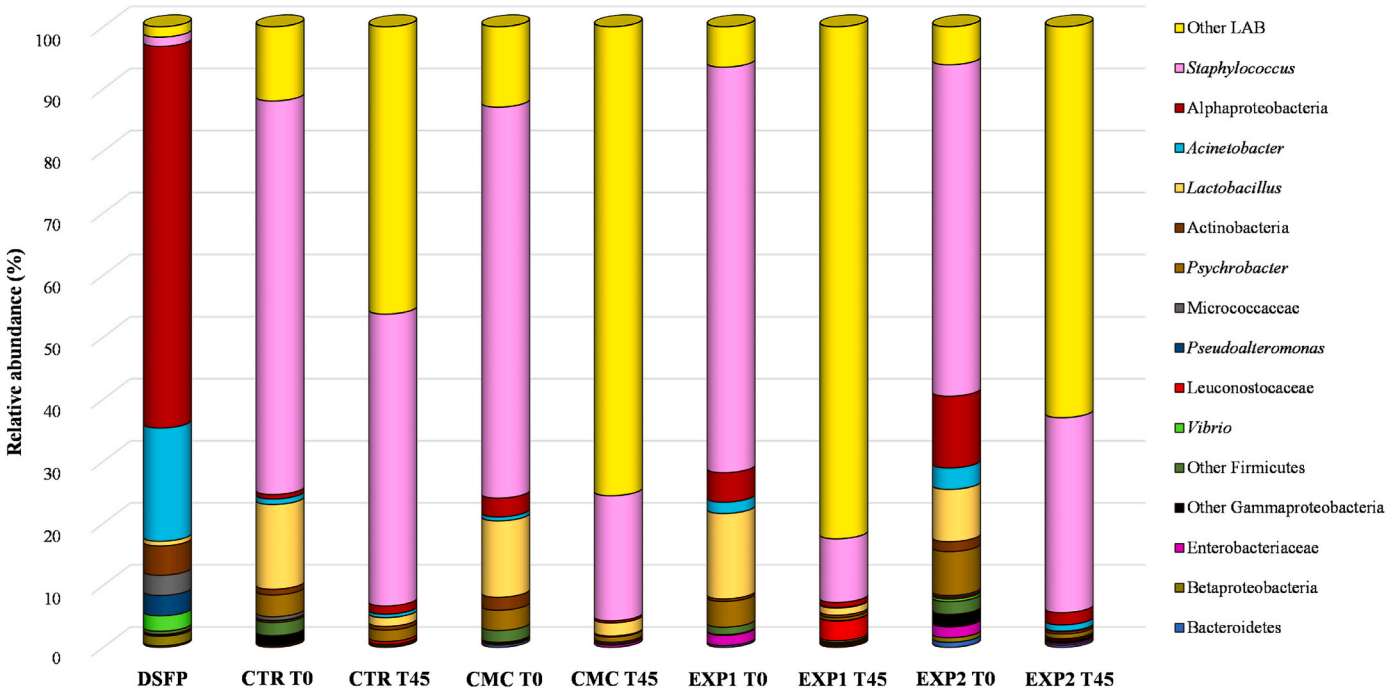


Fig. 1. Relative abundances (%) of bacteria identified by MiSeq Illumina. Abbreviations: DSFP, dried sumac fruit powder; CTR 0 d, control production without nitrate salt and DSFP immediately after stuffing; CTR 45 d, control production without nitrate salt and DSFP after 45 d of ripening; CMC 0 d, commercial control production with nitrate salt but without DSFP immediately after stuffing; CMC 45 d, commercial control production with nitrate salt but without DSFP after 45 d of ripening; EXP1 0 d, experimental production without nitrate salt but with DSFP immediately after stuffing; EXP1 45 d, experimental production without nitrate salt but with DSFP after 45 d of ripening; EXP2 0 d, experimental production with both nitrate salt and DSFP immediately after stuffing; EXP2 45 d, experimental production with both nitrate salt and DSFP after 45 d of ripening.

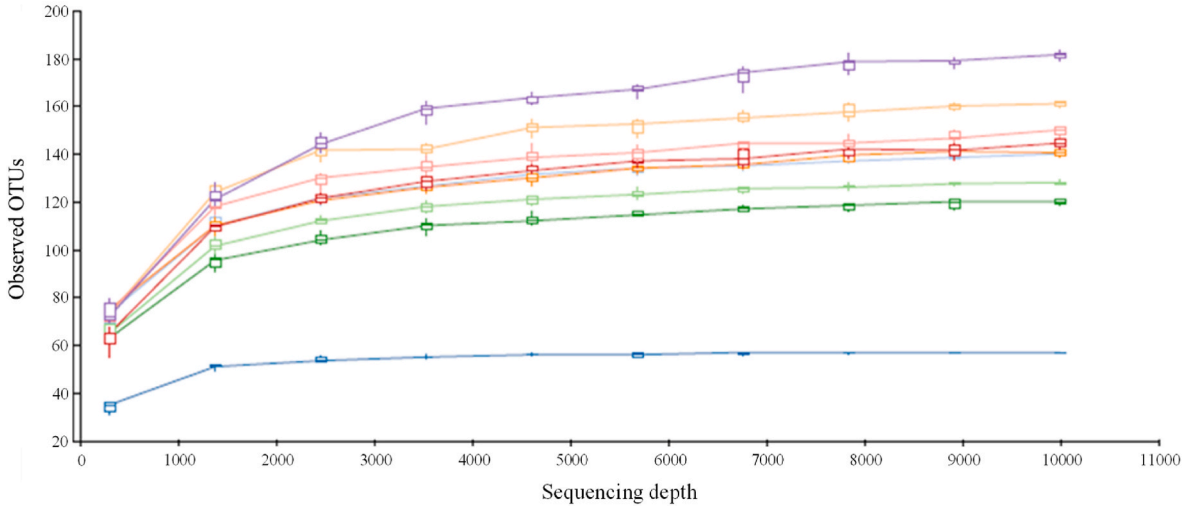


Fig. 2. Alpha diversity observed OTUs rarefaction curves in salamis. Legend of samples: blue line, dried sumac fruit powder (DSFP); light blue line, control production without nitrate salt and DSFP immediately after stuffing (CTR 0 d); orange line, control production without nitrate salt and DSFP after 45 d of ripening (CTR 45 d); light orange line, commercial control production with nitrate salt but without DSFP immediately after stuffing (CMC 0 d); green line, commercial control production with nitrate salt but without DSFP after 45 d of ripening (CMC 45 d); light green line, experimental production without nitrate salt but with DSFP immediately after stuffing (EXP1 0 d); red line, experimental production without nitrate salt but with DSFP after 45 d of ripening (EXP1 45 d); pink line, experimental production with both nitrate salt and DSFP immediately after stuffing (EXP2 0 d); purple line, experimental production with both nitrate salt and DSFP after 45 d of ripening (EXP2 45 d). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

observed by Aliyari et al. (2020) in beef salami produced with the inclusion of pomegranate peel extract and green pistachio hull.

Regarding secondary lipid oxidation, expressed as TBARS, the protection provided by nitrate salt in CMC and EXP2 was more effective than that offered by sumac alone. However, the exclusive presence of dried sumac in EXP1 salami significantly reduced the TBARS value compared to the CTR salami. Thus, during ripening, the protection against oxidation increased progressively from sumac alone (EXP1), to nitrate salt (CMC), and to the combination of sumac with nitrate salt (EXP2). These findings are consistent with those reported by Nowak et al. (2016), who observed a decrease in TBARS values when adding cherry (*Prunus cerasus* L.) and blackcurrant (*Ribes nigrum* L.) leaf extracts

Table 3
Physicochemical traits of raw material and salami samples.

	Samples						SEM	*p value*
	DSFP	PM	CTR	CMC	EXP1	EXP2		
Dry matter (DM), %	95.61	27.36	71.55 ab	74.56 ab	76.04 a	70.51 b	1.071	0.0111
Ash, % DM	2.33	4.17	8.80 c	8.91 b	7.17 d	9.51 a	0.006	<0.0001
Protein, % DM	6.78	66.59	39.83 b	38.73 b	46.45 a	46.37 a	0.274	<0.0001
Fat, % DM	23.19	29.24	44.20 a	45.17 a	40.76 b	41.66 b	0.422	0.0050
pH at T0	n.e.	n.e.	5.87	5.84	5.83	5.91	0.021	n.s.
pH at T45	n.e.	n.e.	5.48	5.40	5.17	5.51	0.068	n.s.
Water activity at T0	n.e.	n.e.	0.984 a	0.949 b	0.901 c	0.979 a	0.004	0.0004
Water activity at T45	n.e.	n.e.	0.759 a	0.731 b	0.679 c	0.761 a	0.004	0.0003
Cross-section area, cm^2	n.e.	n.e.	3.69 b	3.52 b	4.21 a	3.61 b	0.060	<0.0001
Hardness, N/mm^2 at T15	n.e.	n.e.	0.196 a	0.177 ab	0.174 b	0.119 c	0.003	0.0004
Hardness, N/mm^2 at T45	n.e.	n.e.	0.426	0.422	0.407	0.408	0.027	n.s.
Colour	n.e.	n.e.						
Lightness L*	n.e.	n.e.	39.37	37.63	38.74	37.73	2.004	n.s.
Redness a*	n.e.	n.e.	9.02	12.97	9.27	12.20	1.208	n.s.
Yellowness b*	n.e.	n.e.	7.75	7.71	9.74	9.65	1.239	n.s.

Results indicate mean values of determinations carried out in duplicate for each of the two independent productions. Abbreviations: DSFP, dried sumac fruit powder; PM, pork meat; CTR, control production without nitrate salt and DSFP; CMC, commercial control production with nitrate salt but without DSFP; EXP1, experimental production without nitrate salt but with DSFP; EXP2, experimental production with both nitrate salt and DSFP; SEM, standard error of the mean; n.e., not evaluated; n.s., not significant. Within each line for each trial (four salamis for typology), means followed by different letters are significantly different ($p < 0.05$).

Table 4
Antioxidant capacity of salamis.

	Samples				SEM	*p value*
	CTR	CMC	EXP1	EXP2		
Polyphenols, g GAE/kg DM	1.22 b	1.27 b	3.37 a	3.35 a	0.016	<0.0001
TEAC, mmol/kg DM	38.53 b	42.00 b	51.98 a	52.45 a	0.867	<0.0001
POV, mEq O_2/kg fat	4.17 a	2.23 b	1.99 d	2.11 c	0.018	<0.0001
TBARS, mg MDA/kg DM	0.228 a	0.151 c	0.177 b	0.123 d	0.002	<0.0001

Results indicate mean values of determinations carried out in duplicate for each of the two independent productions. Abbreviations: CTR, control production without nitrate salt and dried sumac fruit powder (DSFP); CMC, commercial control production with nitrate salt but without DSFP; EXP1, experimental production without nitrate salt but with DSFP; EXP2, experimental production with both nitrate salt and DSFP; SEM, standard error of the mean; GAE, gallic acid equivalent; TEAC, Trolox equivalent antioxidant capacity; POV, peroxide value; TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde. Within each column for each trial (four salamis for typology), means followed by different letters are significantly different ($p < 0.05$).

to salami, and by Sucu and Turp (2018), who noted a synergistic effect between nitrite and beetroot (*Beta vulgaris*) powder on TBARS reduction in fermented beef sausage.

Salami is prone to lipid oxidation due to its fat content (Hur et al., 2007; Wirth, 1991; Wójciak and Dolatowski, 2012). Incorporating antioxidant substances during processing is an effective strategy to reduce lipid oxidation (Kim et al., 2013). Accordingly, the results obtained highlight how the fortification of salami with sumac powder can represent a promising technique to avoid the use of nitrite salt, thus improve the finished product in terms of both oxidative stability and health quality.

3.5. VOC profiles of salamis

Fig. 3 presents the results of the volatile composition analysis for the four trials. CTR and CMC samples exhibited similar aromatic profiles, indicating that the nitrate salt did not significantly impact the aroma profile of the salamis. However, significant differences were found between the control and experimental salamis. Specifically, sesquiterpenes such as α-caryophyllene, β-caryophyllene, muurolene, and α-cubebene were detected only in the DSFP-enriched salami. These compounds, commonly found in sumac (Ozcan et al., 2021; Zannou et al., 2024), suggest that their presence is due to the addition of DSFP. In all trials, acetic acid was the most abundant acid compound, followed by butyric acid. Acetic acid may originate from carbohydrate catabolism, while butyric acid is associated with fat oxidation (Liu et al., 2023). Among aldehydes, hexanal is the most abundant, consistent with findings from previous studies on similar products (Belleggia et al., 2022; Bianchi et al., 2007; Meynier et al., 1999; Procida et al., 1999). Hexanal originates from the oxidative degradation of unsaturated fatty acids (Frankel, 1980; Meynier et al., 1999) and is recognized as a significant aroma contributor (Gao et al., 2023). Similar to linear aldehydes, ketones also play a role in the aroma of dry sausage (Demeyer et al., 2000). For example, diketones such as 2,3-butanedione and 2,3-octanedione, detected in all salami samples, are known for their buttery and creamy flavors (Bianchi et al., 2007).

Monoterpenes such as pinene, caryophyllene, 3-carene, and limonene were identified in all samples. These compounds can originate from animal feed, but their primary source is the spices used in salami production (Chevance and Farmer, 1998; Chevance et al., 2000; Guadayol et al., 1997). Globally, similar volatile profiles have been reported for various types of dry sausages (Bianchi et al., 2007; Garofalo et al., 2024; Liu et al., 2023; Lorenzo et al., 2012). The inclusion of DSFP resulted in the presence of specific sesquiterpenes and increased the relative percentage of monoterpenes, such as α-pinene and limonene. These results are consistent with those reported by Carballo et al. (2020) on sausages enriched with hop extract. Overall, the results show that adding DSFP significantly impacted the aromatic profile of the enriched salami.

3.6. Sensory evaluation of salamis

Before introducing a novel food product to the market, it is crucial to assess its acceptability among consumers (Fiorentini et al., 2020). A sensory panel test was carried out to evaluate the traits of salami with and without DSFP. Although pathogenic bacteria were never detected in productions without nitrates throughout the ripening process, the sensory analysis specifically focused on CMC and EXP2 salamis at 45 d of ripening. The spider chart in Fig. 4 shows the sensory attributes assessed during taste session. Notably, apart from color intensity and taste intensity, all other sensory attributes showed no significant differences ($p > 0.05$) between the various salami productions. The addition of sumac powder in salami (EXP2) led to increased colour intensity and taste

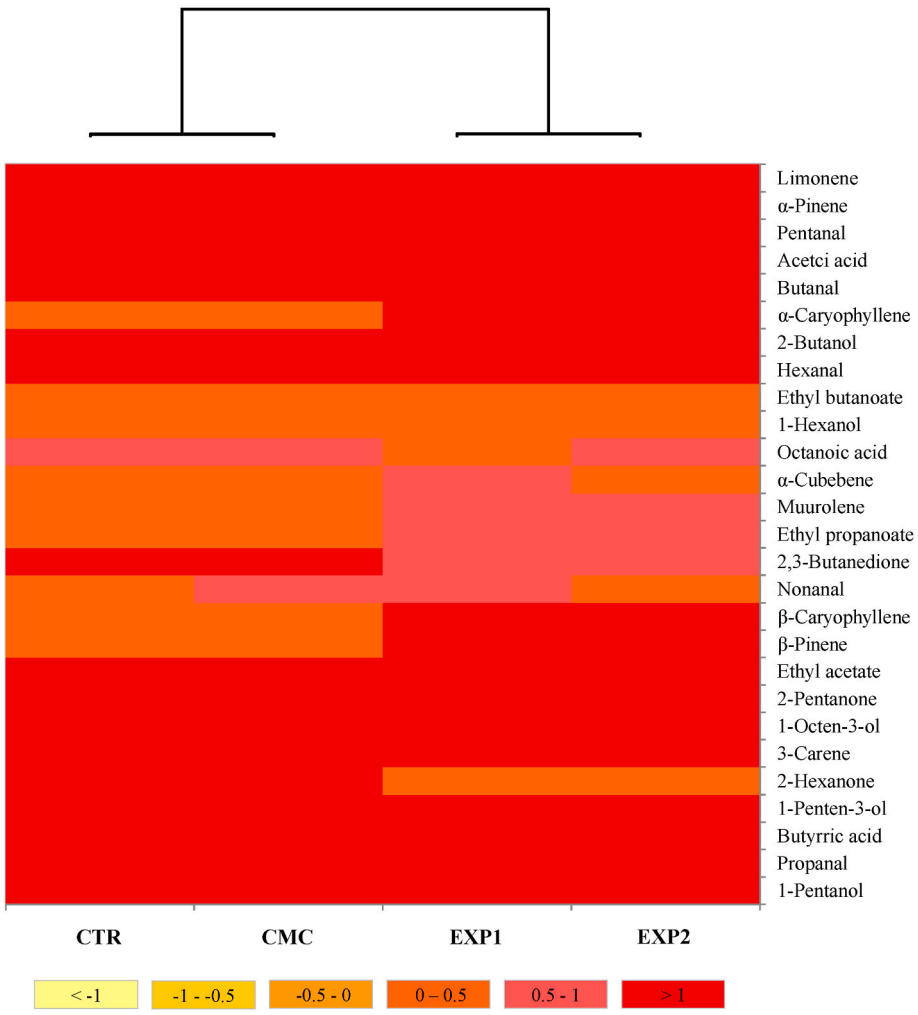


Fig. 3. Distribution of volatile organic compounds in salamis. The heat map plot depicts the relative concentration of each VOC. Abbreviations: CTR, control production without nitrate salt and DSFP; CMC, commercial control production with nitrate salt but without DSFP; EXP1, experimental production without nitrate salt but with DSFP EXP2, experimental production with both nitrate salt and DSFP.

intensity, aligning with previous findings by Grassi et al. (2024), who tested *R. coriaria* in beef burgers. Overall acceptability, determined by considering all sensory attributes (Qasem et al., 2017), remained consistent between CMC and EXP2 salami. These results suggest that incorporating sumac powder during production does not impact the intrinsic characteristics or overall acceptability of the product.

4. Conclusions

This research presents an extensive investigation, for the first time, into the microbiological and physicochemical properties of spontaneously fermented "Suino Nero dei Nebrodi" salami produced under industrial conditions by replacing E 252 potassium nitrate salts with DSFP. This replacement did not affect the development of LAB, CNS, and yeasts during salami production, which all reached values of about 7.0 log CFU/g in the 45-d ripened EXP samples. The absence of unwanted microorganisms, such as *E. coli*, at 30 d in DSFP-enriched salami strongly indicates that this plant-derived product could be a viable alternative to synthetic preservatives in short-ripened salamis. The evaluation of the total bacterial composition in all salami samples using DNA-based Illumina technology revealed that LAB dominated the bacterial community in the final products. The inclusion of DSFP had a positive influence on the chemical properties of the Suino Nero dei Nebrodi salamis, leading to lower fat content, higher phenolic content, enhanced antioxidant activity, and improved oxidation stability. The addition of DSFP in salami production significantly influenced the aromatic profile, introducing unique sesquiterpenes such as α-caryophyllene, β-caryophyllene, muurolene, and α-cubebene, and increasing the relative percentage of monoterpenes.

Interestingly, sensory evaluation showed that DSFP-enriched salamis were not significantly different from the control production in terms of overall acceptability. This research has led to the development of meat products that mitigate the harmful effects of synthetic preservatives on human health by utilizing a rediscovered plant native to the Mediterranean area.

CRediT authorship contribution statement

Gabriele Busetta: Writing – original draft, Formal analysis, Data curation. **Giuliana Garofalo:** Formal analysis, Data curation. **Marialetizia Ponte:** Writing – original draft, Formal analysis, Data curation. **Marcella Barbera:** Writing – original draft, Formal analysis, Data curation. **Antonio Alfonzo:** Visualization, Software, Methodology, Data curation. **Elena Franciosi:** Software, Formal analysis, Data curation. **Nicola Francesca:** Project administration, Funding acquisition. **Giuseppe Frusteri:** Resources, Conceptualization. **Daniela Piazzese:** Visualization, Methodology. **Adriana Bonanno:** Visualization, Software, Methodology. **Rosario Schicchi:** Investigation, Conceptualization. **Giancarlo Moschetti:** Visualization, Methodology. **Raimondo Gaglio:** Writing – review & editing, Writing – original draft, Validation,

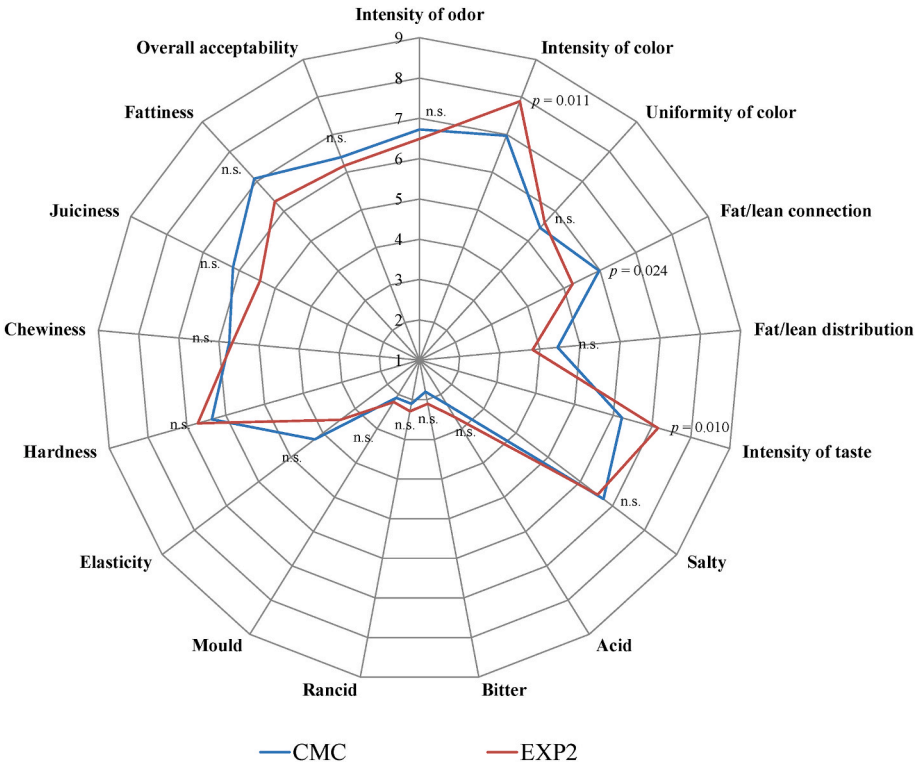


Fig. 4. Spider chart of sensory evaluation of salamis. Abbreviations: CMC, commercial control production with nitrate salt but without DSFP; EXP2, experimental production with both nitrate salt and DSFP; n.s., not significant.

Supervision, Conceptualization. **Luca Settanni:** Writing – review & editing, Conceptualization.

Data availability

Data will be made available on request.

Funding information

This research has been financially supported by the Sicily Region research project "AINEB 50 Living Lab Nebrodi – Comune di Galati Mamertino (Messina Province, Sicily)" CUP: F47H20003430009 and by the PRIMA MEDIET4ALL project "A Transnational movement to support the sustainable transition towards a healthy and Eco-friendly Agri-Food System through the promotion of MEDIET and its lifestyle in modern society" CUP: B73C23000060001.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to the student Giovanna V. Farrauto (University of Palermo) for her help with the microbiological analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2024.104684.

References

Abu-Reidah, I.M., Ali-Shtayeh, M.S., Jamous, R.M., Arráez-Román, D., Segura-Carretero, A., 2015. HPLC–DAD–ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (Sumac) fruits. Food Chem. 166, 179–191. https://doi.org/10.1016/j.foodchem.2014.06.011.

Alahakoon, A.U., Jayasena, D.D., Ramachandra, S., Jo, C., 2015. Alternatives to nitrite in processed meat: up to date. Trends Food Sci. Technol. 45, 37–49. https://doi.org/10.1016/j.tifs.2015.05.008.

Aliyari, P., Bakhshi Kazaj, F., Barzegar, M., Ahmadi Gavlighi, H., 2020. Production of functional sausage using pomegranate peel and pistachio green hull extracts as natural preservatives. J. Agric. Sci. Technol. 22 (1), 159–172.

Alsamri, H., Athamneh, K., Pintus, G., Eid, A.H., Iratni, R., 2021. Pharmacological and antioxidant activities *of Rhus coriaria* L. (Sumac). Antioxidants 10 (1), 73. https://doi.org/10.3390/antiox10010073.

AOAC, 2023. Association of official analytical chemists. In: Off. Methods Anal., twentysecond ed. AOAC, Washington.

Aro, J.M.A., Nyam-Osor, P., Tsuji, K., Shimada, K.I., Fukushima, M., Sekikawa, M., 2010. The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. Food Chem. 119, 279–285. https://doi.org/10.1016/j.foodchem.2009.06.025.

Belleggia, L., Ferrocino, I., Reale, A., Haouet, M.N., Corvaglia, M.R., Milanović, V., Boscaino, F., Di Renzo, T., Di Bella, S., Borghi, M., Farneti, S., Cesaro, C., Garofalo, C., Cardinali, F., Aquilanti, L., Musari, E., Cocolin, L., Osimani, A., 2022. Unravelling microbial populations and volatile organic compounds of artisan fermented liver sausages manufactured in Central Italy. Food Res. Int. 154, 111019. https://doi.org/10.1016/j.foodres.2022.111019.

Bianchi, F., Cantoni, C., Careri, M., Chiesa, L., Musci, M., Pinna, A., 2007. Characterization of the aromatic profile for the authentication and differentiation of typical Italian dry-sausages. Talanta 72, 1552–1563. https://doi.org/10.1016/j.talanta.2007.02.019.

Blaiotta, G., Murru, N., Di Cerbo, A., Romano, R., Aponte, M., 2018. Production of probiotic bovine salami using *Lactobacillus plantarum* 299v as adjunct. J. Sci. Food Agric. 98, 2285–2294. https://doi.org/10.1002/jsfa.8717.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Carballo, D.E., Andrés, S., Giráldez, F.J., Khanjari, A., Caro, I., Llamazares, D., Operta, S., Mateo, J., 2020. The effects of storage and hop extract on aroma and flavour compounds in Balkan-style sausages packed under a CO2-containing anaerobic atmosphere. Heliyon 6 (10), e05251. http://10.1016/j.heliyon.2020.e05251.

Chevance, F.F., Farmer, L.J., Desmond, E.M., Novelli, E., Troy, D.J., Chizzolini, R., 2000. Effect of some fat replacers on the release of volatile aroma compounds from low-fat meat products. J. Agric. Food Chem. 48 (8), 3476–3484. https://doi.org/10.1021/jf991211u.

Chevance, F.F.V., Farmer, L.J., 1998. Influence of fat on the flavour of an emulsified meat product. In: Contis, E.T., Ho, C.T., Mussinan, C.J., Parliament, T.H., Shahidi, F., Spanier, A.M. (Eds.), Developments in Food Science. Elsevier, Amsterdam, pp. 255–270. https://doi.org/10.1016/S0167-4501(98)80052-6.

Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., Cocolin, L., 2005. Characterisation of naturally fermented sausages produced in the North East of Italy. Meat Sci. 69 (3), 381–392. https://doi.org/10.1016/j.meatsci.2004.08.007.

Demeyer, D., Raemaekers, M., Rizzo, A., Holck, A., De Smedt, A., Ten Brink, B., Hagen, B., Montel, C., Zanardi, E., Murbrekk, E., Leroy, F., Vandendriessche, F., Lorentsen, K., Venema, K., Sunesen, L., Stahnke, L., De Vuyst, L., Talon, R., Chizzolini, R., Eerola, S., 2000. Control of bioflavour and safety in fermented sausages: first results of a European project. Food Res. Int. 33, 171–180. https://doi.org/10.1016/S0963-9969(00)00031-4.

Elagbar, Z.A., Shakya, A.K., Barhoumi, L.M., Al-Jaber, H.I., 2020. Phytochemical diversity and pharmacological properties of *Rhus coriaria*. Chem. Biodiversity 17 (4), e1900561. https://doi.org/10.1002/cbdv.201900561.

European Commission (EC), 2023. Regulation (EC) No 2023/2108 of 6 october 2023 amending annex II to regulation (EC) No 1333/2008 of the European parliament and of the council and the annex to commission regulation (EU) No 231/2012 as regards food additives nitrites (E 249-250) and nitrates (E 251-252). Off. J. Eur. Union 295, 11–12.

European Food Safety Authority and European Centre for Disease Prevention and Control, 2019. The European Union one health 2018 zoonoses report. EFSA J. 17 (12), e05926. http://10.2903/j.efsa.2019.5926.

Ferysiuk, K., Wójciak, K.M., 2020. Reduction of nitrite in meat products through the application of various plant-based ingredients. Antioxidants 9 (8), 711. https://doi.org/10.3390/antiox9080711.

Fiorentini, M., Kinchla, A.J., Nolden, A.A., 2020. Role of sensory evaluation in consumer acceptance of plant-based meat analogs and meat extenders: a scoping review. Foods 9 (9), 1334. https://doi.org/10.3390/foods9091334.

Flores, M., Toldrá, F., 2021. Chemistry, safety, and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products-Invited review. Meat Sci. 171, 108272. https://doi.org/10.1016/j.meatsci.2020.108272.

Frankel, E.N., 1980. Lipid oxidation. Prog. Lipid Res. 19 (1–2), 1–22. https://doi.org/10.1016/0163-7827(80)90006-5.

Gaglio, R., Francesca, N., Maniaci, G., Corona, O., Alfonzo, A., Giosuè, C., Di Noto, A., Cardamone, C., Sardina, M.T., Portolano, B., Alabiso, M., 2016. Valorization of indigenous dairy cattle breed through salami production. Meat Sci. 114, 58–68. https://doi.org/10.1016/j.meatsci.2015.12.014.

Gao, H., Liu, M., Zheng, L., Zhang, T., Chang, X., Liu, H., Zhou, S., Zhang, Z., Li, S., Sun, J., 2023. Comparative analysis of key odorants and aroma characteristics in hot-pressed yellow horn (*Xanthoceras sorbifolia bunge*) seed oil via gas chromatography–ion mobility spectrometry and gas chromatography–olfactory-mass spectrometry. Foods 12 (17), 3174. https://doi.org/10.3390/foods12173174.

Garofalo, G., Ponte, M., Busetta, G., Barbera, M., Tinebra, I., Piazzese, D., Franciosi, E., Di Grigoli, A., Farina, V., Bonanno, A., Gaglio, R., Settanni, L., 2024. Microbial dynamics and quality characteristics of spontaneously fermented salamis produced by replacing pork fat with avocado pulp. Food Microbiol. 122, 104536. https://doi.org/10.1016/j.fm.2024.104536.

Grassi, G., Di Gregorio, P., Rando, A., Perna, A.M., 2024. Quality and sensorial evaluation of beef burgers added with Sicilian sumac (*Rhus coriaria* L). Heliyon 10 (5), e26848. https://doi.org/10.1016/j.heliyon.2024.e26848.

Guadayol, J.M., Caixach, J., Ribe, J., Cabanas, J., Rivera, J., 1997. Extraction, separation and identification of volatile organic compounds from paprika oleoresin (Spanish type). J. Agric. Food Chem. 45 (5), 1868–1872. https://doi.org/10.1021/jf960266.

Hammes, W.P., 2012. Metabolism of nitrate in fermented meats: the characteristic feature of a specific group of fermented foods. Food Microbiol. 29 (2), 151–156. https://doi.org/10.1016/j.fm.2011.06.016.

Hur, S.J., Park, G.B., Joo, S.T., 2007. Formation of cholesterol oxidation products (COPs) in animal products. Food Control 18, 939–947. https://doi.org/10.1016/j.foodcont.2006.05.008.

IARC, 2010. Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. IARC Monogr. Eval. Carcinog. Risks Hum. 94, 111–297.

IARC, 2018. Working group on the evaluation of carcinogenic risks to humans. IARC monographs on the evaluation of carcinogenic risks to humans. Red meat and processed meat. IARC Monogr. Eval. Carcinog. Risks Hum. 107–422.

IDF, 1991. Determination of the peroxide value. In: International Standard FIL-IDF No. 74A. International Dairy Federation, Schaerbeek.

ISO 11290-1, 2017. Microbiology of the Food Chain – Horizontal Method for the Detection and Enumeration of *Listeria Monocytogenes* and of *Listeria Spp.* Part 1: Detection Method. International Organization for Standardization, Geneva, Switzerland.

ISO 14502-1, 2005. Determination of Substances Characteristic of Green and Black Tea. Part 1: Content of Total Polyphenols in Tea. Colorimetric Method Using Folin-Ciocalteu Reagent. International Organization for Standardization, Geneva, Switzerland.

ISO 15214, 1998. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Mesophilic Lactic Acid Bacteria – Colony-count Technique at 30° C. International Organization for Standardization, Geneva, Switzerland.

ISO 21527-1, 2008. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Yeasts and Molds. Part 1: Colony-count Technique in Products with Water Activity Greater than 0,95. International Organization for Standardization, Geneva, Switzerland.

ISO 21528-2, 2017. Microbiology of the Food Chain – Horizontal Method for the Detection and Enumeration of Enterobacteriaceae. Part 2: Colony-count Technique. International Organization for Standardization, Geneva, Switzerland.

ISO 4833, 2003. Microbiology of Food and Animal Feeding Stuffs: Horizontal Method for the Enumeration of Microorganisms: Colony-Count Technique at 30° C. International Organization for Standardization, Geneva, Switzerland.

ISO 6579-1, 2017. Microbiology of the Food Chain – Horizontal Method for the Detection, Enumeration and Serotyping of *Salmonella*–Part 1: Detection of *Salmonella* Spp. International Organization for Standardization, Geneva, Switzerland.

ISO 6888-2, 1999. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Coagulase Positive Staphylococci (*Staphylococcus aureus* and Other Species): Part 2: Technique Using Rabbit Plasma Fibrinogen Agar Medium. International Organization for Standardization, Geneva, Switzerland.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Kim, S.J., Cho, A.R., Han, J., 2013. Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. Food Control 29, 112–120. https://doi.org/10.1016/j.foodcont.2012.05.060.

Leroy, S., Giammarinaro, P., Chacornac, J.P., Lebert, I., Talon, R., 2010. Biodiversity of indigenous staphylococci of naturally fermented dry sausages and manufacturing environments of small-scale processing units. Food Microbiol. 27 (2), 294–301. https://doi.org/10.1016/j.fm.2009.11.005.

Liu, Y., Cao, Y., Yohannes Woldemariam, K., Zhong, S., Yu, Q., Wang, J., 2023. Antioxidant effect of yeast on lipid oxidation in salami sausage. Front. Microbiol. 13, 1113848. https://doi.org/10.3389/fmicb.2022.1113848.

Logares, R., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R., Claverie, J.M., Decelle, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Gobet, A., Kooistra, W.H.C.F., Mahe, F., Not, F., Ogata, H., Pawlowski, J., Pernice, M.C., Romac, S., Shalchian-Tabrizi, K., Simon, N., Stoeck, T., Santini, S., Siano, R., Wincker, P., Zingone, A., Richards, T.A., de Vargas, C., Massana, R., 2014. Patterns of rare and abundant marine microbial eukaryotes. Curr. Biol. 24, 813–821. https://doi.org/10.1016/j.cub.2014.02.050.

Lorenzo, J.M., Montes, R., Purriños, L., Franco, D., 2012. Effect of pork fat addition on the volatile compounds of foal dry-cured sausage. Meat Sci. 91 (4), 506–512. https://doi.org/10.1016/j.meatsci.2012.03.006.

Lücke, F.K., 1998. Fermented sausages. In: Wood, B.J.B. (Ed.), Microbiology of Fermented Foods. Springer, Boston, pp. 441–483. https://doi.org/10.1007/978-1-4613-0309-1_14.

Mahdavi, S., Hesami, B., Sharafi, Y., 2018. Antimicrobial and antioxidant activities of Iranian sumac (*Rhus coriaria* L.) fruit ethanolic extract. J. Appl. Microbiol. Biochem 2 (5), 2576. https://doi.org/10.21767/2576-1412.100021, 1412.

Majou, D., Christieans, S., 2018. Mechanisms of the bactericidal effects of nitrate and nitrite in cured meats. Meat Sci. 145, 273–284. https://doi.org/10.1016/j.meatsci.2018.06.013.

Mele, M., Contarini, G., Cercaci, L., Serra, A., Buccioni, A., Povolo, M., Conte, G., Funaro, A., Banni, S., Lercker, G., Secchiari, P., 2011. Enrichment of Pecorino cheese with conjugated linoleic acid by feeding dairy ewes with extruded linseed: effect on fatty acid and triglycerides composition and on oxidative stability. Int. Dairy J. 21, 365–372. https://doi.org/10.1016/j.idairyj.2010.12.015.

Menegas, L.Z., Colombo Pimentel, T., Garcia, S., Prudencio, S.H., 2013. Dry-fermented chicken sausage produced with inulin and corn oil: physicochemical, microbiological, and textural characteristics and acceptability during storage. Meat Sci. 93 (3), 501–506. https://doi.org/10.1016/j.meatsci.2012.11.003.

Metaxopoulos, J., Genigeorgis, C., Fanelli, M.J., Franti, C., Cosma, E., 1981. Production of Italian dry salami: effect of starter culture and chemical acidulation on staphylococcal growth in salami under commercial manufacturing conditions. Appl. Environ. Microbiol. 42 (5), 863–871. https://doi.org/10.1128/aem.42.5.863-871.1981.

Meynier, A., Novelli, E., Chizzolini, R., Zanardi, E., Gandemer, G., 1999. Volatile compounds of commercial Milano salami. Meat Sci. 51 (2), 175–183. https://doi.org/10.1016/S0309-1740(98)00122-3.

Moretti, V.M., Madonia, G., Diaferia, C., Mentasti, T., Paleari, M.A., Panseri, S., Pirone, G., Gandini, G., 2004. Chemical and microbiological parameters and sensory attributes of a typical Sicilian salami ripened in different conditions. Meat Sci. 66 (4), 845–854. https://doi.org/10.1016/j.meatsci.2003.08.006.

Nikolova, A.S., Belichovska, D., 2022. Possibilities of nitrite replacement in meat products. Knowledge-Int. J. 51, 465–470.

Nowak, A., Czyzowska, A., Efenberger, M., Krala, L., 2016. Polyphenolic extracts of cherry (Prunus cerasus L.) and blackcurrant (Ribes nigrum L.) leaves as natural preservatives in meat products. Food Microbiol. 59, 142–149. https://doi.org/10.1016/j.fm.2016.06.004.

Ozcan, A., Susluoglu, Z., Nogay, G., Ergun, M., Sutyemez, M., 2021. Phytochemical characterization of some sumac (*Rhus coriaria* L.) genotypes from southern part of Turkey. Food Chem. 358, 129779. https://doi.org/10.1016/j.foodchem.2021.129779.

Özcan, M.M., Akbulut, M., 2008. Estimation of minerals, nitrate and nitrite contents of medicinal and aromatic plants used as spices, condiments and herbal tea. Food Chem. 106 (2), 852–858. https://doi.org/10.1016/j.foodchem.2007.06.045.

Połka, J., Rebecchi, A., Pisacane, V., Morelli, L., Puglisi, E., 2015. Bacterial diversity in typical Italian salami at different ripening stages as revealed by high-throughput sequencing of 16S rRNA amplicons. Food Microbiol. 46, 342–356. https://doi.org/10.1016/j.fm.2014.08.023.

Ponte, M., Maniaci, G., Di Grigoli, A., Gannuscio, R., Ashkezary, M.R., Addis, M., Pipi, M., Alabiso, M., Todaro, M., Bonanno, A., 2022. Feeding dairy ewes with fresh

or dehydrated sulla (*Sulla coronarium* L.*)* forage. 2. Effects on cheese enrichment in bioactive molecules. Animals 12, 2462. https://doi.org/10.3390/ani12182462.
Price, M.N., Dehal, P.S., Arkin, A.P., 2009. FastTree: computing large minimum evolution,trees with profiles instead of a distance matrix. Mol. Biol. Evol. 26, 1641–1650. https://doi.org/10.1093/molbev/msp077.
Procida, G., Conte, L.S., Fiorasi, S., Comi, G., Favretto, L.G., 1999. Study on volatile components in salami by reverse carrier gas headspace gas chromatography–mass spectrometry. J. Chromatogr. A 830 (1), 175–182. https://doi.org/10.1016/S0021-9673(98)00792-4.
Qasem, A.A.A., Alamri, M.S., Mohamed, A.A., Hussain, S., Mahmood, K., Ibraheem, M.A., 2017. Soluble fiber-fortified sponge cakes: formulation, quality and sensory evaluation. J. Food Meas. Char. 11, 1516–1522. https://doi.org/10.1007/s11694-017-9530-5.
Rashidinejad, A., Birch, E.J., Sun-Waterhouse, D., Everett, D.W., 2013. Effects of catechin on the phenolic content and antioxidant properties of low-fat cheese. Int. J. Food Sci. Technol. 48, 2448–2455. https://doi.org/10.1111/ijfs.12234.
Sanders, E.R., 2012. Aseptic laboratory techniques: plating methods. J. Vis. Exp. 63, e3064. https://doi.org/10.3791/3064.
Settanni, L., Barbaccia, P., Bonanno, A., Ponte, M., Di Gerlando, R., Franciosi, E., Di Grigoli, A., Gaglio, R., 2020. Evolution of indigenous starter microorganisms and physicochemical parameters in spontaneously fermented beef, horse, wild boar and pork salamis produced under controlled conditions. Food Microbiol. 87, 103385. https://doi.org/10.1016/j.fm.2019.103385.
Stoica, M., Antohi, V.M., Alexe, P., Ivan, A.S., Stanciu, S., Stoica, D., Zlati, M.L., Stparu-Cretu, M., 2022. New strategies for the total/partial replacement of conventional sodium nitrite in meat products: a review. Food Bioprocess Technol. 15, 514–538. https://doi.org/10.1007/s11947-021-02744-6.
Sucu, C., Turp, G.Y., 2018. The investigation of the use of beetroot powder in Turkish fermented beef sausage (sucuk) as nitrite alternative. Meat Sci. 140, 158–166. https://doi.org/10.1016/j.meatsci.2018.03.012.
Sueprasarn, J., Reabroy, S., Pirak, T., 2017. Antioxidant properties of Karanda (*Carissa carandas* Linn.) extracts and its application in Thai traditional fermented pork sausage (Nham). Int. Food Res. J. 24 (4).
Sukumaran, A.T., Holtcamp, A.J., Englishbey, A.K., Campbell, Y.L., Kim, T., Schilling, M. W., Dinh, T.T., 2018. Effect of deboning time on the growth of *Salmonella, E. coli*, aerobic, and lactic acid bacteria during beef sausage processing and storage. Meat Sci. 139, 49–55. https://doi.org/10.1016/j.meatsci.2018.01.012.
Tabanelli, G., Coloretti, F., Chiavari, C., Grazia, L., Lanciotti, R., Gardini, F., 2012. Effects of starter cultures and fermentation climate on the properties of two types of typical Italian dry fermented sausages produced under industrial conditions. Food Control 26 (2), 416–426. https://doi.org/10.1016/j.foodcont.2012.01.049.
Tack, D.M., Ray, L., Griffin, P.M., Cieslak, P.R., Dunn, J., Rissman, T., Jervis, R., Lathrop, S., Muse, A., Duwell, M., Smith, K., Tobin-D'Angelo, M., Vugia, D.J., Kufel, J.Z., Wolpert, B.J., Tauxe, R., Payne, D.C., 2020. Preliminary incidence and trends of infections with pathogens transmitted commonly through food—foodborne diseases active surveillance network, 10 US sites, 2016–2019. MMWR morb. Mortal. Wkly Rep 69 (17), 509–514. https://doi.org/10.15585/mmwr.mm6917a1.
Tarladgis, B.G., Watts, B.M., Younathan, M.T., Dugan, L., 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37, 44–48. https://doi.org/10.1007/BF02630824.
Viola, E., Mannino, G., Serio, G., La Rosa, L., Garofalo, G., Schicchi, R., Settanni, L., Gentile, C., Gaglio, R., 2024. Phytochemical profiling and investigation of antioxidant, anti-proliferative, and antibacterial properties in spontaneously grown Sicilian sumac (*Rhus coriaria* L.) fruits. Food Biosci. 61, 104704. https://doi.org/10.1016/j.fbio.2024.104704.
Wirth, F., 1991. Reducing the fat and sodium content of meat products. What possibilities are there? Fleischwirtschaft 71, 294–297.
Wójciak, K.M., Dolatowski, Z.J., 2012. Oxidative stability of fermented meat products. Acta Sci. Pol. Technol. Aliment. 11, 99–109.
Wood, B.J., 2012. Microbiology of fermented foods. In: Wood, B.J.B. (Ed.), Microbiology of Fermented Foods. Springer Science & Business Media, New York, p. 852.
Zannou, O., Oussou, K.F., Chabi, I.B., Alamou, F., Awad, N.M., Miassi, Y.E., Loké, F.C.V., Abdoulaye, A., Pashazadeh, H., Redha, A.A., Kpoclou, Y.E., Guclu, G., Koca, I., Selli, S., Ibrahim, S.A., 2024. Phytochemical and nutritional properties of sumac (*Rhus coriaria*): a potential ingredient for developing functional foods. J. Future Foods 5 (1), 21–35. https://doi.org/10.1016/j.jfutfo.2024.01.002.