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Sustainable poultry meat valorization: Microbiological, physicochemical, and sensory characterization of salami made from spent egg-laying hens

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ABSTRACT

Valorizing spent egg-laying hens offers a sustainable solution to reduce waste and recover nutritional resources from poultry production, embracing the principle of "from waste to taste". This study advances the concept of closing the loop in poultry systems by exploring salami production as a circular economy approach. To achieve this, meat from spent hens was processed into salami, comparing two formulations: one using fat derived from spent hens (SHF) and another using traditional pork fat (SPF). Microbiological analyses confirmed the safety of both productions, with lactic acid bacteria (LAB) dominating fermentation and pathogenic species remaining undetected. Next generation sequencing (NGS) revealed a clear succession of microbial communities, highlighting the prevalence of LAB at the end of ripening. The SHF salami showed higher protein content and good antioxidant capacity, but a slightly higher susceptibility to lipid peroxidation. SPF salami showed better fat-lean cohesion and lower rancidity. The fatty acid profile indicated a more favorable nutritional composition for the SHF salami, which was richer in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Sensory evaluation revealed good overall acceptability, with SPF salami preferred for structural and flavor stability, while SHF salami enhanced odor intensity. In both salamis, aldehydes were the most abundant volatiles (59.8 % SHF, 40.7 % SPF), followed by alcohols, acids, and monoterpenes, with SHF salamis showing higher levels of key lipid-derived aldehydes such as hexanal, pentanal, and propanal, reflecting the influence of fat type on volatile profile. Overall, the findings demonstrate that meat from spent egg-laying hens can be successfully employed in fermented meat products, offering a promising avenue for circular economy valorization and improved sustainability in poultry production.

Introduction

The global chicken population exceeds 26 billion heads and continues to show a steady upward trend over the past decade. In this context, Italy has approximately 150-160 million chickens and an annual egg production of approximately 13-14 million tons, confirming the country's role as one of the leading egg producers within the European Union (FAO, 2023). Commercial laying hens exhibit high productivity between 60 and 72 weeks of age, beyond this period, they are classified as "spent egg-laying hens" due to a marked decline in both egg-laying capacity and egg quality (El-Tarabany et al., 2022; Wang et al., 2025). The fate of spent egg-laying hens varies significantly depending on the geographical context. In several Asian countries, they are used in the preparation of soups, snacks, and traditional dishes (De Souza et al., 2011; Kumar et al., 2015; Sabikun et al., 2021). In contrast, in Western countries, their meat is generally undervalued by consumers, mainly due to its poor organoleptic properties such as toughness and limited palatability. These defects are attributed to the high collagen content (Katemala et al., 2021) and the extensive cross-linking of connective tissues (Giri et al., 2018). However, Shin et al. (2020) demonstrated that processing spent hen meat through grinding, comminution, or incorporation into further processed products (e.g., sausages, patties,

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or emulsified formulations) can enhance its sensory attributes by mitigating the structural effects of connective tissue and collagen cross linking.

Currently, the majority of spent egg-laying hens are culled on farms (Cheng et al., 2004) or destined for alternative uses such as the production of protein meals and oils for feed and pet food, or for composting (Pirsich et al., 2017; Amicarelli et al., 2023). Nevertheless, such practices not only represent an additional cost for producers but also raise concerns regarding animal welfare and environmental impact (Freeman et al., 2009). Within a circular economy framework, the valorization of spent egg-laying hens may represent a sustainable solution capable of reducing waste while recovering residual value for the poultry industry. Although in some European countries these hens are still considered waste or by-products, their meat shows an interesting nutritional profile, being rich in protein, omega-3 fatty acids, and characterized by a superior essential amino acid composition compared to broiler meat (Ajuyah et al., 1992; El-Tarabany et al., 2022). Despite this potential, studies evaluating the use of spent egg-laying hen meat in processed products remain limited. Although some research has investigated the use of spent hens in dry-cured or fermented poultry sausages (Anisa et al., 2023; Boodhoo et al., 2014), their application in traditional fermented meat systems such as salami remains comparatively limited. In this context, the working hypothesis was that spent egg-laying hens could be valorised through their use in salami production. It was further hypothesised that the type of fat included in the formulation (spent hen fat vs. pork fat) could influence these properties while still ensuring comparable microbiological safety and fermentation dynamics. Future perspectives indicate the potential for full recovery and utilisation of this raw material, both to support egg producers and to promote fermented meat products as local, low-cost options within a circular-economy framework. Therefore, the aim of the present study was to evaluate the feasibility of this valorisation strategy by assessing the microbiological, physicochemical and sensory parameters of salami produced using meat and fat from spent egg-laying hens.

Materials and methods

Production of hen's salamis

In this study, meat from spent egg-laying hens, derived from commercial Hy-Line Brown and Hy-Line white hybrids, was obtained from a private laying hens farm (Palermo, Italy). Twenty hens at the end of production cycle were transported to a slaughterhouse for processing. The hens were electrically stunned using 86 V for 10 s (Maino Industries, Como, Italy) followed immediately by exsanguination through severing of the carotid arteries and jugular veins. After 5 min of bleeding, the carcasses were immersed in a water bath at 60°C for 2 min, mechanically defeathered in a rotating drum for 30 s, and subsequently washed with white vinegar water solution (5 % v/v). After slaughter, the carcasses were transported under refrigerated conditions in ice box to the Department of Agricultural, Food and Forest Sciences (University of Palermo, Italy). There, they were stored overnight at 2°C in a cooling chamber. The following day, the meat was manually deboned with a knife, while spent egg-laying hens fat was simultaneously recovered.

The experimental plan included two independent salami productions: one made with meat and fat from spent egg-laying hens (SHF), and the other with meat from spent egg-laying hens and pork fat (SPF). Pork fat (*Sus domesticus*) was as sourced from the butchery section of a local supermarket (Decò-Gruppo Arena, Palermo, Italy). In both productions, the salamis were manufactured in the laboratory under controlled conditions and in accordance with traditional curing practices for long ripened fermented sausages, nitrate was included as a long term precursor of nitrite, allowing nitrate reducing starter cultures to gradually generate nitrite during fermentation. This ensures a controlled and sustained curing effect, complementing the immediate action of the nitrite added at the beginning of processing. Each salami formulation was prepared using the following proportions: 800 g/kg meat, 200 g/kg fat, 30 g/kg salt (Italkali, Palermo, Italy), 3 g/kg ground black pepper (ITALPEPE s.r.l. Roma, Italy), 0.25 g/kg of TCS D8 200 starter culture (Tec-Al, Traversetolo, Italy) and 0.1 g/kg potassium nitrate, 0.1 g/kg nitrite (Tec-Al, Traversetolo, Italy). These levels fall within the maximum ingoing limits permitted by Commission Regulation (EC) no. 1333/2008 for cured meat products (European Commission, 2008). The meat was minced once using meat grinder with a 6-mm plate (Minerva Omega Group S.r.l., Bologna, Italy) and the ingredients were manually mixed in aluminium vats. The mixtures were stuffed into 4.0 cm diameter swine bowels, previously washed with a 50 % v/v solution of water and white wine vinegar, using a horizontal manual sausage filler (Tre Spade mod. 5, FACEM S.p.A., Turin, Italy). The drying was performed for 9 days (d) under the following conditions: progressive temperature decrease, from 20°C to 12°C, and concomitant progressive relative humidity (R.H.) decrease from 87 % to 68 %. Ripening of the sausages was carried out at 13°C and 85 % R.H. for 21 days in (GTEST-FCA Fratelli Galli, Milan, Italy).

Samples of minced meat from spent egg-laying hens, spent hen fat, pork fat, natural casings, mixtures immediately after stuffing, and salami after 30 d of ripening were collected for analysis. The two salami production trials were repeated twice at a one-month interval under comparable operating conditions to ensure reproducibility. Both raw-material batches were sourced from the same farm and exhibited similar characteristics (breed, age, type of feed). Ambient temperature during the trials remained within a consistent range (15–18°C), with no fluctuations expected to influence the microbiota or volatilome profiles.

Microbiological counts

Twenty-five grams of each sample were transferred into sterile bags (BagFilter P, Interscience, Saint Nom, France) and homogenized with 225 mL sterile peptone (1 g/L) (Oxoid, Milan, Italy) water using a stomacher (BagMixer® 400, Interscience) at the highest speed for 2 min. The microbiological viable counts were performed on the same solution after serial decimal dilutions. The following microbial populations were monitored in this study: total mesophilic microorganism was enumerated on Plate Count Agar (PCA) incubated for 72 h at 30°C (ISO 4833, 2003); mesophilic lactic acid bacteria (LAB) were enumerated in Man-Rogosa-Sharpe (MRS) agar medium (Oxoid, Basingstoke, United Kingdom) supplemented with cycloheximide (10 mg/mL) and the plates were incubated in accordance with ISO 15214, 1998; coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (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); yeasts and moulds on Dicloran Rose-Bengal Chloramphenicol (DRBC) agar incubated at 25°C for 5 d (ISO 21527–1:2008). *Escherichia coli* was enumerated on Hektoen Enteric Agar (HEA) incubated for 24 h at 37°C; Enterobacteriaceae enumerated on Violet Red Bile Glucose Agar (VRBGA) incubated for 24 h at 37°C (ISO 21528–2, 2017). *Listeria monocytogenes* was enumerated on Agar *Listeria* according to Ottaviani and Agosti (ALOA) incubated for 24 h at 37°C (ISO 11290-2, 2017); *Salmonella* spp. was enumerated on Xylose Lysine Desoxycholate agar (XLD) incubated for 24 h at 37°C (ISO 6579-2, 2017); *Campylobacter* spp. was enumerated on modified Charcoal Cefoperozone Deoxycholate agar (mCCD) incubated for 48 h at 41.5°C in a micro-aerobic condition (ISO 10272-2, 2017). The media used in the study were supplied by Oxoid (Milan, Italy). In addition, the detection of *L. monocytogenes, Salmonella* spp. and *Campylobacter* spp. was performed according to ISO 11290-1, 2017, ISO 6579-1, 2017 and ISO 10272-1, 2017 guidelines, respectively. The results of viable counts, expressed as the log of colony-forming units (CFU) per gram of sample, were reported as mean value of the two biological and three technical replicates ± standard deviation (S.D).

Culture-independent analysis of total bacterial community

A next generation sequencing (NGS) approach was applied to analyse the bacterial communities present in salami samples. For each sample, approximately 1.0 g of pool salami were required for total genomic DNA extraction. Following the manufacturer's instructions, DNA was extracted using the DNeasy PowerFood Microbial Kit (QIAGEN, Hilden, Germany).

The extracted DNA was then quantified with a Nanodrop 8800 Fluorospectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, an amplicon library was prepared, and the quality of the libraries was checked. Finally, pair-end sequencing was performed using the Illumina MiSeq system (Illumina, USA) at the Sequencing Platform of Fondazione Edmund Mach (FEM, San Michele all' Adige, Italy). Salami genomic DNAs were amplified to investigate the bacterial community composition with primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'GACTACHVGGGTATCTAATCC-3') corresponding to the V3–V4 variable region of the 16S rRNA gene of *E. coli* (Baker et al., 2003; Claesson et al., 2010). PCR was performed by the GeneAmp PCR System 9700 (Thermo Fisher Scientific). Amplicons were checked by gel electrophoresis and then purified using the Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA) to prevent preferential sequencing of the smaller amplicons. 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).

Illumina data analysis and sequences identification by QIIME2

Raw paired-end FASTQ files underwent demultiplexing using the idemp tool (https://github.com/yhwu/idemp/blob/master/idemp.cpp). Subsequently, they were imported into Quantitative Insights Into Microbial Ecology (QIIME2, version 2018.2) (Bolyen et al., 2019). The sequences, then, underwent quality-filtering, trimming, denoising, and merging using the DADA2 program (Callahan et al., 2016). Chimeric sequences were detected and removed using the consensus method within DADA2. Representative bacterial sequence was aligned with MAFFT and utilized for phylogenetic reconstruction via FastTree using plugin alignment and phylogeny (Price et al., 2009, 2010; Katoh and Standley, 2013). The taxonomy and composition of bacterial community was analysed using the feature classifier plugin (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, which had previously been trimmed to the V3-V4 region of 16S rDNA and bound by the 341F/805R primer pair, was applied to paired-end sequence reads to generate taxonomy tables. The data generated by Illumina sequencing were deposited in the NCBI Sequence Reads Archive (SRA), and are accessible under Accession Number PRJNA1369161.

Physicochemical analysis of salamis

Colorimetric parameters were measured on the salami samples using a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan). The pH was determined with a HI 9025 pH meter (Hanna Instruments, Ann Arbor, MI, USA), while water activity (a_w) was assessed using a HygroPalm indicator (Rotronic, Bassersdorf, Switzerland), following the procedure reported by Gaglio et al. (2016).

Textural properties were assessed after 30 d to evaluate product tenderness. Measurements were carried out in duplicate using an Instron 5564 testing device (Instron, Trezzano sul Naviglio, Milan, Italy). Compressive strength (N/mm^2) was calculated at the point of maximum deformation. Before testing, samples were standardised (2.5 cm in both diameter and height), casings removed, and brought to room temperature.

For compositional analysis, salamis were frozen at –20 °C and subsequently subjected to freeze-drying by a SCANVAC Coolsafe 55–9 (Labogene Aps, Lynge Denmark). The proximate composition, including dry matter (DM), fat, and ash content, was determined in accordance with AOAC (2023) guidelines, whereas protein content was calculated by difference (100 - water - fat - ash).

Assessment of antioxidant activity

The antioxidant potential of freeze-dried salami extracts was evaluated following a modified version of the protocol described by Rashidinejad et al. (2013). Briefly, 0.5 g of sample was extracted in 25 mL of 95 % aqueous methanol containing 1 % HCl. The mixture was vortexed, sonicated at 40°C for 30 min (LBS1 Sonicator; Falc Instruments, Treviglio, Italy) with intermittent agitation, cooled, filtered through linen, centrifuged at 7000 RPM for 10 min at 9°C, and stored at -18°C until use.

Antioxidant capacity was quantified using the Trolox Equivalent Antioxidant Capacity (TEAC) assay, performed in duplicate according to Ponte et al. (2022). The ABTS$\bullet^+$ radical was generated by incubating 14 mM ABTS with 4.9 mM potassium persulfate in the dark for 16 h at 22°C. The working solution was diluted in 5 mM phosphate-buffered saline (PBS) at pH 7.4 to an absorbance of 0.7–0.8 at 734 nm.

A volume of 75 μL of PBS was mixed with 1425 μL of the diluted ABTS$\bullet^+$ solution, read on the spectrophotometer, incubated for 6 min at 30°C and re-read to measure the background absorbance. Similarly, 75 μL of each sample extract with 1425 μL of diluted ABTS$\bullet^+$ solution was read at 734 nm. Antioxidant activity was calculated against a Trolox calibration curve ($R^2 = 0.99$) and expressed as mmol Trolox equivalents per kg of DM (mmol TE/kg DM).

Lipid oxidation was evaluated by measuring both primary and secondary products. The peroxide value (POV, meq O_2/kg fat) was determined according to IDF (1991), while secondary oxidation was quantified as thiobarbituric acid reactive substances (TBARS), expressed as mg malondialdehyde (MDA)/kg DM, following the procedure of Tarladgis et al. (1960), with modifications by Mele et al. (2011) and Ponte et al. (2022).

Fatty acid profile determination

The fatty acid (FA) composition was assessed in the salami at 0 and 30 d of ripening. The lipid extraction and preparation of fatty acid methyl esters (FAME) were performed on 0.5 g of lyophilized samples, following the methodology reported by O'Fallon et al. (2007). A known amount of C23:0 (Sigma-Aldrich, Milan, Italy) was added into each sample as internal standard to facilitate the accurate quantification of total FA content. The FAME were dissolved in 1.5 mL of hexane and 1 μL of each sample was then injected by an autosampler into a gas chromatography system (HP 6890, Agilent Technologies, Santa Clara CA, USA) equipped with a flame ionization detector. Chromatographic separation of the FAME was obtained using a CP-Sil 88 capillary column (Chrompack, Middelburg, Netherlands). This column was 100 m in length, with an internal diameter of 0.25 mm and a film thickness of 0.25 μm. Gas chromatography operating conditions, and the procedures for identification of FA, were performed as previously described by Bonanno et al. (2013). The concentration of individual FA was reported as a percentage of the total identified FA (g/100 g total identified FA).

Volatile organic compounds emitted from salamis

The volatile organic compounds (VOC) profile of salami samples was determined using solid-phase microextraction (SPME) combined with gas chromatography–mass spectrometry (GC–MS). Salami samples were finely chopped, and 5 g of each sample was exposed to SPME fibre (DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) at 25°C for 60 min. After

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

Samples	Bacterial counts						
	TMM	LAB	CNS	Yeast	Molds	*E. coli*	Enterobacteriaceae
Casing	4.7	<1	<2	3.9	<2	<2	<1
Spent egg-laying hens fat	5.7	5.2	4.8	5.1	<2	2.7	2.9
Pork fat	4.6	4.4	4.3	4.1	3.1	2.1	3.2
Spent egg-laying hens' meat	5.7	4.2	3.8	2.4	3.5	2.8	3.9
Salamis at 0 d							
SHF	7.1	6.6	4.6	3.2	3.6	2.7	3.0
SPF	7.0	6.9	5.0	3.4	3.2	2.5	2.9
SEM	0.06	0.05	0.07	0.04	0.07	0.06	0.04
p value	0.735	0.170	0.188	0.316	0.188	0.511	0.591
Salamis at 30 d							
SHF	8.4	8.3	6.6	6.2	6.5	<2	<1
SPF	8.6	8.4	6.8	6.0	6.4	<2	<1
SEM	0.06	0.04	0.08	0.09	0.04	n.e	n.e
p value	0.511	0.591	0.591	0.653	0.593	n.e	n.e

Results are expressed as log CFU/g and indicate mean values of four plate counts (carried out in duplicate for two independent productions). Abbreviations: TMM, total mesophilic microorganisms; LAB, lactic acid bacteria, CNS, coagulase-negative staphylococci; *E.*, *Escherichia*; SHF, salami made with meat and fat from spent egg-laying hens; SPF salami made with meat from spent egg-laying hens and pork fat; SEM, standard error of the mean; n.e., not evaluated. On the column: a, b = $p < 0.05$.

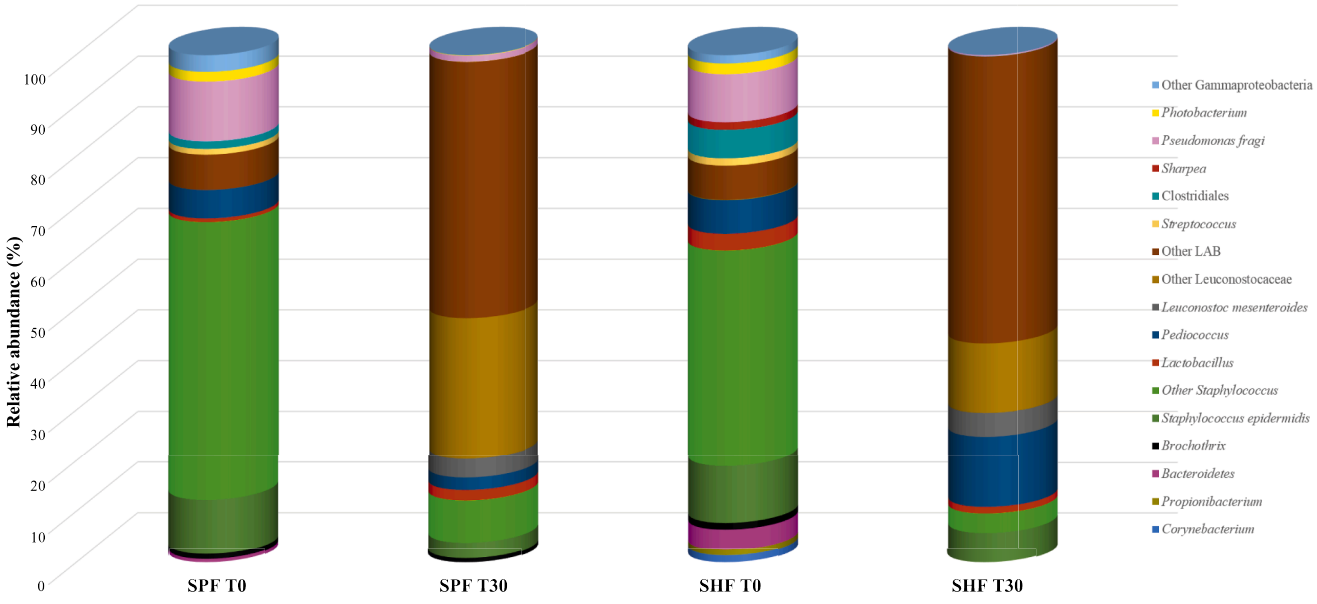

Fig. 1. Relative abundances (%) of bacteria identified by MiSeq Illumina. Abbreviations: SPF T0, salami made with meat from spent egg-laying hens and pork fat after stuffing; SPF T30, salami made with meat from spent egg-laying hens and pork fat after 30 d of ripening; SHF T0, salami made with meat and fat from spent egg-laying hens after stuffing; SHF T30, salami made with meat and fat from spent egg-laying hens after 30 d of ripening.

adsorption, the fibre was thermally desorbed in the GC injection port at 250°C for 5 min. Separation of volatile compounds was achieved on a DB-624 capillary column (Agilent Technologies, Santa Clara, CA, USA; 60 m × 0.25 mm × 1.40 μm). The GC oven was programmed from 40 to 230°C at 4°C/min, followed by a 40-min isothermal hold and a final temperature hold of 2 min. Mass spectrometric detection was performed in full scan mode (m/z 40–400) with an interface temperature of 230°C. VOCs were identified by matching their mass spectra with the NIST05 library. Relative abundances were calculated by normalizing the peak area of each compound against the total area of all significant peaks. Each salami sample was analysed in duplicate.

Sensory evaluation

Sensory evaluation was performed following the protocol of Chiavari et al. (2007). Twelve judges (7 men and 5 women), aged between 22 and 63 and trained in the sensory analysis of salamis, were used for the sensory evaluation. Each judge was given a slice of salami 4 mm thick placed in a polyethylene container, which was hermetically sealed and presented individually. The final evaluation was conducted through a descriptive analysis, assessing the perceived intensity of 17 different attributes including three aspects: intensity of odor (odor), intensity of color, uniformity of color, fat/lean connection, fat/lean distribution (appearance), intensity of taste, salty, acid, bitter, rancid, mould, elasticity, hardness, chewiness, juiciness, fattiness (taste) and overall acceptability. Each attribute was scored on a 9-point linear scale, with 1 representing low perception and 9 representing high perception.

Statistical analysis

Microbiological data were subjected to One-Way Variance Analysis (ANOVA) using XLStat software version 7.5.2 for Excel (Addinsoft, New

Table 2
The effect of different salami mixtures and ripening time on physicochemical traits and oxidative status of salami.

Parameters	Salamis at 0 d		Salamis at 30 d		SEM	*p* value		
	SHF	SPF	SHF	SPF		SA	RT	SA*RT
Dry matter (DM), %	35.76^{c}	34.57^{c}	54.61^{b}	65.15^{a}	0.61	<0.0001	<0.0001	<0.0001
Ash, % DM	9.69	9.54	10.23	9.98	0.20	0.2980	0.0223	0.8025
Protein, % DM	54.06^{b}	56.07^{a}	57.22^{a}	56.34^{a}	0.28	0.1356	0.0085	0.0138
Fat, % DM	36.25^{a}	34.40^{b}	32.55^{c}	33.68^{b}	0.24	0.0769	0.0005	0.0016
pH	5.83^{a}	5.78^{b}	5.73^{c}	5.36^{d}	0.018	<0.0001	<0.0001	<0.0001
Water activity, a_w	0.98^{a}	0.97^{a}	0.91^{b}	0.87^{c}	0.004	0.0064	0.0002	0.0335
Hardness, N/mm^2			0.13	0.18	0.026	0.0016		
Lightness L*	52.47	53.04	50.39	42.50	2.51	0.1725	0.0288	0.1201
Redness *a**	5.83	5.77	13.24	10.40	0.98	0.0685	<0.0001	0.0777
Yellowness *b**	6.98	6.05	12.40	9.93	1.01	0.1216	0.0008	0.4637
TEAC, mmol/kg DM	22.87^{c}	35.45^{ab}	45.98^{a}	26.11^{bc}	3.89	0.2183	0.0312	0.0001
POV, mEq O_2/kg fat	1.15^{b}	1.43^{b}	2.55^{a}	2.24^{a}	0.089	0.5222	<0.0001	0.0249
TBARS, mg MDA/kg DM	0.92^{a}	0.88^{a}	0.48^{b}	0.15^{c}	0.029	<0.0001	<0.0001	<0.0001

Abbreviations: SHF, salami made with meat and fat from spent egg-laying hens; SPF salami made with meat from spent egg-laying hens and pork fat. TEAC, Trolox equivalent antioxidant capacity; POV, peroxide value; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; SEM, standard error of the mean; SA, salamis; RT, ripening time. On the row: a, b, c = $p < 0.05$

Table 3
The effect of different mixtures and ripening time on salami fatty acid (FA) profile.

Fatty acids	0 d		30 d		SEM	*p* value		
	SHF	SPF	SHF	SPF		SA	RT	SA*RT
Total FA, % DM	31.16	30.82	29.57	28.52	0.58	0.3209	0.0458	0.5891
C12:0	0.22^{a}	0.16^{b}	0.18^{b}	0.15^{b}	0.006	0.0049	0.0244	0.0411
C14:0	1.29	1.95	1.26	1.98	0.058	0.0007	0.9470	0.4973
C15:0 *iso*	0.092	0.048	0.088	0.037	0.004	0.0007	0.1164	0.3688
C15:0 *anteiso*	0.046	0.019	0.049	0.024	0.002	0.0002	0.0538	0.5869
C15:0	0.28	0.27	0.21	0.20	0.022	0.6793	0.6284	0.8610
C16:0	34.18	33.46	34.09	32.74	0.80	0.2676	0.6346	0.7094
C17:0 *iso*	0.125	0.093	0.095	0.083	0.022	0.3894	0.4325	0.6704
C17:0 *anteiso*	0.52	0.37	0.49	0.40	0.017	0.0067	0.8797	0.1394
C17:0	0.23	0.31	0.20	0.33	0.011	0.0025	0.5117	0.1449
C18:0	8.61	14.29	8.80	13.79	0.23	0.0002	0.5544	0.2422
C20:0	0.09^{c}	0.23^{a}	0.10^{c}	0.18^{b}	0.011	0.0006	0.0691	0.0245
Saturated FA	45.70	51.20	45.56	49.92	1.01	0.0155	0.5273	0.6063
C14:1 *c9*	0.20	0.10	0.18	0.10	0.007	0.0015	0.4184	0.2391
C16:1 *c9*	5.23	2.98	5.37	3.27	0.087	0.0001	0.0901	0.4503
0.C17:1 *c9*	0.14	0.22	0.11	0.25	0.012	0.0024	0.8623	0.0961
C18:1 *c9*	37.20	35.47	38.23	37.41	0.71	0.1697	0.1276	0.5685
Monounsaturated FA	42.77	38.77	43.90	41.03	0.77	0.0214	0.1168	0.5176
C18:2 n-6	4.20	3.53	3.42	3.52	0.39	0.5006	0.3710	0.3866
C18:3 n-3	0.22	0.30	0.18	0.16	0.044	0.4945	0.0880	0.2660
C20:2 n-6	1.53	1.09	1.67	0.39	0.21	0.0256	0.2715	0.1359
C20:3 n-3	0.22	0.24	0.11	0.06	0.030	0.4520	0.0068	0.2477
C20:5 n-3, EPA	0.16	0.12	0.09	0.09	0.061	0.5571	0.2401	0.5198
Polyunsaturated FA	6.33	5.27	5.47	4.22	0.43	0.0163	0.0269	0.7208
Undefined FA	5.20	4.76	5.08	4.83	0.19	0.1611	0.8890	0.6512

Abbreviations: SHF, salami made with meat and fat from spent egg-laying hens; SPF salami made with meat from spent egg-laying hens and pork fat. EPA, eicosapentaenoic acid; SEM, standard error of the mean; SA, salamis; RT, ripening time. Data within a row followed by different letters are significantly different according to Tukey's test ($p < 0.05$). On the row: a, b, c = $p < 0.05$.

York, USA). Physicochemical parameters of salamis were analysed using SAS software version 9.2 (2010) through the MIXED procedure. Fixed effects included the type of salamis (SA: SHF, salami made with meat and fat from spent egg-laying hens; SPF salami made with meat from spent egg-laying hens and pork fat; ripening time (RT: 0 and 30 d), and the SA*RT interaction. The production batch (first and second) was treated as a random effect. The Tukey's test was applied for pairwise comparison. Statistical significance was attributed to p values of $p < 0.05$.

Results and discussion

Microbiological evolution

Table 1 reports the microbial loads observed during the production of the two different types of salami (SHF, SPF), from the raw materials to the final salami after 30 d of ripening. The natural casing showed a TMM count at 30°C of approximately 5.0 log CFU/g. Similar results were previously reported by Busetta et al. (2025) for natural casings used in the production of Nebrodi salami. Pork fat exhibited a lower microbial load compared to spent egg-laying hens fat, with TMM, LAB and CNS exceeding 5.0 log CFU/g. The microbial counts observed are in line with those reported by Garofalo et al. (2024). In both productions, immediately after stuffing, the initial levels of LAB were around 7.0 log CFU/g, while those of CNS had initial values of 4.6–5.0 log CFU/g. This trend is very similar to the results observed by Rocchetti et al. (2023) in aged pork Italian salamis. After 30 d of ripening, in both productions, the levels of LAB and CNS reached values of approximately 9 log CFU/g and 7 log CFU/g, respectively (Casaburi et al., 2008). Although undesirable microorganisms, such as *E. coli* and Enterobacteriaceae, were detected in

Table 4
Volatile organic compounds emitted from salami.

VOC	Samples		SEM	*p* value
	SHF (%)	SPF (%)		
Aldehydes				
Propanal	7.54[a]	4.14[b]	0.97	0.015
Butanal	7.43	8.53	0.52	0.357
Benzaldeide	0.83	1.25	0.14	0.103
Pentanal	12.41	7.87	1.39	0.055
Hexanal	29.00	18.00	3.37	0.055
Heptanal	1.17[a]	n.d[b]	0.32	<0.0001
Octanal	0.30[a]	n.d[b]	0.08	0.022
Nonanal	1.08[a]	0.86[b]	0.06	0.014
Ketones				
2-Pentanone	1.99[b]	2.87[a]	0.26	0.022
2,3-Butanedione	0.79[b]	1.07[a]	0.08	0.011
2-Heptanone	1.55[b]	2.29[a]	0.22	0.036
2,3-Octanedione	n.d	0.84	0.23	<0.0001
2-Hexanone	0.45	n.d	0.14	0.071
Alcohols				
2-Butanol	11.52	13.80	0.96	0.274
1-Propanol	n.d[b]	2.78[a]	0.76	<0.0001
1-Penten-3-ol	1.85[b]	3.05[a]	0.36	0.039
1-Pentanol	2.40[b]	4.61[a]	0.66	0.036
1-Hexanol	n.d[b]	0.30[a]	0.08	0.001
1-Octen-3-ol	2.69[b]	3.65[a]	0.29	0.050
Esters				
Ethyl acetate	0.82[b]	2.66[a]	0.52	0.009
Ethyl propanoate	0.20[b]	0.31[a]	0.03	0.019
Ethyl butanoate	0.15[b]	0.33[a]	0.05	0.002
Acids				
Acetic acid	3.56	4.76	0.69	0.466
Butyric acid	2.60	4.09	0.50	0.110
Octanoic acid	0.88[a]	0.64[b]	0.07	0.015
Monoterpenes				
α-Pinene	2.47	4.02	0.48	0.056
Caryophyllene	3.01	2.29	0.24	0.106
Limonene	2.38	3.82	0.49	0.122
α-Cubebene	0.90	1.17	0.16	0.489

Data are means percentage four determination (carried out in duplicate for two independent productions expressed as (peak area of each compound/total area of significant peaks) x100. Abbreviations: SHF, salami made with meat and fat from spent egg-laying hens; SPF salami made with meat from spent egg-laying hens and pork fat; n.d., not detected. On the row: a, b = $p < 0.05$.

the raw materials (except for the casing) and in the salamis immediately after stuffing, previous studies have demonstrated that the use of starter cultures is effective in significantly reducing these microbial populations (Cenci-Goga et al., 2008). Moreover, it can be observed that after 30 d of ripening, these levels were below the detection limit (< 2 log CFU/g) in both productions. Pathogenic microorganisms including *L. monocytogenes, Salmonella* spp. and *Campylobacter* spp., were not detected in any of the 25 g sample aliquots analysed (data not shown).

Characterization of salami microbiota by Illumina analysis

To investigate bacterial community dynamics during fermentation, samples were collected on 0 d (T0) and 30 d (T30) of ripening. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was sequenced from four samples using Illumina technology. OTUs with a relative abundance (RA) higher than 0.1 %, a threshold commonly used to describe abundant bacterial populations (Logares et al., 2014), are presented in Fig. 1.

Due to the intrinsic taxonomic resolution limits of 16S rRNA gene sequencing at the V3–V4 region, not all detected taxa could be reliably assigned to the species level. Consequently, several phylogenetically related taxa were grouped into higher-level categories, such as “other lactic acid bacteria (LAB)” or “other *Staphylococcus*.” These groupings reflect dominant functional bacterial groups rather than individual starter strains and were used to describe overall microbial succession during fermentation.

Overall, 17 taxonomic groups were identified as predominant primarly at the genus level. Although *Staphylococcus*, Leuconostocaceae, and *Pediococcus* accounted for the majority of the total RA in salamis, their relative abundances differed markedly among the samples.

In SPF T0, the bacterial community was largely dominated by *Staphylococcus* spp. with “other *Staphylococcus*” representing 43.78 % of the total RA and *Staphylococcus epidermidis* accounting for 8.61 %. By contrast, in SPF T30, a notable shift in microbial composition was observed. LAB associated taxa became dominant, with “other LAB” reaching 49.33 %, accompanied by substantial increases in “other Leuconostocaceae” (27.08 %), *Pediococcus* (2.43 %), and *Leuconostoc mesenteroides* (3.65 %). Conversely, the RA of *Staphylococcus* spp., including *S. epidermidis*, declined markedly. This LAB driven succession is characteristic of fermented meat products and reflects the selective pressure of ripening conditions, in agreement with previous observations reported by Busetta et al. (2025). A similar, although not identical, microbial evolution was observed in SHF salami. At T0, the microbiota was again dominated by *Staphylococcus* spp. with “other *Staphylococcus*” accounting for 37.89 %, and *Staphylococcus epidermidis* for 10.07 % of the total RA. LAB associated taxa were present at lower levels (“other LAB” 5.99 %), together with a broader diversity of minor bacterial groups including *Clostridiales* (4.99 %), *Bacteroidetes* (3.56 %) and *Sharpea* (1.34 %). After 30 days of fermentation, in SHF T30, a clear LAB predominance was established with “other LAB” increasing to 55.81 %, accompanied by a marked expansion of *Pediococcus* (13.65 %) and “other Leuconostocaceae” (13.5 %). As observed in SPF salami, the RA of *Staphylococcus* spp. was strongly reduced at the end of ripening.

This observed microbial succession highlights the competitive advantage of LAB during the late stage of fermentation. This process contributes to acidification, flavor development, and microbiological safety as widely documented for fermented meat products (Seleshe and Kang, 2021). However, given the taxonomic resolution of the 16S rRNA gene approach, the present data describe overall community dynamics and dominant functional groups rather than the technological performance of individual starter strains. Species and strain level characterization of starter cultures was therefore assessed using complementary culture dependent methods.

Notably, none of the samples analysed tested positive for pathogenic bacteria such as *L. monocytogenes, Campylobacter* spp., and *Salmonella* spp., confirming the results obtained through the culture dependent method.

Physicochemical characterization of salamis

The analysis of physicochemical parameters, presented in Table 2, showed significant differences between SHF and SPF salamis and fresh and 30 d ripened products. Also the interactions between these two factors were significant. Dry matter increased to statistically significant levels after 30 d, when was higher in SPF; these trends are consistent with the reduction in a_w values. During ripening, SHF salamis showed a more pronounced decrease in fat, accompanied by a concomitant increase in protein. This reduction may be attributed to the fatty acid profile of poultry fat, which contains higher levels of polyunsaturated fatty acids (PUFA) that are more prone to lipid oxidation. In addition, both endogenous and microbial lipolytic activities, as well as physical fat loss from the matrix during drying, may have contributed to the observed decrease (Visessanguan et al., 2006; Apriliyani et al., 2025). As expected, pH decreased during ripening in both salami types, confirming the good activity of LAB for the product safety and quality (Settanni et al., 2020). Regarding tenderness (hardness, N/mm^2), SHF salamis were found to be softer, probably due to the minor consistency of the fat. The softer texture observed in SHF salami could be related to the higher percentage of PUFA in spent egg-laying hens fat and its lower melting temperature compared to pork fat, which implies greater softness (Abbasiliasi et al., 2024). Color parameters did not show statistically significant differences due to salami mixture, while ripening was

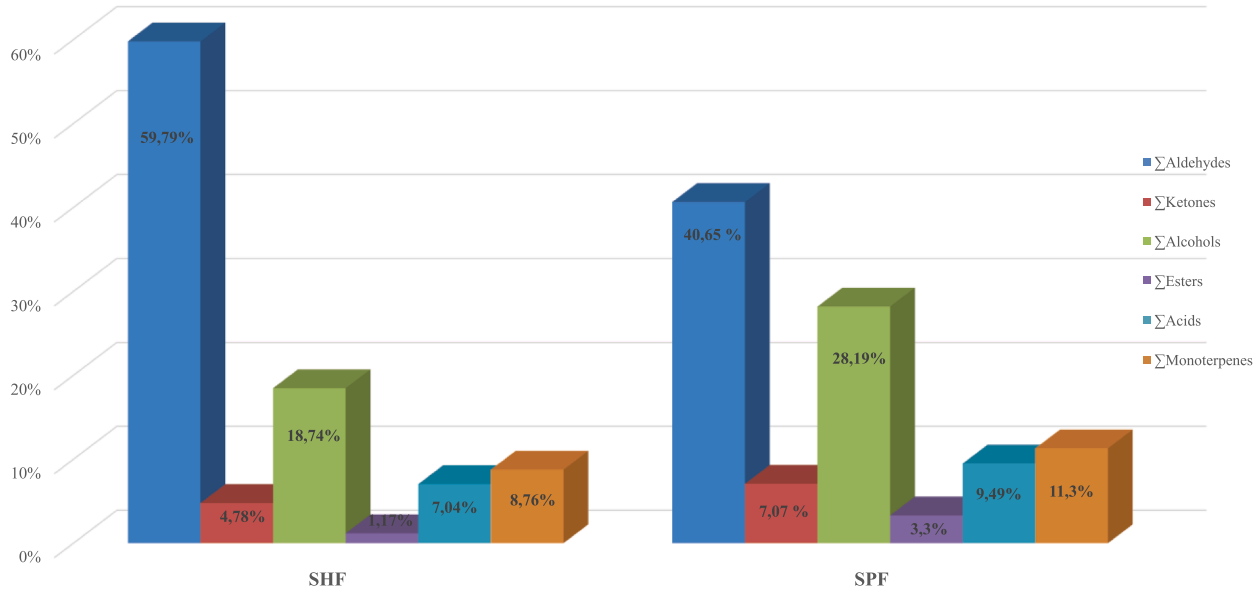

Fig. 2. Percentage composition of major volatile chemical groups. Abbreviations: SHF, salami made with meat and fat from spent egg-laying hens; SPF salami made with meat from spent egg-laying hens and pork fat.

responsible for the lightness reduction and the increase of red and yellow indexes.

Antioxidant capacity of salamis

TEAC was significantly affected by ripening time and the interaction (Table 2). Indeed, during ripening its value greatly increased in salami with poultry fat (from 22.9 to 46.0 mmol TE/kg DM), while it decreased in SPF salami (from 35.4 to 26.1 mmol TE/kg DM). This result may suggest that poultry lipids are richer in endogenous antioxidant components mobilized or activated during ripening and originating from feeding, among which can be hypothesized the presence of vitamins, such as α-tocopherol and β-carotene. Instead, the decreasing antioxidant capacity in salami with pork fat could be due to the lower levels of these protective compounds. Oxidative markers showed contrasting trends, although salami is known to be a food product particularly susceptible to lipid oxidation (Hur et al., 2007; Wójciak and Dolatowski, 2012). Indeed, POV increased at 30 d of storage, presumably due to the instability of peroxides over time (Lee et al., 2010); on the contrary, TBARS decreased at 30 d in both mixtures, with a more marked reduction in the SPF salami. The decrease in TBARS could be associated with its lower content in PUFA, but can reflect the degradation or binding of secondary oxidation products with other molecules, reducing their detectability. Similar trends were observed in fermented salami and sausages by other authors, highlighting the complexity of oxidative reactions in meat systems (Domínguez et al., 2019). Overall, the data indicates that SHF salami exhibits higher antioxidant capacity but also a slightly greater susceptibility to peroxide formation, whereas SPF salami shows a decrease in antioxidant protection and lower TBARS values. These results clearly reflect the different lipid composition and suggest that the choice of fat source strongly influences oxidative stability and the final quality of salami; therefore, further investigation in this regard is warranted.

Fatty acid profile

The FA profile of salami (Table 3) was significantly influenced by the type of fat used in the initial mixture, while the ripening time had only a limited impact. The SPF salami exhibited a significantly higher percentage of saturated FA compared to the SHF salami. This difference is primarily attributable to the higher content of stearic acid (C18:0) and myristic acid (C14:0) due to the inclusion of pork fat. The SPF salami also showed significantly higher concentrations of arachidic acid (C20:0) and certain branched-chain FA such as C15:0 *iso* and C15:0 *anteiso*.

Conversely, SHF salami displayed a more nutritionally favorable profile, presenting significantly higher percentages of monounsaturated fatty acids (MUFA) and PUFA compared to SPF. It is well-known that the content of unsaturated FA is generally much higher in poultry fat compared to pork or beef fat (Jin et al., 2007). Specifically, the spent hen fat contributed to a markedly superior content of C16:1c9 (palmitoleic acid) and C20:2n-6 in SHF salami compared to SPF. The ripening time also contributed to a significant decrease in the overall PUFA content in salamis, particularly in SPF. This finding suggests, as previously noted by Hur et al. (2007) and Wójciak and Dolatowski (2012), a certain vulnerability of PUFA to oxidation or hydrolysis during the maturation process. Overall, the FA profile suggests that salamis including spent hen fat may possess a superior nutritional quality due to its higher content of MUFA and PUFA. This result strongly supports the attempt of valorization of spent egg-laying hen meat, indicating that using the poultry's intrinsic fat allows for the production of a final product with potentially improved nutritional characteristics compared to the use of traditional pork fat. However, the significant decrease in PUFA observed during ripening highlights the necessity of further investigation into the mechanisms of lipid oxidation to fully preserve the final product's nutritional value.

VOC profiles of salamis

Table 4 reports the VOC profiles of salamis produced with hen meat combined either with chicken fat (SHF) or pork fat (SPF). In both formulations (Fig. 2), the identified volatile compounds belonged to the chemical classes of aldehydes, alcohols, acids, monoterpenes, ketones, and esters. All these are commonly found in dry-fermented meat

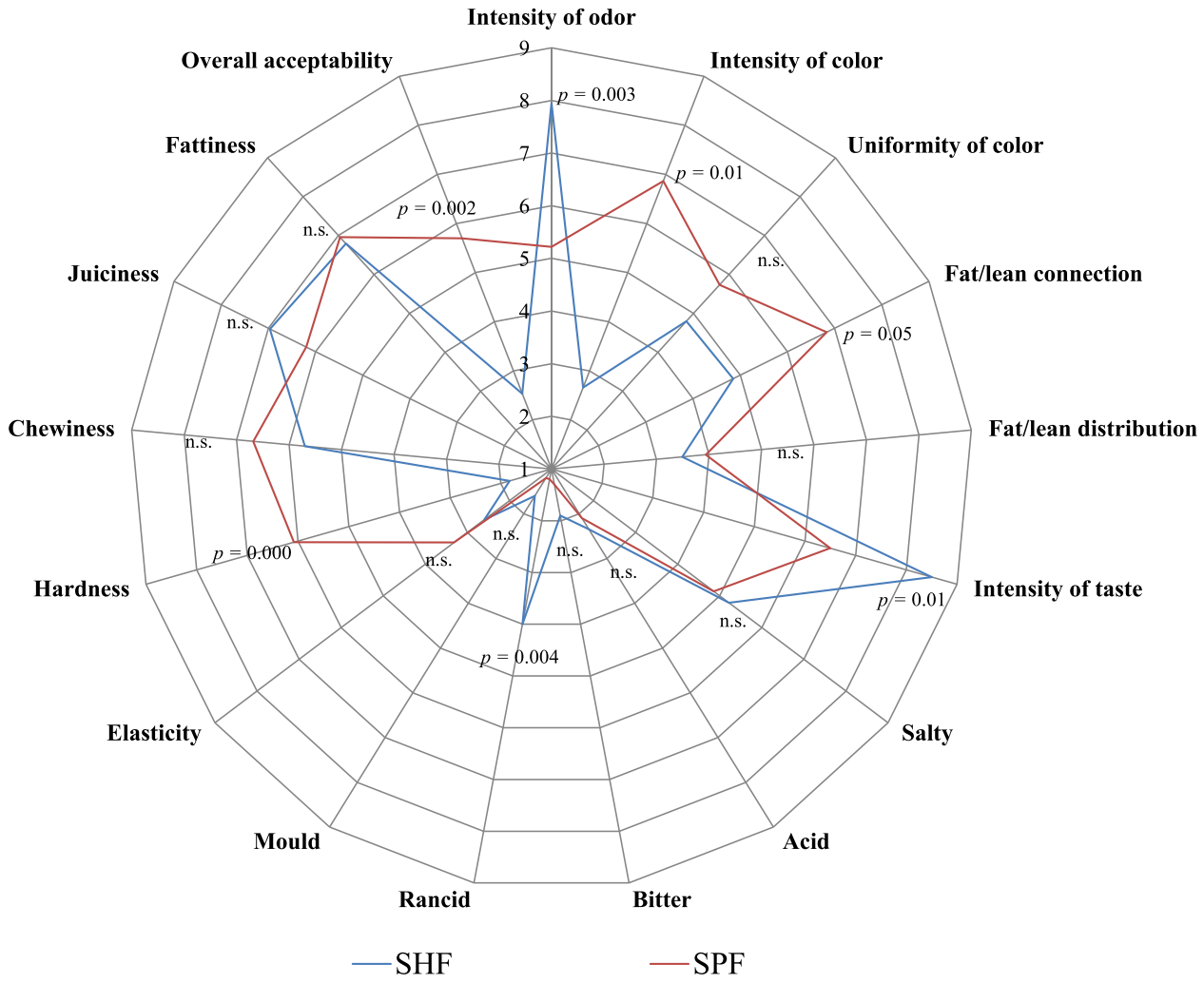

Fig. 3. Spider plot of descriptive sensory analysis of salamis. Abbreviations: SHF, salami made with meat and fat from spent egg-laying hens; SPF salami made with meat from spent egg-laying hens and pork fat. n.s., not significant ($p > 0.05$).

products and are collectively responsible for their complex aromatic profiles (Bianchi et al., 2007; Lorenzo et al., 2012; Liu et al., 2023; Woldemariam et al., 2024). Aldehydes were the most abundant group in both salami types, accounting for 59 % in SHF and 41 % in SPF. These compounds primarily result from lipid oxidation processes and are among the most odor-active volatiles in fermented meat, typically associated with green, fatty, or rancid notes (Sánchez-Peña et al., 2005; Rivas-Cañedo et al., 2012; Vandendriessche et al., 2013; Domínguez et al., 2019). Alcohols represented the second most abundant class, contributing 18 % in SHF and 28 % in SPF. These compounds mainly originate from the reduction of aldehydes or hydroperoxide degradation and contribute with herbaceous, woody, and fatty notes (García and Timón, 2001; Lorenzo et al., 2013, 2014), as well as sweet, fruity, onion-like, or mushroom-like aromas (Bosse et al., 2017). Acids and monoterpenes followed, with comparable relative abundances (acids: 7 % in SHF and 9 % in SPF; monoterpenes: 8 % in SHF and 11 % in SPF). Among the acids, acetic acid was the most prominent in both formulations, followed by butyric acid. These compounds likely result from carbohydrate fermentation and fatty acid degradation, respectively (Liu et al., 2023). Monoterpenes such as α-pinene, limonene, and 3-carene, consistently detected in all samples, are mainly derived from the spices used in the formulation, particularly black pepper, though they may also partially originate from animal feed (Guadayol et al., 1997). Ketones and esters were the least abundant groups (ketones: 5 % in SHF and 7 % in SPF; esters: 1 % in SHF and 3 % in SPF). Despite their lower concentrations, both classes play important roles in aroma development. Ketones, which derive from lipid oxidation and amino acid catabolism, display a wide range of sensory attributes such as buttery, fruity, blue cheese-like, or solvent-like notes (Pastorelli et al., 2003; García--González et al., 2008; Purriños et al., 2012; Petričević et al., 2018). Esters like ethyl acetate and ethyl butanoate, formed through reactions between alcohols and acids, contribute with fruity and sweet nuances (Purriños et al., 2012; Lorenzo and Carballo, 2015). Although both formulations shared similar volatile compounds due to their common raw meat base and same processing production process, notable compositional differences were observed, particularly in the aldehyde content. SHF production showed substantially higher relative levels of key lipid oxidation products. In particular, the levels of hexanal, pentanal, and propanal in SHF salamis were approximately 61 %, 58 %, and 82 % higher, respectively, than in those made with pork fat (SPF). This compositional difference is consistent with the fatty acid profiles of the two formulations, as SHF salamis exhibited significantly higher proportions of PUFAs ($p = 0.0163$), confirming the greater oxidative susceptibility of poultry lipids and explaining the higher abundance of lipid-derived aldehydes (Frankel, 1980; Mancinelli et al., 2021).

Sensory evaluation of salamis

The spider plot showed in Fig. 3 illustrates the outcomes of the descriptive sensory analysis conducted on the salami samples during the

tasting session by expert panellists. According to Tukey's test, no statistically significant differences ($p > 0.05$) were found for uniformity of color, fat/lean distribution, salty, acid, bitter, mould, elasticity, chewiness, juiciness, fattiness. In contrast, there were statistically significant differences ($p < 0.05$) were observed for intensity of odor, intensity of color, fat/lean connection, intensity of taste, hardness, rancid and overall acceptability. In particular, the incorporation of fat derived from laying hens resulted in an increased odor intensity and a heightened perception of rancidity, consistent with the higher oxidative susceptibility of poultry lipids, as also reflected by the chemical indicators of lipid oxidation. These oxidative processes may lead to the formation of lipid peroxidation products, which are known to contribute to off-flavors and negatively impact the sensory quality of the final product (Peña-Saldarriaga et al., 2020; Shahidi et al., 2022). In terms of color intensity, the SPF showed higher values, indicating greater stability. Moreover, the SPF salami demonstrated superior connection between fat/lean respect to SHF salami. These results suggest that pork fat contributes positively to visual appeal and structural cohesion, whereas spent egg-laying hens fat tends to amplify olfactory attributes (Domínguez et al., 2019). The sensory evaluation revealed that salami produced with spent egg-laying hen meat and pork fat received high scores in terms of overall acceptability from the trained panel, indicating a favorable sensory profile for this formulation. Although these results derive from expert assessors rather than consumers, the positive sensory performance suggests promising potential for future market introduction, pending dedicated consumer studies.

Conclusions

This study demonstrates that valorizing spent egg-laying hens through salami production is a viable strategy to reduce waste and promote circular economy principles in poultry systems. Physicochemical analysis revealed significant differences between salami types SHF and SPF and ripening times, particularly in texture and color development. Microbiological assessments confirmed the safety of both products, with pathogenic microorganisms absent and starter cultures effectively reducing undesirable bacteria. Sensory evaluation indicated that SPF salami showed higher color intensity and better fat/lean connection, while both formulations achieved acceptable scores for flavor and overall quality. These findings highlight the potential of spent hens as a raw material for high-value meat products, supporting sustainability goals and reducing environmental impact.

Further optimization, such as using selected starter cultures, natural antioxidants, and refined ripening conditions, could improve texture and lipid stability. Targeted consumer strategies, including informative packaging, tasting sessions, and promotion of nutritional and environmental benefits, may enhance market acceptance. Overall, this approach exemplifies how integrated valorization can transform low-value by-products into functional foods, fostering innovation and resource efficiency in the agri-food sector.

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Data availability

Data will be available on request.

CRediT authorship contribution statement

Gabriele Busetta: Writing – original draft, Software, Formal analysis, Data curation. **Marialetizia Ponte:** Writing – original draft, Software, Formal analysis, Data curation. **Marcella Barbera:** Writing – original draft, Formal analysis, Data curation. **Giuliana Garofalo:** Writing – original draft, Software, Formal analysis, Data curation. **Daniela Piazzese:** Methodology. **Elena Franciosi:** Formal analysis, Data curation. **Adriana Bonanno:** Software, Methodology. **Luca Settanni:** Writing – review & editing, Validation. **Raimondo Gaglio:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Disclosures

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.

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