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Effect of selected starter cultures on microbial dynamics, lipid profile, volatile aroma compounds and sensory properties in buffalo meat salami

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ABSTRACT

Mediterranean buffalo meat is a nutrient-rich, low-fat resource with high protein and favorable lipid profiles. Its use in fermented products, like buffalo salami, remains still limited, despite its economic and cultural importance in regional livestock systems. This study evaluated the impact of four treatments on microbiological succession, lipid composition, volatile compounds, and sensory properties of buffalo salami. The treatments were: not inoculated control (CTR), commercial starter culture (S1), commercial starters combined with *Lacticaseibacillus rhamnosus* E26 and *Lactiplantibacillus plantarum* M5.1 strains (S2), and a combination of *L. rhamnosus* E26 and *L. plantarum* M5.1 strains (S3). Results showed that all treatments did not alter nutritional composition but significantly enhanced lipid quality. S2 and S3 samples reduced cholesterol levels compared to the inoculated control and increased polyunsaturated fatty acids. Atherogenic and thrombogenic indices were lower S1 samples, suggesting a potentially more favorable lipid profile from a nutritional perspective. Microbiological analyses confirmed the absence of *Salmonella* spp. and *Listeria monocytogenes*, a drastic reduction of staphylococci, after 40 days of ripening, and a quite stable level of lactic acid bacteria. Volatile profiles revealed modulation of acids, aldehydes, ketones, and terpenes, consistent with sensory outcomes, with S2 and S3 samples having the highest acceptability, improved aroma and texture. Collectively, targeted use of *L. plantarum* and *L. rhamnosus* strains, as starter cultures, improved microbiological safety, influenced lipid-related nutritional parameters, and improved sensory characteristics, highlighting their potential for the development of high-value buffalo salami. These findings underscore the strategic role of lactic acid bacteria in optimizing functional and organoleptic properties of fermented meat products.

1. Introduction

The Mediterranean Italian buffalo breed plays a strategic role in the national livestock sector, with 437,265 heads distributed across 2271 farms, according to official data from the Italian National Database (BDN, 2025). The buffalo supply chain is traditionally oriented toward milk production for the renowned Mozzarella di Bufala DOP (Denominazione di Origine Protetta; Protected Designation of Origin, PDO), while buffalo meat, mainly from male animals and culled females, remains an underutilized by-product despite its valuable nutritional properties (Berlese et al., 2019).

Buffalo meat is characterized by a low-fat content (approximately 1.37 g/100 g), high protein levels (20.4 g/100 g), and significant amounts of micronutrients such as iron and potassium (Naveena & Kiran, 2014; Tamburrano et al., 2019). Furthermore, it exhibits a favorable lipid profile, with a substantial proportion of unsaturated fatty

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acids and low cholesterol content, making it suitable for the development of nutritionally oriented products (Calabrò et al., 2014; Tamburrano et al., 2019). These features align with current consumer trends favoring foods with high nutritional value and reduced environmental impact. The development of buffalo meat salami represents a strategic opportunity to diversify product offerings, reduce waste, and enhance the profitability of the buffalo supply chain (Coppola et al., 2025; Haque et al., 2024). However, the manufacturing process typically requires the addition of pork fat to ensure proper texture and palatability, which may compromise the nutritional advantage of lean buffalo meat.

To address this challenge, selected starter cultures of lactic acid bacteria (LAB), particularly strains such as *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Lactobacillus garciae*, *Lactiplantibacillus pentosus*, *Limosilactobacillus fermentum* among others, can actively modulate the lipid profile of fermented meat products, thereby enhancing and diversifying their overall nutritional value (Xia et al., 2023). Through their lipolytic and fermentative metabolism, LAB can produce extracellular lipases and esterases that hydrolyze triacylglycerols and phospholipids in meat, releasing free fatty acids, glycerol, and diglycerides (García-Cano et al., 2019).

These intermediates can subsequently undergo desaturation, re-esterification, or incorporation into new lipid classes, shifting the lipid profile toward a higher proportion of nutritionally relevant unsaturated fatty acids, including monounsaturated fatty acids (MUFA) such as oleic acid (C18:1 n-9), and polyunsaturated fatty acids (PUFA) such as linoleic acid (C18:2 n-6), alpha-linolenic acid (C18:3 n-3), and conjugated linoleic acid (CLA, C18:2 cis-9, trans-11). These changes enhance the nutritional profile of the meat while also contributing to flavor, oxidative stability, and the formation of bioactive lipid-derived compounds. Additionally, certain staphylococcal species, such as *Staphylococcus xylosus* and *Staphylococcus carnosus*, are commonly employed as starter cultures due to their key role in nitrate reduction, which enhances the characteristic red color of fermented meat products. Moreover, these species also contribute significantly to lipolytic and proteolytic activities, influencing both the flavor development and textural properties of the final product (Stegmayer et al., 2023).

In general, the selection of bacterial combinations as starter cultures for meat matrices is an important consideration, as they not only promote microbiological stability and sensory development but also contribute to the formation of metabolites with potential functional and organoleptic effects, including short-chain fatty acids and bioactive compounds in the final product. In this context, the aim of the present study was to evaluate the effect of specific starter cultures on microbial succession, as well as on the lipid profile, volatile compounds and sensory properties of buffalo meat salami. This approach seeks to integrate technological requirements with nutritional and functional objectives, positioning buffalo salami as an innovative product with potential nutritional value aligned with current market trends.

2. Materials and methods

2.1. Microorganisms and culture conditions

L. rhamnosus E26 (isolated from Sicilian pecorino cheese) and *L. plantarum* M5.1 (isolated from donkey milk) were selected from the culture collection of the Food and Agricultural Microbiology Laboratory (University of Catania, Sicily, Italy) based on preliminary screening for biotechnological potential according to analyses of the strains' capacity to inhibit pathogenic microorganisms, maintain sensory attributes, and produce bioactive compounds (unpublished data). The selected starter cultures were propagated in Man, Rogosa, and Sharpe (MRS) broth and incubated at 37 °C for 24 h, prior to inoculation. A commercial starter culture, Lyocarni SBL-48 (SACCO, Cadorago, Lombardia, Italy), containing *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Latilactobacillus curvatus*, and *Latilactobacillus sakei*, was acquired and used as experimental group. All starter cultures were standardized to 10^9 CFU/mL, yielding an initial inoculation level of 10^6 CFU/g in samples.

2.2. Sample formulation and processing

Male Mediterranean Italian buffaloes, slaughtered at 23 ± 4 months of age with an average live weight of 551 ± 66 kg, were used for salami production. A total of 40 salamis were manufactured in a commercial processing facility (Masserie del Sole, Ragusa, Italy) under traditional industrial conditions (Fig. 1).

The formulation consisted of 80% buffalo meat (hind leg muscles) and 20% pork belly. Visible fat was trimmed, and both meat and fat were minced using a cutter to obtain particles of approximately 10 mm. Salt (2.5%), whole and ground black pepper (0.10%), natural pork flavor (0.35%) (Mix Dry 1000 AG 17131), salt flavor (0.10%) (Salamix P10, Suprarom extra 46502, Fratelli Pagani, Milan, Italy) and a commercial mix (0.8%) containing dextrose, salt, and antioxidants (Composal 3/B NA 17037and Composal P6, Fratelli Pagani, Milan, Italy) were added before stuffing into collagen artificial casings.

After preparation, salamis were stored at 16 °C and 55% relative humidity (RH) for 6 days, followed by a 40-day drying phase in a ripening room under controlled conditions (11 °C and 76% RH). After ripening, samples were collected for microbiological analyses, nutritional composition, fatty acid profiling, volatile aroma compounds and sensory analysis.

Each salami was considered an independent biological replicate. The salamis were randomly divided into four homogeneous groups of 10 salamis each (n = 10 biological replicates per treatment), designated as S1, S2, S3, and C (control). Each salami represented one experimental unit. For each experimental unit, analytical determinations were performed in triplicate (n = 3 technical replicates). All salamis had a uniform weight (0.9–1.0 kg) and diameter (5.5 cm). They were randomly divided into four homogeneous groups of 10 salamis each, designated as S1, S2, S3, and C (control). The experimental groups differed in the type of starter culture applied, whereas the control group was produced under the same conditions without any starter addition, representing the standard formulation.

- **S1**: Lyocarni SBL-48 commercial starter culture containing *Staphylococcus carnosus*, *Staphylococcus xylosus, Latilactobacillus curvatus, Latilactobacillus saquei* (SACCO, Cadorago, Lombardia, Italy);
- **S2**: Lyocarni SBL-48 commercial starter culture previously described combined with strains *L. rhamnosus* E26 and *L. plantarum* M5.1;
- **S3**: only strains *L. rhamnosus* E26 and *L. plantarum* M5.1.

2.3. Chemical composition and fatty acid analyses

Five salami samples were collected from each experimental group at the end of the 40-day ripening period. All analyses were performed in triplicate. Moisture content was determined by oven-drying (Memmert UF 110, Memmert GmbH + Co. KG, Schwabach, Germany) at 105 °C until a constant weight was achieved. Crude protein was quantified using the Kjeldahl method (AOAC, 2005) and the results were expressed as % nitrogen × 6.25. Ash content was measured by incinerating samples in a muffle furnace at 550 °C for 6 h. Total lipids were extracted following the method described by Folch et al. (1957), based on a chloroform–methanol solvent system, and expressed as a percentage of fresh tissue.

The composition of fatty acid was assessed by gas chromatography (GC) after methylation of fatty acids according to Christie (1982). Fatty acid methyl esters (FAMEs) were separated and quantified using a GC equipped with a flame ionization detector (FID) and a capillary column suitable for FAME analysis. Results were expressed as the percentage of total identified fatty acids.

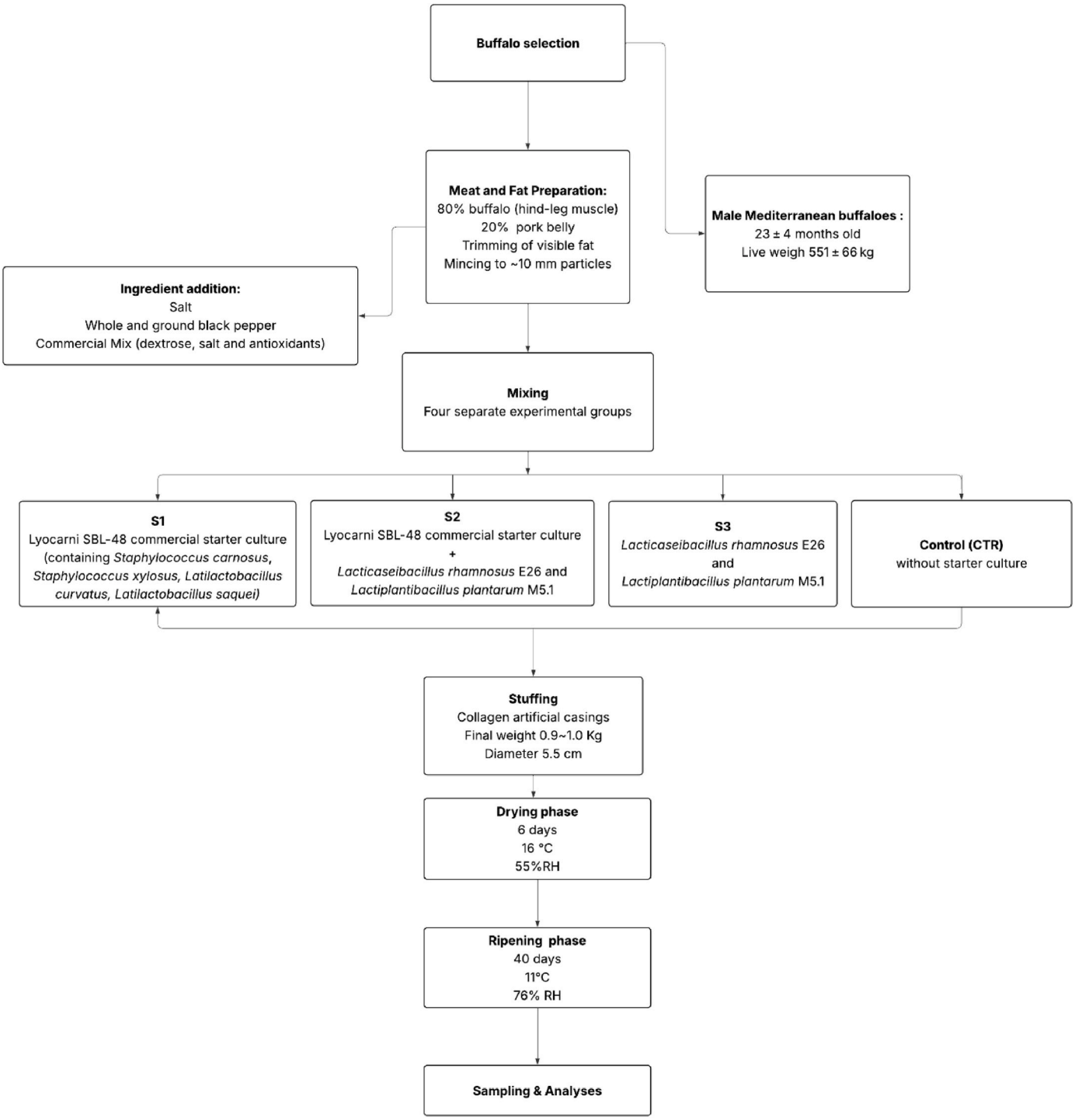

Fig. 1. Flowchart of Buffalo Salami production.

2.4. Nutritional indicators and lipid health indices

The analysis included the main fatty acid classes: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), as well as the nutritional indices, which is considered relevant for cardiovascular health.

To better assess the nutritional quality of fat, two indices were calculated according to Ulbricht and Southgate (1991):

Atherogenic Index (AI), which estimates the potential of fatty acids to promote atheroma formation.

$$AI = (C12{:}0 + (4 \times C14{:}0) + C16{:}0)/(\Sigma MUFA + \Sigma n{-}6\ PUFA + \Sigma n{-}3\ PUFA) \quad (1)$$

Thrombogenic Index (TI), which reflects the tendency of fatty acids to favor clot formation.

$$TI = (C14{:}0 + C16{:}0 + C18{:}0)/[(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n{-}6\ PUFA) + (3 \times \Sigma n{-}3\ PUFA) + (\Sigma n{-}3\ PUFA/\Sigma n{-}6\ PUFA)] \quad (2)$$

Additionally, the Hypocholesterolemic Fatty Acids (DFA) were calculated following Osmari et al. (2011), representing fatty acids that

tend to lower blood cholesterol.

(3) DFA = UFA + C18:0

where UFA is the sum of unsaturated fatty acids.

Conversely, Hypercholesterolemic Fatty Acids (OFA), associated with increased cholesterol levels, were calculated as follows.

(4) OFA = C12:0 + C14:0 + C16:0

2.5. Microbiological and physicochemical analyses

The microbiological analyses were performed in accordance with reg. EC 2073/2005 (European Commission, 2005). Changes over time were assessed at two sampling points (20 and 40 days). Specifically, 25 g of each sample was dissolved in 225 mL of sterile saline solution and homogenized in a stomacher (BagMixer® 400, Interscience, France) for 90 s. After appropriate serial dilutions, they are plated on agar media and incubated at 37 °C for 24–48 h. *Escherichia coli* β-glucuronidase positive on Chromatic EC-X Gluc Agar (Ec-x gluc Agar, Biolife, Italy), *Escherichia coli* O157 on CHROMagar/Colorex *E. coli* 0157 (Biolife, Italy), members of the Enterobacteriaceae family on Violet Red Bile Glucose Agar (VRBGA, Liofilchem, Italy), *Clostridium perfringens* on Sulphite Polymixin Sulphadiazine Agar (SPS, Liofilchem, Italy), total mesophilic count on Plate Count Agar (PCA, Liofilchem, Italy), mesophilic LAB in Man-Rogosa-Sharpe (MRS, Biolife, Italy) and presumptive staphylococci on Mannitol Salt Agar (MSA, Biolife, Italy). The results were expressed as log CFU/g.

To detect *Listeria monocytogenes* and *Salmonella* spp., two sequential pre-enrichments were performed for each pathogen. For *L. monocytogenes*, samples were incubated in Listeria Half Fraser Broth followed by Listeria Broth (Liofilchem, Italy), and then streaked onto Listeria Palcam Agar (Liofilchem, Italy). For *Salmonella* spp., samples were first inoculated into peptone water (10 g/L peptone, 5 g/L NaCl) and subsequently into Rappaport–Vassiliadis Broth (Liofilchem, Italy), before being streaked onto Hektoen Enteric Agar (Liofilchem, Italy). All plates were incubated at 37 °C for 24 h and the presence or absence of *L. monocytogenes* and *Salmonella* spp. was assessed, respectively.

The pH was measured using a pH meter pH 8+ DHS BASIC (XS Instruments, Carpi, Italy) on a homogenate of 10 g of sample in 90 mL of distilled water.

2.6. Volatile aroma compounds analysis

The volatile aroma compounds of buffalo meat salami samples were analyzed at the end of the 40-day ripening period by Headspace Solid-Phase Microextraction coupled with Gas Chromatography–Mass Spectrometry (HS-SPME-GC-MS). For each salami, 5 g were homogenized and added with 15 mL of saturated NaCl solution, transferred into a 40 mL vial, and equilibrated for 30 min at 40 °C under continuous stirring. A triphasic DVB/CARB/PDMS fiber was then exposed to the headspace for 30 min to extract volatile compounds under the same conditions, followed by thermal desorption in the GC injector at 260 °C for 3 min.

GC-MS analyses were performed using a Shimadzu GC-2010 Plus system coupled to a TQMS 8040 triple-quadrupole mass spectrometer (Shimadzu, Milan, Italy), equipped with a Vf-WAX-ms capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Separation was performed under the following chromatographic conditions: injector temperature 260 °C in splitless mode; oven program starting at 45 °C (held for 5 min), ramped to 80 °C at 5 °C/min, then to 150 °C at 2 °C/min, then to 240 °C at 10 °C/min and held for 15 min; carrier gas helium at a constant flow of 1 mL/min; transfer line temperature at 250 °C; mass acquisition range 40-300 m/z, scan speed 1250 amu/s; Electron Ionization (EI) at 70 eV.

Volatile compounds were identified by comparison with the NIST 2024 mass spectral library (NIST/EPA/NIH, Wiley, Gaithersburg, MD, USA) and the FFNSC 3.0 database. Identification was further confirmed by calculating linear retention indices (LRIs) by using a C7-C30 homologous series of n-alkanes (Supelco Inc., Bellefonte, PA, USA) under the same chromatographic conditions, according to the Van den Dool and Kratz equation, and comparing them with the NIST database (https://webbook.nist.gov/chemistry) based on published reference data (Cincotta et al., 2025). For each experimental trial, three salami were analyzed, and each sample was analyzed in triplicate. The results were expressed as mean relative peak area percentages.

2.7. Sensory analysis

2.7.1. Qualitative descriptive analysis

Qualitative Descriptive Sensory Analysis (QDA) was performed on salami at the end of the 40-day ripening period. A trained panel of 8 judges was selected based on prior experience in salami evaluation and trained according to ISO 8586:2023. The participants have given their written consent according to the principles of the Declaration of Helsinki.

During preliminary sessions, panelists tasted commercial salami slices and described taste, odor, flavor, appearance, and texture. Through discussion, a list of 24 sensory attributes was generated, and from these, 16 attributes with >60% citation frequency were selected for final evaluation (Fig. 6a). Reference standards were provided for each attribute to anchor intensity and to establish a shared vocabulary. All evaluations were conducted in a controlled sensory laboratory equipped with individual booths. Samples were served at room temperature (20-23 °C). Panelists evaluated each sample in triplicate in different sessions. Attributes were rated using a structured 1-9 scale (1 = absence, 9 = extremely intense). Samples were presented in randomized order, coded with three-digit numbers, and served as slices of 0.4 mm thickness. Panelists cleansed their palate with water at the beginning of each session and with unsalted crackers between samples. The final score for each attribute was calculated as the average of three replicates from three independent samples per trial. Panel performance was confirmed by ensuring that the coefficient of variation for each attribute did not exceed 20%.

2.7.2. Consumer acceptability

Seventy-two regular salami consumers (mean age 38 years; 46% men, 54% women) participated in the consumer's sensory acceptability test as described by Tripodi et al. (2025). Each consumer evaluated a 0.4 cm-thick slice of each salami sample, presented in a randomized order, and cleansed their palate with water at the beginning of the session and with unsalted crackers between samples. Consumers rated the overall acceptability by using a 9-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely).

2.8. Statistical analyses

Data from chemical composition, fatty acid profile, nutritional indicators, and lipid health indices were analyzed using one-way analysis of variance (ANOVA). When significant effects were detected, means were compared using Tukey's multiple comparison test (adjusted Tukey HSD), with significance defined as $P < 0.05$. To compare mean microbiological counts among groups, one-way ANOVA was performed. Data normality was assessed using the Shapiro–Wilk test, and Tukey's HSD post hoc test was applied to identify differences among microbial counts. For volatile aroma compounds and sensory data, one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test was applied to determine significant differences among treatments ($P < 0.05$). Statistical analyses were performed using XLSTAT software, version 2024.1 (Addinsoft, New York, NY, USA).

3. Results and discussion

3.1. Chemical composition, fatty acid profile, and lipid-related health indices

The macro-nutritional composition of buffalo meat salami was not significantly affected by the use of different starter cultures ($P > 0.05$), as moisture, protein, fat, and ash contents remained similar among treatments (Table 1). These results suggest that technological formulation played a predominant role in determining the structural characteristics of the product, whereas the starter cultures mainly affected biochemical changes occurring during the ripening process. Similar findings have been reported in fermented meat systems, where LAB guide fermentative metabolism and enhance safety without substantially altering formulation-driven compositional features (Bis-Souza et al., 2019).

A key finding was the significant reduction in cholesterol content in salami inoculated with selected starter cultures, particularly in treatments S2 and S3 compared to the control (CTR). Cholesterol decreased from 104 mg/100 g in CTR to 79.9 mg/100 g in S3 and to 85.6 mg/100 g in S2 ($P = 0.01$), which represents a nutritionally relevant improvement. Several mechanisms may account for this effect. LAB strains such as *L. plantarum* and *L. rhamnosus* can assimilate cholesterol by incorporating it into their membranes or binding it to cell surfaces (Ahmed et al., 2025; Guo et al., 2024). Their activity is also associated with bile salt hydrolase (BSH) enzymes, which deconjugate bile salts and indirectly enhance cholesterol removal. Some strains further convert cholesterol into coprostanol, a sterol with reduced intestinal absorption (Netter et al., 2025). These biological processes, combined with microbial lipolysis and fat redistribution during fermentation, likely contributed to the reductions observed in S2 and S3.

Although the cholesterol-lowering capacity of several LAB strains has been demonstrated *in vitro* and *in vivo* (Ahmed et al., 2025; Lv et al., 2024), evidence of such effects within fermented meat matrices remains limited. To our knowledge, no peer-reviewed study has clearly shown cholesterol reduction in salami through LAB inoculation, suggesting that this outcome may depend on specific strains or synergistic combinations. Notably, even though these strains are not currently classified as probiotics in meat products, their viability in fermented sausages suggests potential post-consumption functionality.

Starter cultures also influenced the fatty acid profile of buffalo salami. Palmitoleic acid (C16:1) increased significantly, while linoleic acid (C18:2 n-6) and total PUFA were higher in S1 and S3 compared to CTR. In contrast, when the same strains were combined in S2, metabolic interactions appeared to suppress PUFA accumulation, possibly due to competition or altered metabolic fluxes. Total PUFA was significantly greater in S1 (3.94%) than in CTR (3.20%) ($P = 0.01$), whereas MUFA tended to increase and SFA slightly decreased, although without statistical significance. These modifications align with the lipolytic and fermentative potential of LAB, which release free fatty acids and contribute to bioactive lipid formation during ripening.

The metabolic behavior observed in S1 is consistent with the succession typical of mixed starter cultures. LAB (*L.curvatus* and *L. sakei*) dominate early fermentation by rapidly utilizing sugars, producing lactic acid, and reducing pH. As ripening progresses, conditions become favorable for coagulase-negative staphylococci (CNS), including *S. carnosus* and *S. xylosus*, which mediate lipolytic and proteolytic activities and modulate the sensory and lipid characteristics of the final product (Ye et al., 2024). Moreover, health-related indices further confirmed the functional benefits of starter cultures. The atherogenic index (AI) was significantly lower in S1 (0.64) compared to CTR (0.69) ($P = 0.02$), and the thrombogenic index (TI) showed a marked reduction in S1 (1.84) versus CTR (2.01) ($P = 0.002$). These indices are widely recognized as predictors of cardiovascular risk, and their improvement highlights the potential of microbial starters to enhance the nutritional quality of fermented meat products.

Overall, while starter cultures did not alter the macro-nutritional composition of buffalo meat salami, they significantly improved lipid quality by reducing cholesterol, increasing PUFA, and lowering AI and TI. These effects, driven by microbial lipolytic activity and cholesterol assimilation, demonstrate that selected starter cultures can enhance both the nutritional and functional attributes of buffalo salami. This approach provides an innovative strategy for adding value to buffalo meat while aligning with current consumer demands for foods with improved lipid profiles and greater sustainability.

Table 1
Effect of experimental treatments (CTR, S1, S2, and S3) on proximate composition, fatty acid methyl esters (FAME), and nutritional indices in the seasoned Salami of Italian Mediterranean buffaloes (mean ± SEM).

Item	Treatment				SEM[a]	*P*-value Tukey adj.
	CTR	S1	S2	S3		
Moisture, %	43.79	43.33	43.53	44.77	0.59	0.21
Protein, %	25.35	25.65	25.57	25.17	0.35	0.56
Fat, %	23.59	24.12	24.74	23.64	0.35	0.10
Ash, %	5.50	5.35	5.57	5.30	0.09	0.09
Cholesterol, mg/100 g	104.00^{b}	89.73^{ab}	85.60^{a}	79.90^{a}	6.51	**0.01**
Fatty Acids, g/100 g of total fat						
C14:0	1.72	1.87	1.83	1.97	0.12	0.14
C15:0	0.24	0.28	0.34	0.29	0.05	0.20
C16:0	26.65	24.84	25.37	25.49	0.88	0.17
C16:1	1.24^{a}	1.71^{b}	1.80^{b}	1.74^{b}	0.27	**0.05**
C17:0	0.92	1.00	0.92	0.91	0.04	0.19
C17:1	0.47^{ab}	0.54^{a}	0.38^{b}	0.41^{ab}	0.06	**0.03**
C18:0	21.96^{ab}	21.24^{a}	23.01^{b}	22.23^{ab}	0.44	**0.01**
C18:1	43.60^{ab}	44.28^{a}	42.83^{b}	43.31^{ab}	0.47	**0.05**
C18:2 n-6	2.96^{a}	3.63^{b}	3.06^{a}	3.18^{ab}	0.19	**0.01**
C18:3 n-3	0.23	0.31	0.28	0.24	0.04	0.22
C20:0	0.33	0.13	0.14	0.33	0.24	0.56
SFA	51.58	49.36	51.62	51.21	1.11	0.09
MUFA	45.30	46.54	45.01	45.46	0.47	0.08
PUFA	3.20^{a}	3.94^{b}	3.33^{a}	3.42^{ab}	0.21	**0.01**
UFA	50.48^{b}	48.34^{a}	48.87^{a}	48.50^{a}	0.47	**0.001**
UFA/SFA	1.02^{b}	0.94^{a}	0.95^{a}	0.94^{a}	0.01	**0.003**
DFA	71.72	71.36	71.49	71.49	0.53	0.83
OFA	46.50	46.42	50.12	45.87	2.72	0.38
Atherogenic Index (AI)	0.69^{b}	0.64^{a}	0.68^{ab}	0.68^{ab}	0.01	**0.02**
Thrombogenic Index (TI)	2.02^{a}	1.84^{b}	2.01^{a}	1.98^{a}	0.03	**0.002**

[a-b] Different letters indicate statistically significant differences among treatments ($P \leq 0.05$).
SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; UFA = Unsaturated Fatty Acids; UFA/SFA = Unsaturated Fatty Acids/Saturated Fatty Acids; DFA = Desirable Fatty Acids; OFA = Other Fatty Acids; TI = Thrombogenic index; AI = Atherogenic index.
[a] Greatest standard error of mean.

3.2. Microbiological and physicochemical analyses

In the microbiological assessment, *Salmonella* spp. and *Listeria monocytogenes* were absent in 25 g for all samples. Moreover, none of the samples presented quantifiable levels (<1 $CFUg^{-1}$) of *Clostridium* spp., *Escherichia coli* β-glucuronidase positive, *Escherichia coli* O157 and Enterobacteriaceae. Collectively, these results underscore the effectiveness of the processing conditions and the need for rigorous control measures throughout the production chain to maintain microbiological quality and ensure food safety. However, as noted by Coppola et al. (2025), it is important to emphasize that studies have reported detectable levels of coliforms and Enterobacteriaceae family at the beginning of the ripening period, which generally decline significantly over time (Busetta et al., 2025; Coppola et al., 2025). Also, during the first days of salami ripening, a pH declines to around 5.2 or lower typically occurs.

Regarding our pH results, at the end of ripening, the pH values of the salami samples ranged between 5.18 and 5.52 (Fig. 2). These values are

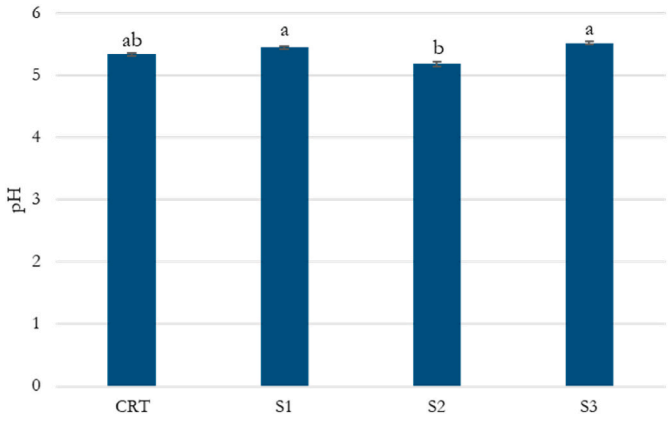

Fig. 2. pH value of the seasoned Salami of Italian Mediterranean buffaloes. Different letters indicate statistically significant differences among treatments ($P \leq 0.05$).

in agreement with findings on the pH of buffalo salami (Conte et al., 2012). Statistically significant differences were observed across the different samples, with S2 showing the lowest value, 5.18. This could be due to the synergy of the commercial starter cultures combined with *L. rhamnosus* E26 and *L. plantarum* M5.1 strains, used in the production process. Moreover, the observed reduction in pH contributes to microbiological safety by creating conditions that inhibit the growth of total and fecal coliforms and other undesirable microorganisms, as has been demonstrated in fermented meat products where acidification during ripening leads to significant decreases in coliform counts (Dalzini et al., 2022; Hospital et al., 2014; López et al., 2004). Despite statistical differences, pH showed only minor variation among samples and did not correlate with sensory perception of acidity, as discussed in the sensory section. This confirms that pH mainly reflects free H^+ ions from predominant acids, especially lactic acid in salamis (Kim et al., 2021).

In relation to mesophilic microbial counts, mesophilic LAB, and presumptive staphylococci, all treatments (S1, S2, and S3) significantly modified microbial ecology after 40 days of ripening compared with the control (CTR) (Fig. 3). The treatments S2 and S3 were the most effective, showing a significant reduction of approximately 1.5 log cycles in total mesophilic counts compared with the CTR and S1 ($P < 0.05$). The statistical stability observed between T20 and T40 in these groups suggests that mesophilic inhibition occurs early and remains stable throughout the evaluated period.

An increase in mesophilic LAB was observed across all treatments, as expected, since all formulations included starter cultures. This finding confirms the effectiveness of the formulations in supporting starter viability. A time-dependent growth pattern was evident, with T40 counts exceeding those of T20 either numerically or statistically. The highest levels were observed in treatment S3 (>8 Log CFU/g), which contained strains E26 and M5.1. This population increase represents the metabolic requisite for the antimicrobial effects observed against the pathogens. Moreover, as these strains were selected for use as starter cultures, their growth demonstrates a favorable microbial development in fermented products. It should be noted that the enumeration of lactic acid bacteria performed in the present study reflects the total LAB population rather than the specific dynamics of the inoculated starter strains. In fermented meat systems, the added starter cultures may coexist with autochthonous microbiota naturally present in the raw material, and these populations can overlap under conventional culture conditions. As a result, traditional plating methods using standard selective media (e.g., MRS agar) do not allow reliable differentiation between inoculated strains and indigenous lactic acid bacteria. Therefore, total LAB counts were used as an indicator of the overall fermentation dynamics, which is a common approach in studies of fermented meat products. A more precise monitoring of individual strains would require strain-specific molecular tools, such as polymerase chain reaction (PCR) based methods or sequencing approaches.

For ripening products, it is desirable for starter cultures to remain viable and metabolically active throughout the entire phase, and the results support this requirement (Wilkinson & LaPointe, 2020). Even in the CTR, the presence of LAB can be observed at around 4 CFU/g, which may be considered part of the native microbiota. This indigenous microbiota also plays an important role in the ripening of salamis that are not inoculated with starter cultures. Similar findings were reported by Busseta et al. (2025), who observed LAB counts of 7 CFU/g at the end of the ripening phase. The authors also detected comparable levels of coagulase-negative staphylococci and yeasts, which further confirms that the addition of starter cultures effectively modulates both staphylococcal populations and other microorganisms.

Overall, all treatments demonstrated an antimicrobial antagonism against staphylococci compared with the control. From 20 to 40 days of ripening, treatments S1 and S2 exhibited a significant reduction in staphylococci counts. This decline was not immediate but progressive, indicating a notable interaction between treatment and time. While the control (CTR) maintained consistently high and stable populations, S1 and S2 showed pronounced and statistically significant reductions at T40. The progressive reduction of staphylococci observed in our study is consistent with previous findings in fermented meat systems. In a classical sausage fermented model, *L. plantarum* starter culture and their bacteriocin, significantly reduced, for example, *Staphylococcus aureus* growth, demonstrating the capacity of LAB starters to modulate

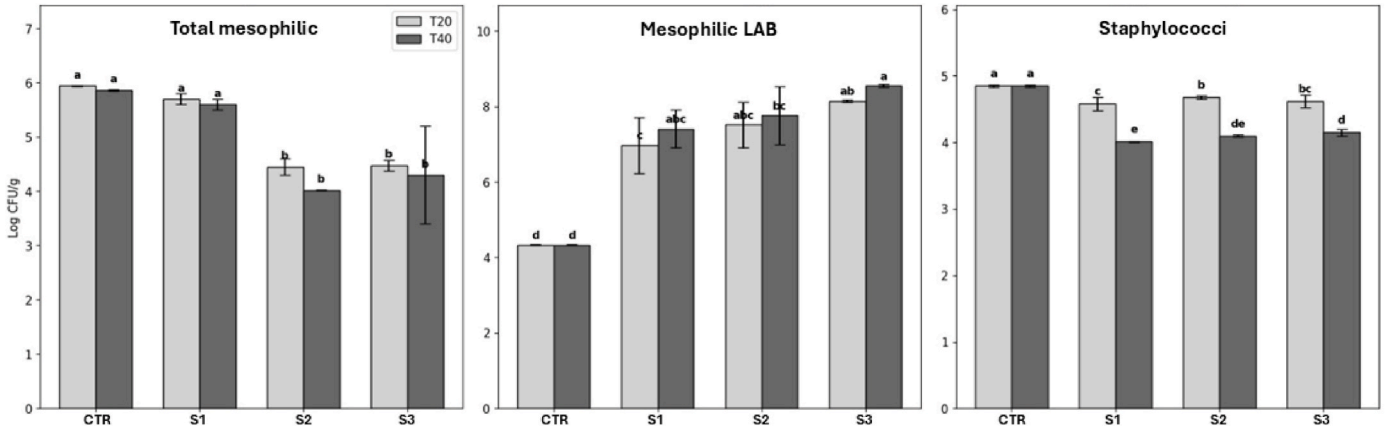

Fig. 3. Changes in total mesophilic, mesophilic LAB and presumptive staphylococci counts across treatments (CTR, S1, S2, and S3) at 20 (T20) and 40 (T40) days of buffalo salami ripening. Error bars correspond to the mean standard deviation. [a-e] Different letters indicate statistically significant differences among treatments ($P \leq 0.05$).

pathogen behavior through competitive and metabolic mechanisms (Li et al., 2024). Similarly, investigations in salami-type fermented meat products have shown that the establishment of a dominant LAB population during ripening is strongly associated with a marked decline in *S. aureus*, driven by acidification, nutrient competition, and the accumulation of antimicrobial metabolites produced by active starter cultures (Mani-López et al., 2024).

These observations align with our data, supporting the hypothesis that the inhibitory effect at T40 results from the sustained metabolic activity of the starter cultures throughout ripening. Notably, even in the buffalo meat matrix, for which few studies are available, our findings indicate that microbiota modulation through specific starter culture combinations plays a critical role during in the salami process. Furthermore, the potential impact of our findings is significant. The search for starter cultures with proven antagonistic activity against undesirable microorganisms, while also contributing to improvements in the nutritional profile and sensory characteristics of meat products, represents a promising strategy for the meat industry and an important market opportunity.

3.3. Volatile aroma compounds

A broad range of volatile aroma compounds was identified in all salami samples produced with different starter cultures. Across all treatments (CTR, S1, S2, and S3), terpenes were the most represented chemical class, followed by acids, alcohols, aldehydes, ketones, esters, sulfur compounds, and furans. Statistically significant differences ($P \leq 0.05$) were observed among treatments for all classes of substances (Fig. 4). These differences reflect not only the direct effects of starter cultures on microbial metabolism but also the indirect modulation of enzymatic reactions and oxidative processes, which together shape the final aroma profile. Changes in the abundance of volatile compounds in fermented salamis are mainly driven by the meat matrix composition, spice addition, and ripening time, which promote terpene release, basal lipid oxidation, and endogenous enzymatic reactions (Ewelina et al., 2025). The observed variations suggest that starter cultures are likely responsible for differences in the levels of pre-existing aroma compounds, thereby shaping the overall sensory profile of the product without generating entirely new volatile compounds (Talon et al., 2007).

Regarding the volatile acids, a significantly higher amount ($P = 0.02$) was observed in samples S1 (15.94%) and S3 (15.79%) compared to the CTR (8.21%) and S2 (12.61%) (Table 2). These results indicate that the added selected starter cultures modulate acid production by redistributing metabolic fluxes rather than suppressing it entirely, reflecting a dynamic interaction between starter and autochthonous microbiota (Polizzi et al., 2024). As reported by Blaya et al. (2018), the interaction between starter cultures and non-starter cultures may lead to competition for residual carbohydrates, which can alter the expression of genes involved in acid metabolism in both populations.

The highest concentrations of 2-methyl-propanoic and 3-methyl-butanoic acids observed in S1 and S3 indicate an enhanced catabolism of branched-chain amino acids, particularly valine and leucine. In S1, this is likely facilitated by synergistic activity of LAB and coagulase-negative staphylococci, such as *S. carnosus* and *S. xylosus,* which are known to possess alcohol and aldehyde dehydrogenase activities, promoting efficient conversion of amino acid-derived aldehydes into acids, which may enhance characteristic savory notes (Madsen et al., 2002). This metabolic pathway, closely related to the Ehrlich pathway suggests a finer modulation of flavor precursors that could affect the perception of mild versus intense aroma attributes. This pathway, described in yeasts and certain LAB, involves amino acid transaminases and decarboxylases and has been shown to underlie the generation of key odor compounds in meat fermentation systems (Liu et al., 2022). In S3, *L. plantarum* M5.1 and *L. rhamnosus* E26 may also contribute to branched-chain acid formation, although through a more restricted metabolic pathway, leading to the accumulation of simpler and more stable metabolites. Accordingly, the low total aldehydes content observed in S1 and S3 suggests an enhanced microbial conversion of these reactive intermediates, whereas the low amount of acids detected in the CTR samples indicates a reduced microbial transformation of proteolysis-derived compounds.

Distinct ketone profiles were observed among treatments, demonstrating that the selected LAB starter cultures specifically modulate

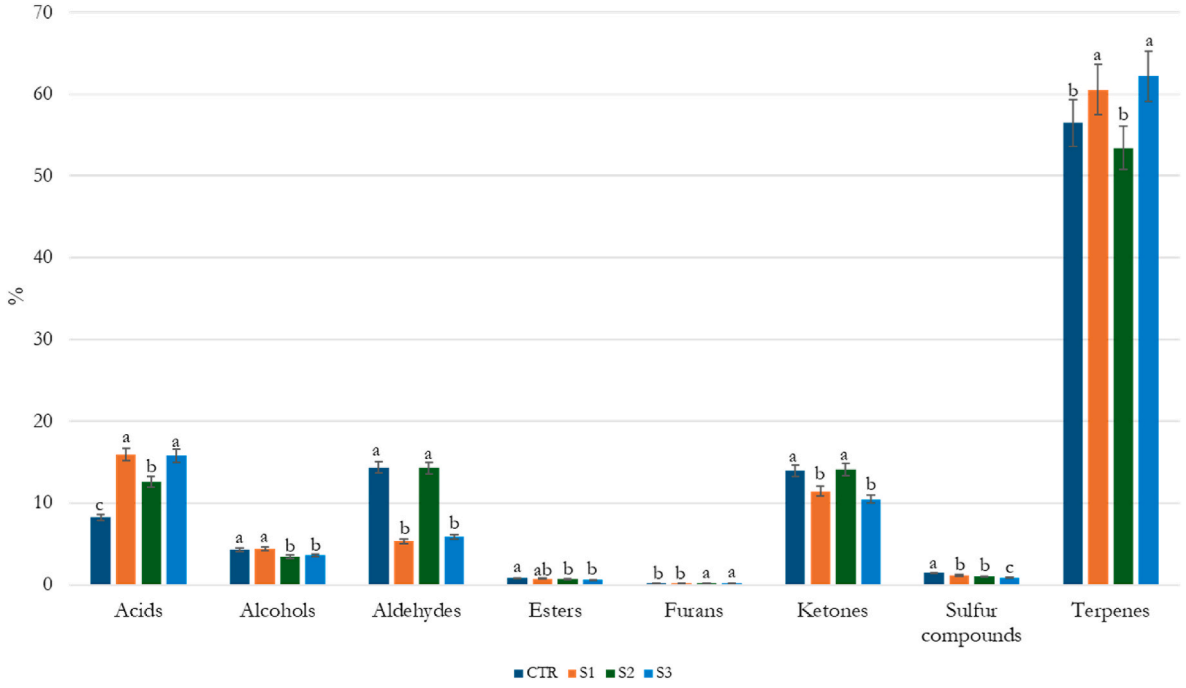

Fig. 4. Volatile aroma compounds amount percentage for class of substances of buffalo meat salami samples. Different letters in the same class of substances indicate statistically significant differences among treatments ($P \leq 0.05$).

Table 2
Volatile aroma compounds content (average area %) of Italian Mediterranean buffalo meat salami under different treatments.

Compound	LRI*	CTR	S1	S2	S3
Acids					
2-Methyl-propanoic acid	1563	0.93b	1.79a	1.83a	2.12a
3-Methyl-butanoic acid	1664	7.28c	14.15a	10.78b	13.67a
All		*8.21c*	*15.94a*	*12.61b*	*15.79a*
Alcohols					
Methyl Alcohol	903	0.74c	1.43a	0.62c	0.91b
Ethanol	936	0.14b	0.24a	0.18a	0.09b
1-Propanol	1038	0.28a	0.12b	0.11b	0.10b
1-Butanol	1142	0.27a	0.04b	0.08b	0.08b
1-Penten-3-ol	1155	0.29a	0.14b	0.35a	0.20b
Isoamyl alcohol	1201	1.47a	1.65a	1.16b	1.45a
1-Pentanol	1238	0.81	0.74	0.90	0.76
1-Octen-3-ol	1434	0.30a	-**b	-b	-b
All		*4.28a*	*4.37a*	*3.41b*	*3.59b*
Aldehydes					
Butanal	880	0.49a	0.03b	0.12b	0.03b
2-Methyl-butanal	917	0.01b	0.05a	0.04a	0.01b
3-Methyl-butanal	920	0.05b	0.05b	0.09a	0.05b
Hexanal	1081	5.92b	0.79d	8.94a	3.64c
(*E*)-2-Pentenal	1131	0.12b	0.10b	0.38a	0.07b
Heptanal	1180	1.78a	1.08b	2.33a	0.54c
(*E*)-2-Hexenal	1215	0.14b	0.12b	0.43a	0.09b
(*Z*)-2-Heptenal	1312	0.55a	0.13c	0.18c	0.28b
Nonanal	1380	5.29a	2.98b	1.73c	1.14c
All		*14.35a*	*5.32b*	*14.26a*	*5.85b*
Esters					
Methyl isobutyrate	924	0.05	0.09	0.03	0.06
2-Methyl-butanoic acid methyl ester	1011	0.12	0.12	0.10	0.12
3-Methyl-1-butanol acetate	1117	0.02b	0.04b	0.07a	0.04b
Hexyl formate	1334	0.37a	0.37a	0.39a	0.21b
Methyl octanoate	1374	0.22a	0.13b	0.10b	0.19a
All		*0.78a*	*0.75 ab*	*0.69b*	*0.62b*
Furans					
2-Pentyl furan	1220	0.14b	0.12b	0.18a	0.19a
All		*0.14b*	*0.12b*	*0.18a*	*0.19a*
Ketones					
Acetone	821	1.69c	1.66c	2.74b	3.62a
2-Butanone	907	0.11c	1.29a	0.19b	0.16b
Diacetyl	982	3.50a	1.47b	3.38a	1.84b
2,3-Pentadione	1061	0.13b	0.12b	0.21a	0.15b
3-Heptanone	1148	0.04a	-b	-b	-b
2-Heptanone	1178	0.21a	0.21a	0.14b	0.21a
3-Octanone	1243	0.12	0.13	0.11	0.11
2-Octanone	1271	0.10	0.09	0.07	0.06
Acetoin	1277	7.76a	6.24a	6.89a	3.91b
2,5-Octanedione	1308	0.33b	0.23c	0.37a	0.40a
All		*13.98a*	*11.44b*	*14.10a*	*10.46b*
Sulfur compounds					
Allyl mercaptan	894	0.42a	0.36a	0.32b	0.28b
Allyl methyl sulfide	957	0.78a	0.61a	0.49b	0.39b
Diallyl sulfide	1139	0.11a	0.05b	0.05b	0.08a
Diallyl disulfide	1467	0.12	0.12	0.17	0.09
All		*1.42a*	*1.14b*	*1.03b*	*0.85c*
Terpenes					
α-Pinene	1020	1.69b	2.23a	1.50b	1.62b
α-Thujene	1025	0.07b	0.17a	0.15a	0.13a
β-Pinene	1097	3.20	2.95	2.78	3.35
3-Carene	1135	10.12	10.93	10.26	11.85
Myrcene	1151	4.44	4.80	4.18	4.79
α-Terpinene	1167	0.03	0.06	0.10	0.09
Limonene	1188	19.10	18.18	17.89	18.71
β-Phellandrene	1198	0.31a	0.34a	0.26b	0.36a
γ-Terpinene	1232	0.73a	0.34b	0.19c	0.29b
p-Cymene	1254	2.49b	2.66b	3.12a	2.35b
(+)-4-Carene	1261	0.30b	0.52a	0.54a	0.36b
Isoterpinolene	1266	0.84	0.90	0.86	0.93
δ-Elemene	1457	0.46b	1.03a	0.64b	0.95a
α-Copaene	1480	0.91a	1.13a	0.74b	1.26a
Linalool	1536	2.15a	0.67b	0.60b	0.61b
Caryophyllene	1587	9.16b	12.93a	9.13b	13.94a
α-Humulene	1659	0.38b	0.51a	0.38b	0.39b
δ-Cadinene	1746	0.09b	0.20a	0.14b	0.24a
All		*56.48b*	*60.57a*	*53.45b*	*62.20a*

*LRI= Linear retention index; **Not detected; Different letters indicate statistically significant differences among treatments ($P \leq 0.05$). LRI were confirmed by comparing those present in the NIST database (https://webbook.nist.gov/chemistry/).

metabolic pathways. The progressive increase in acetone from CTR to S3 indicates that these strains enhance pyruvate metabolism and the generation of intermediates derived from lipids and amino acids, thereby playing a key role in shaping the volatile profile and overall aroma of the fermented salami. 2-Butanone, responsible for fruity notes, was significantly higher in S1, indicating that the combined activity of LAB and coagulase-negative staphylococci increases its formation (Flores, 2018). In contrast, diacetyl, associated with buttery aromas, was more abundant in CTR and S2 samples, suggesting that the combination of the commercial starter with *L. plantarum* M5.1 and *L. rhamnosus* E26 strains may regulate or further metabolize this compound. Ketones such as diacetyl and acetone, which have low odor thresholds, contribute markedly to buttery and fruity notes, whereas longer-chain ketones add aromatic complexity (Flores, 2018). However, aroma perception in fermented meat products cannot be attributed to ketones alone, as it results from the combined effects of matrix composition, physicochemical properties, microbial metabolism, and lipolytic and proteolytic reactions during fermentation and ripening (Bleicher et al., 2022).

Aldehyde differed markedly among treatments, indicating variable lipid oxidation and amino acid catabolism. CTR and S2 showed the highest total aldehyde content (14.35% and 14.26%), while S1 and S3 presented significantly lower levels, suggesting improved oxidative stability. Hexanal, commonly used as a key marker of linoleic acid oxidation (Liu et al., 2023), was more present in S2 and less in S1 samples. Heptanal and nonanal followed a similar trend, with higher values in CTR and S2, in line with studies reporting reductions in heptanal and nonanal when antioxidant-active starter cultures or yeast strains are used (Domínguez et al., 2019). Branched aldehydes such as 2- and 3-methyl-butanal, derived from amino acid degradation, were generally more abundant in S1 and S2, supporting previous observations that proteolytic activity and formulation factors influence these compounds in dry fermented salami (Amaral et al., 2018).

Regarding terpenes, a high prevalence of terpenes was found across all treatments. Although statistically significant differences ($P = 0.01$), these compounds are not primarily produced through microbial metabolism. Most of them, such as limonene, α-terpinene, 3-carene, isoterpinolene, and myrcene, originate from the spices used in salami formulation (Flores, 2018; Kaya & Kaban, 2024). The role of starter cultures and LAB in this context may appear to be related to the preservation and stabilization of terpenes by reducing oxidation or minimizing degradation during ripening. Consequently, microbial metabolic activity may contribute to the stabilization and modulation of volatile compounds, influencing the overall sensory attributes of the fermented product. Our results indicate that, despite the overall high terpene abundance in all samples, S1 and S3 treatments slightly modulate volatile composition by limiting oxidative reactions and enhancing terpene retention, thereby maintaining a higher relative amount of specific terpenes, such as caryophyllene, δ-elemene, and α-copaene. Similar results were reported by Kaya and Kaban (2024) in fermented sausages by using mixed cultures of *L. plantarum* and *S. xylosus*, which led to an increase of terpenes such as caryophyllene and copaene.

Sulfur compounds such as allyl mercaptan, allyl methyl sulfide, diallyl sulfide, and diallyl disulfide were detected at low relative amounts. These volatiles were commonly identified in fermented dry sausages and sucuk, and they may originate both from spice ingredients, such as the addition of garlic, and from microbial metabolism of sulfur-containing amino acids during fermentation and ripening (Kaya & Kaban, 2024; Sallan et al., 2022). These compounds have very low odor thresholds and can perceptibly contribute to the aroma of the final product even at low concentrations, impacting sensory notes such as pungency or savory. Significant differences were observed among

treatments ($P = 0.02$); notably, in CTR samples, allyl methyl sulfide and allyl mercaptan showed the highest values of 0.78% and 0.42%, respectively. Similar results were reported by Belleggia et al. (2020), who also found allyl methyl sulfide in *Ciauscolo* salami. Differences among treatments imply that starter cultures may modulate sulfur compound accumulation, potentially balancing pungency and savory notes in the final product.

The heatmap (Fig. 5) clearly shows that the treatments significantly influence the volatile profiles of buffalo salamis. The CTR samples showed a higher relative intensity of several compounds typically associated with spontaneous fermentation and lipid oxidation, such as alcohols, aldehydes, and ketones. This finding suggests a more heterogeneous aromatic profile, indicating that the absence of a selected starter culture results in not controlled and distinctly different volatiles composition. Treatments S1 and S3 clustered more closely, indicating greater similarity in their aromatic profiles. S1 exhibited a volatile profile typical of salami produced with commercial starter cultures, which are well validated and widely used in the meat industry due to their proven technological performance and consistency.

In contrast, S2 showed an intermediate intensity profile but with greater metabolic diversity, supporting the hypothesis that the combination of a commercial starter with *L. plantarum* M5.1 and *L. rhamnosus* E26 enhances and diversifies the aromatic profile of fermented salamis by activating more complex metabolic pathways. Treatment S3 showed a higher amount of branched aldehydes, alcohols, and ketones, indicating strong activation of carbohydrate and amino acid metabolic pathways. These pathways are known to be highly active in *L. plantarum* M5.1 and *L. rhamnosus* E26, which possess versatile metabolic capabilities related to carbohydrate fermentation and amino acid catabolism. The results suggest that these strains have a strong potential to modulate aroma development in meat products even in the absence of a commercial starter culture, indicating their suitability as promising candidates for future use as starter or adjunct cultures in fermented meat processing.

3.4. Sensory analysis

The sensory analysis of buffalo salami enabled the systematic characterization of their key sensory attributes, providing an integrated assessment of its visual, olfactory, textural, and flavor properties. This approach also offered valuable insights into how microbial metabolism and chemical transformations occurring throughout fermentation shape the final sensory profile.

The sensory results, as illustrated in the spider plot (Fig. 6a), are consistent with the difference in volatile aroma compounds observed among the different treatments. Samples inoculated with starter cultures (S1, S2, and S3) showed different sensory profiles when compared to the CTR, reflecting the impact of microbial metabolism on aroma development and overall acceptability.

S1 samples inoculated with the commercial starter culture exhibited a sensory profile characterized by higher scores for desirable aroma attributes such as odor intensity, seasoned and fermented odor, and black pepper odor. These sensory features align with the higher relative abundance of acids, ketones, and terpenes observed in the volatile analysis, coupled with lower aldehyde levels, suggesting efficient microbial conversion of reactive intermediates into more stable, aroma-active compounds, as previously reported for fermented sausages inoculated with coagulase-negative staphylococci (Li et al., 2025). Furthermore, commercial starter cultures are composed of well-characterized microorganisms that undergo careful technological selection and performance evaluation prior to industrial application. This ensures consistent metabolic activity that reliably shapes aroma and flavor, explaining the standardized sensory profile observed in S1. Consequently, even when applied to different meat matrices, these cultures tend to generate more standardized and homogeneous sensory profiles (García-Díez & Saraiva, 2021).

Despite the presence of LAB, S2 and S3 samples showed a reduced perception of acid taste, which may be attributed to metabolic interactions and competition for fermentable substrates, which can redirect carbohydrate metabolism and reduce the sensory impact of organic acid. Similar effects have been reported when adjunct LAB are combined with established starter cultures, resulting in altered acidification kinetics and reduced accumulation of sensory-active acids (Blaya et al., 2018; Mani-López et al., 2024). Moreover, the combination of selected LAB with commercial ones containing staphylococci, may promote further conversion of organic acids and aldehydes into less sensory-impactful metabolite contributing to smoother flavor perception and increased overall acceptability.

The S3 samples, which were fermented exclusively with *L. plantarum* M5.1 and *L. rhamnosus* E26, exhibited improved sensory attributes compared to the control. Their pronounced acidification and branched-chain acid formation capacity enhanced odor complexity, while their enzymatic activity contributes to textural attributes such as gumminess, reflecting proteolytic modulation. These samples also showed higher scores for black pepper odor and pepper taste related to the higher relative amount of terpenes observed in S3, and support a protective effect against oxidative degradation of spice-derived compounds, directly correlating with the sensory perception (Gutiérrez-del-Río et al., 2021).

The CTR samples, fermented exclusively by the autochthonous microbiota without the addition of selected starter cultures, showed a distinctive sensory and chemical profile compared to S1 and S3. These samples were characterized by a higher amount of aldehydes and lower concentrations of branched-chain acids, which may contribute to the perception of a less distinct odor. Aldehydes are often associated with pungent, fatty, or green notes and can negatively affect aroma quality, as reflected in the higher rancid odor scores observed. Hexanal and nonanal are products of fatty acid oxidation and are frequently associated with sensory perceptions of rancidity and undesirable flavors in meats (Karabagias, 2018). These compounds act as markers of lipid oxidation and sensory degradation when present at high levels, as demonstrated by the volatile aroma compounds findings (Table 2). CTR samples shows a high score for bitter and acidic taste, a stronger perception of saltiness, and greater hardness. These attributes likely arise from limited microbial activity, where many compounds remain unprocessed and undergo only natural enzymatic or lipid oxidation, resulting in stronger basic tastes, firmer texture, and off-flavors.

The differences in sensory characteristics reflected the findings in the consumer acceptability scores obtained across the experimental treatments (Fig. 6b). Samples from treatments S2 and S3 showed significantly higher acceptability ($P \leq 0.05$) compared with the CTR and S1. These findings indicate that targeted microbial modulation, through its ability to enhance flavor complexity, aroma, and texture, leads to a more appealing and consumer-preferred product. These findings also highlight that even with the addition of different LAB strains, microbial metabolism can follow complex pathways, and overall consumer acceptability is multifactorial, depending on product composition, texture, flavor, odor, color, and other sensory attributes.

Although few studies have specifically focused on buffalo salami, recent work by Coppola et al. (2025) demonstrated that formulation adjustments, such as the inclusion of functional ingredients, can positively affect sensory attributes and consumer acceptability by improving flavor and overall product quality. Moreover, variations in buffalo meat-to-fat ratios have been shown to significantly change juiciness and texture, correlating with overall sensory acceptability (Frangopoulos et al., 2025). Consumer preference studies in fermented salamis with different spice additions further highlight aroma, flavor, and texture as key determinants of acceptability (Freschi et al., 2023). In light of this, in fermented meat products, the balance between fat content, spice composition, and fermentation time interacts with microbial metabolism to shape sensory characteristics. Even subtle changes in microbial composition can drive pronounced differences in aroma complexity,

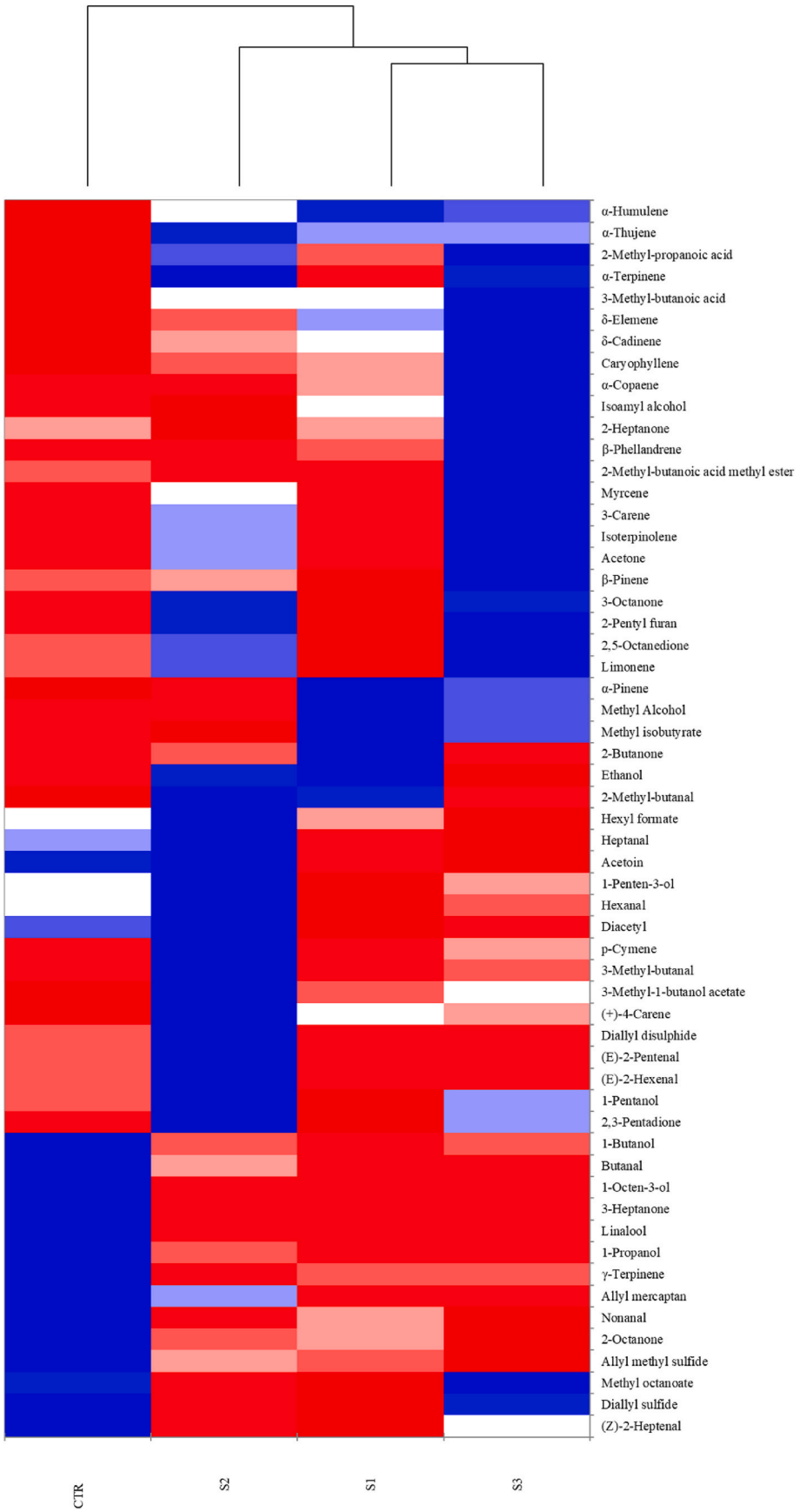

Fig. 5. Heatmap visualization based on the volatile compounds of seasoned buffalo meat salami samples. The color of each tile of the heatmap presents the abundance of each volatile compound among the different samples. The red color indicates major abundance, while the blue color indicates minor abundance.

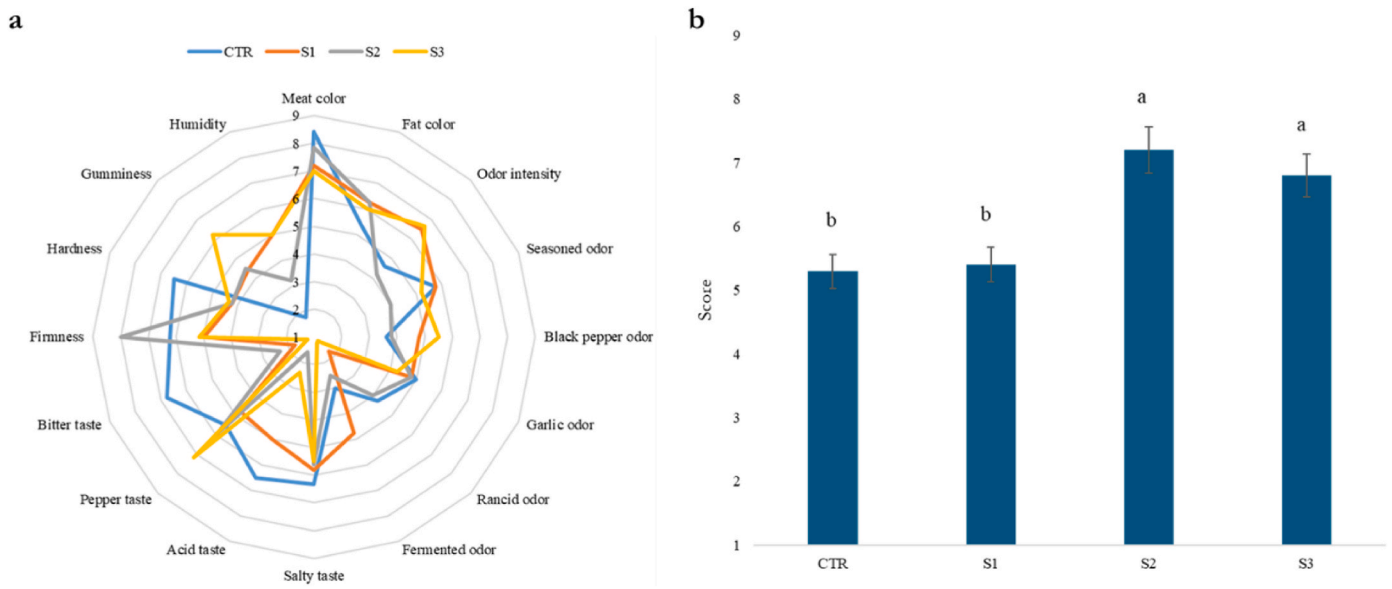

Fig. 6. a) Radar chart showing the sensory attributes at the end of ripening time of Salami of Italian Mediterranean buffaloes; **b)** Sensory acceptability of Salami of Italian Mediterranean buffaloes. Different letters indicate statistically significant differences among treatments ($P \leq 0.05$).

acid perception, and textural attributes, underscoring that sensory acceptability is multifactorial and strongly driven by the interplay between chemical and microbiological factors.

Our results demonstrate that specific starter cultures, particularly *L. plantarum* M5.1 and *L. rhamnosus* E26, can enhance sensory acceptability by promoting efficient metabolic conversions, stabilizing aroma-active compounds, and preventing undesirable oxidative reactions. These findings suggest that, beyond ingredient adjustments, the strategic use of starter cultures, not only to control fermentation but also to optimize aroma, flavor, and texture, may represent an effective approach for improving the overall quality of buffalo salami. In this context, their application could support the competitiveness and growth potential of buffalo salami within the Mediterranean market.

4. Conclusions

The strategy used in the present study, based on the application of selected starter cultures markedly enhanced the safety, nutritional quality, and sensory profile of Italian Mediterranean buffalo salami. Treatments S2 and S3 achieved significant cholesterol reduction, improved PUFA content, and lowered atherogenic and thrombogenic indices, suggesting a more favorable lipid profile from a nutritional perspective. Starter cultures, particularly *L. plantarum* M5.1 and *L. rhamnosus* E26, promoted rapid dominance, suppressed undesirable microbiota, and shaped the volatile aroma profile, resulting in higher consumer acceptability. These strains exhibit strong technological versatility and hold promise as future starters or adjunct cultures. Overall, the findings demonstrate that deliberate selection of starter cultures can simultaneously ensure microbiological safety, improve lipid-related nutritional quality, and enhance the sensory appeal of buffalo salami. This approach represents a practical strategy for adding value to buffalo meat products while supporting more sustainable production and responding to growing market interest in buffalo-based fermented products in the Mediterranean region.

CRediT authorship contribution statement

Luana Virgínia Souza: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Fabrizio Cincotta:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Georgiana Bosco:** Writing – original draft, Validation, Methodology, Formal analysis. **Antonio Fernandes de Carvalho:** Writing – review & editing, Visualization, Validation. **Cinzia Caggia:** Writing – review & editing, Visualization, Validation, Conceptualization. **Luís Augusto Nero:** Writing – review & editing, Visualization, Validation. **Vincenzo Lopreiato:** Writing – review & editing, Visualization, Validation, Data curation, Conceptualization. **Danilo Scalone:** Writing – review & editing, Visualization, Validation, Conceptualization. **Orazio Peluso:** Writing – review & editing, Visualization, Validation, Resources. **Nicoletta Paparone:** Writing – review & editing, Visualization, Validation. **Cinzia Lucia Randazzo:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Luigi Liotta:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Ethics statement

The experimental protocol, including all sensory evaluation procedures involving human participants, was reviewed and approved by the Ethical Committee of the Department of Veterinary Science, University of Messina, Italy (approval code: 07/2024bis). All participants provided written informed consent prior to participation, in accordance with the principles of the Declaration of Helsinki. The study was conducted ensuring the protection of participants' rights, privacy, and voluntary participation.

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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.

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Data availability

Data will be made available on request.

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