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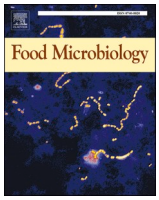

Microbial dynamics and quality characteristics of spontaneously fermented salamis produced by replacing pork fat with avocado pulp

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ABSTRACT

The aim of this study was to develop a novel and healthier fermented meat product by replacing pork fat with avocado pulp (AVP) during salami production. Experimental salamis were produced under laboratory conditions by substituting pork fat with AVP partially (10-AVP) and totally (20-AVP), while control salamis (CTR) remained AVP-free. The microbial composition of control and experimental salamis was assessed using a combined culture-dependent and -independent approach. Over a 20-days ripening period, lactic acid bacteria, coagulase-negative staphylococci, and yeasts dominated the microbial community, with approximate levels of 9.0, 7.0 and 6.0 log CFU/g, respectively. Illumina technology identified 26 taxonomic groups, with leuconostocs being the predominant group across all trials [constituting 31.26–59.12 % of relative abundance (RA)]. Gas Chromatography–Mass Spectrometry (GC-MS) analysis revealed changes in fatty acid composition and volatile organic compounds due to the substitution of pork fat with AVP. Specifically, monounsaturated fatty acids and terpene compounds increased, while saturated fatty acids and lipid oxidation products decreased. Although AVP influenced the sensory characteristics of the salamis, the highest overall satisfaction ratings were observed for the 10-AVP salamis. Consequently, substituting pork fat with AVP emerges as a viable strategy for producing healthier salamis and diversifying the meat product portfolio.

1. Introduction

Salamis, traditional foods with a rich history, owe their production techniques to empirical methods developed for meat preservation (Leroy et al., 2015). These globally consumed products are traditionally made from pork meat and fat, seasoned with salt and spices, and devoid of nitrate, nitrite, or starter cultures (Lupu, 2021). The fermentation process in salami production, when starter cultures are absent, relies on indigenous microorganisms present in the raw materials or introduced from the processing environment (Francesca et al., 2013). These beneficial microorganisms must proliferate rapidly to outcompete unwanted spoilage or pathogenic bacteria, ensuring the quality and safety of the final products (Settanni et al., 2020).

Pork fat, a key ingredient in the formulation of fermented salamis, is rich in saturated fatty acids (SFA) and cholesterol (Parunović et al., 2017). Research has consistently demonstrated that diets high in SFA and cholesterol, coupled with low unsaturated fatty acids (UFA), can adversely impact human health, particularly contributing to non-communicable diseases (Li and Sun, 2019; WHO, 2018). Presently, consumers associate full-fat meat products with unhealthiness (Jezewska-Zychowicz et al., 2020). In response to this challenge, both academic researchers and salami producers are actively seeking innovative formulations that reduce SFA and cholesterol levels in salamis while maintaining their quality attributes (Kumar, 2021). A promising strategy involves substituting pork fat with plant-derived fats, either partially or entirely. This approach not only lowers SFA and cholesterol content but also enhances antioxidant properties, ultimately rebuilding consumer trust in meat products (Rodríguez-Carpena et al., 2012).

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Among the alternatives explored, extra-virgin olive oil and emulsified seed oils have emerged as primary substitutes for animal fat in fermented salamis (Del Nobile et al., 2009; Martínez et al., 2023; Monteiro et al., 2017).

To our knowledge, no previous research has investigated the partial or complete replacement of pork fat with fruit pulp in fermented salamis. In this study, avocado (*Persea americana*) pulp (AVP) was used to create a novel type of salami with reduced SFA content. However, it is widely acknowledged that this tropical fruit is abundant in UFA, vitamins, minerals, dietary fibers, and bioactive compounds, all of which positively impact human health (Alkhalaf et al., 2019). Salami productions were conducted under laboratory conditions without the addition of starter cultures. The specific objectives of this study were to: (i) assess the primary microbial populations during salami productions using a combined culture-dependent and -independent approach; (ii) evaluate the physicochemical and antioxidant properties throughout salami productions; and (iii) analyse the composition of volatile organic compounds and sensory characteristics of the final salami products.

2. Materials and methods

2.1. Avocado fruit characteristics

Avocado (*Persea americana*) fruits from the cultivar Hass were provided by the farm "Sicilia Avocado®" located in Giarre (Catania, Italy, 37.69′ N, 15.17′ E). After harvesting, the fruits were stored at room temperature (20 ± 1 °C) until they reached full ripeness, which was evaluated based on their dry matter content, as described by Magwaza and Tesfay (2015). A representative sample of five avocado fruits was used to analyse the dry matter content and determine the ideal ripeness for creating salamis. The seeds and skin were removed from the fruits, and the pulp was cut into smaller pieces and processed into a paste with a mechanical blender. The resulting sample was placed in three pre-weighed ceramic pods and the initial weight of the fresh sample was recorded. The samples were then placed in an oven at 103.5 °C for 12 h to remove the entire water content. The final weight of the dried sample was measured, and the dry matter was calculated according to the following equation (1) (Osuna-García et al., 2010):

$$DM\ (\%) = \frac{[1 - (FW - DW)]}{FW} x\ 100 \quad (1)$$

where *DM* is dry matter, *FW* is fresh weight, *DW* is dry weight.

Dry matter content was evaluated three times at monthly intervals after the date of harvest.

2.2. Avocado pulp preparation

Before salami productions, ripe avocado fruits were washed using a two-step procedure as described by Alfonzo et al. (2018). This technique involved immersing the fruits in a chlorinated water solution (0.2%, v/v) for 2 min, followed by a rinse in cold water (4 °C) to remove the residual chlorine. After drying, the fruits were manually peeled with a sterilized stainless-steel sharp knife. The avocado pulp (AVP) was then cut into pieces approximately 0.5 × 0.5 cm^2 in size.

2.3. Production of salamis

Salami productions were performed under laboratory conditions using minced meat and fat from pork (*Sus domesticus*) purchased at a local supermarket (Decò - Gruppo Arena, Palermo, Italy) without the addition of starter cultures. The experimental plan consisted of three different trials: one control (CTR) and two experimental (10-AVP and 20-AVP) productions (Table 1). Cooking salt (Italkali, Petralia Soprana, Italy), black pepper (Conad, Scarperia e San Piero, Italy), and nitrate/nitrite (Tec-Al S.r.l., Traversetolo, Italy) were added to all trials. The ingredients were manually mixed in aluminium vats, previously sanitized with an antimicrobial solution (DTH101, Trezzo sull'Adda, Italy). The resulting mixtures were stuffed into 3.5 cm diameter natural pork casing (Mediterranea Budella, Alcamo, Italy) using a horizontal manual sausage filler (Tre Spade mod. 5, FACEM S.p.A., Turin, Italy). The salamis were then ripened for 20 d in a storage chamber at 13 °C and 90% relative humidity. Samples of AVP, minced meat, pork fat, natural pork casing, mixtures soon after stuffing, and salami after 20 d of ripening were collected for analysis. Two independent salami productions were performed two weeks apart.

Table 1
Salami recipes.

Trials	Composition (g)					
	Minced meat	Pork fat	Avocado pulp	Salt	Black pepper	Nitrate/ Nitrite
CTR	1600	400	–	60	6	0.2
10-AVP	1600	200	200	60	6	0.2
20-AVP	1600	–	400	60	6	0.2

Abbreviations: CTR, control salami prepared without avocado pulp (AVP) addition; 10-AVP, experimental salami prepared with the partial replacement of pork fat with AVP; 20-AVP, experimental salami prepared with the total replacement of pork fat with AVP.

2.4. Microbiological analysis

Twenty-five grams of each sample were first homogenized in 225 mL of Ringer's solution (Sigma-Aldrich, Milan, Italy), by a stomacher and then subjected to the serial dilutions in the same isotonic solution (Settanni et al., 2020). Appropriate dilutions were plated on selective agar media to enumerate the main microbial populations associated with salami productions as reported in Table 2.

Baird Parker was supplemented with Rabbit Plasma Fibrinogen (Oxoid) for the differentiation of CPS and CNS during plate counting. CPS are identified by the production of a fibrin precipitation halo (ISO 6888-2, 1999). Except for the mesophilic LAB cocci and rods and members of Enterobacteriaceae family, inoculated by pour plate method, all other microbial populations were plated using the spread

Table 2
Microorganisms and growth conditions.

Microorganisms	Media	Incubation conditions	Company
TMM	Plate Count Agar	30 °C for 72 h	Microbiol Diagnostici, Uta, Italy
Mesophilic coccusLAB	Medium 17 agar	30 °C for 48 h	Biotec, Grosseto, Italy
Mesophilic rod LAB	de Man-Rogosa-Sharpe agar	30 °C for 48 h	Oxoid, Hampshire, UK
Yeasts	Dichloran Rose Bengal chloramphenicol	30 °C for 48 h	Microbiol Diagnostici, Uta, Italy
Enterobacteriaceae	Violet Red Bile Glucose Agar	37 °C for 24 h	Condalab, Madrid, Spain
Escherichia coli	Chromogenic Medium Agar	37 °C for 24 h	Condalab, Madrid, Spain
Listeria monocytogenes	*Listeria* Selective Agar Base	37 °C for 24 h	Oxoid, Hampshire, UK
Salmonella spp.	Xylose Lysine Deoxycholate	37 °C for 24 h	Liofilchem, Roseto degli Abruzzi, Italy
CPS and CNS	Baird Parker Agar	37 °C for 48 h	Oxoid, Hampshire, UK

Abbreviations: TMM, total mesophilic microorganisms; LAB, lactic acid bacteria; CPS, coagulase-positive staphylococci; CNS, coagulase-negative staphylococci.

plate technique (Guida et al., 2022). Anaerobiosis conditions were applied through hermetically sealed jars using the AnaeroGen AN25 system (Oxoid) only for LAB. Microbiological counts were performed in duplicates for all samples at each collection time.

2.5. MiSeq illumina data analysis

An aliquot (1 mL) from the first serial decimal dilution (10^{-1}) of AVP and salamis soon after stuffing and after 20 d of ripening was centrifuged at 13000 rpm for 3 min (Botta et al., 2022). Cell pellets were used for total genomic DNA extraction using the DNeasy PowerFood Microbial Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The purified DNA was subjected to Illumina MiSeq using 250 bp paired-end kits (Illumina, Inc., San Diego, CA, USA). The amplification of bacterial V3–V4 hypervariable region of the 16S rRNA was performed as reported by Claesson et al. (2010). The purification, quantity, and quality assessments of the purified DNA were performed at the Fondazione Edmund Mach (FEM, San Michele a/Adige, Italy) sequencing platform. The demultiplexed, FASTQ formatted sequences were then deposited to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under Bioproject ID PRJNA1012883.

The datasets were demultiplexed into FASTQ files and then imported into Quantitative Insights Into Microbial Ecology version 2020.11 (QIIME 2, Bolyen et al., 2019) using the cassava 1.8 paired-end demultiplexed FASTQ format. The raw sequence reads were quality-checked using the "qiime demux summarize" plug-in and denoised using DADA2 (Callahan et al., 2016). The "q2-feature-table tabulate-seqs" command was used to generate representative sequences (rep-seqs). The multiple sequence alignment program (MAFFT ver.7) aligned the amplicon sequence variants and the sequence output was piped into the Approximate-Maximum-Likelihood Trees (Katoh and Standley, 2013). A pre-trained Naive Bayes classifier based on the Greengenes 13_8 99% Operational Taxonomic Units (OTUs) database which had been previously trimmed to the V4 region of 16S rDNA, bound by the 341F/805R primer pair, was applied to paired-end sequence reads to generate taxonomy tables.

2.6. Physicochemical analysis of salamis

The cross-sectional area of salamis was measured using a caliper immediately after stuffing and after 20 d of ripening. Salamis were then subjected to the determination of colorimetric parameters by a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan). Samples taken from salamis were analysed for pH by a HI 9025 pH meter (Hanna Instrument, Ann Arbor, MI, USA), and water activity (a_w) by a HygroPalm water activity indicator (Rotronic, Bassersdrof, Germany), as described by Gaglio et al. (2016a).

The tenderness of the salamis was assessed by measuring the compressive strength (N/mm^2) at the point of maximum deformation of a sample with a diameter of 2.5 cm and a height of 2.5 cm, without casing, kept at room temperature. The measurements were taken in duplicate using an Instron 5564 tester (Instron, Trezzano sul Naviglio, Milan, Italy).

All salami samples were frozen at −20 °C and then lyophilized to be analysed for centesimal chemical composition. Dry matter (DM), protein, fat and ash content were determined according to AOAC standard method (AOAC, 2023).

2.7. Antioxidant analysis of salamis

Extracts of salamis were obtained from the lyophilized samples using the method of Rashidinejad et al. (2013), with minor modifications. Briefly, 0.5 g of each sample was suspended in 25 mL of methanol aqueous solution (95% v/v) containing 1% HCl. The mixture was shaken by vortex for 30 s and then placed in an ultrasonic bath (LBS1 Sonicator; Falc Instruments, Treviglio, Italy) at 40 °C for 30 min. The mixture was vortexed every 10 min for a few seconds. After cooling, the suspension was filtered with linen cloth and centrifuged at 7000 RPM/min for 10 min at 9 °C. The extracts were then stored at −18 °C until analysis.

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

The Trolox equivalent antioxidant capacity (TEAC) of the salami extracts was determined by analysing them in duplicate using Trolox as standard, as described by Ponte et al. (2022). The ABTS radical cation was obtained by reacting 14 mM ABTS aqueous solution with an equal volume of 4.9 mM persulfate of potassium and incubating the mixture in the dark for 16 h at 22 °C (room temperature). The ABTS radical cation solution was then diluted with 5 mM phosphate-buffered saline (PBS) at pH 7.4 to an absorbance of 0.795 (±0.02) at 734 nm. The absorbance of a mixture of 75 μL of PBS with 1425 μL of a diluted ABTS solution was read at 734 nm after incubation for 6 min at 30 °C. Similarly, 75 μL of each extracted sample were mixed with 1425 μL diluted ABTS radical cation solution, and after incubation at 30 °C for 6 min, the absorbance read at 734 nm was used to calculate the percentage decrease of the absorbance due to decolorization in comparison with the absorbance read with PBS. Trolox solutions in PBS, between 0 and 2.5 mM, were used to develop a calibration curve ($R^2 = 0.99$). The results were expressed in mmol Trolox equivalent/kg DM.

The oxidative stability of salami fat was evaluated by determining the peroxide values (POV, meq O_2 kg/fat), as a primary lipid oxidation index (IDF, 1991), and the thiobarbituric acid reactive substances [TBARS, mg malondialdehyde (MDA)/kg DM] as secondary lipid oxidation index, performed according to the method proposed by Tarladgis et al. (1960) and modified by Mele et al. (2011) as described by Ponte et al. (2022).

2.8. Determination of salami fatty acids

The determination of fatty acid (FA) composition involved raw materials (avocado pulp, pork meat and pork fat) and the salami at 20 d of ripening of the two productions. The extraction of the fat from the lyophilized samples (0.5 g) and the preparation of FA methyl esters (FAME) were carried out according to O'Fallon et al. (2007). Each sample was added with C23:0 (Sigma-Aldrich, Milan, Italy) as internal standard (0,8 mg/g lyophilized sample) to quantify total FA. The FAME were recovered in 1.5 mL hexane and 1 μl of each sample was injected by an autosampler into a HP 6890 gas chromatography system equipped with a flame ionization detector (Agilent Technologies, Santa Clara CA, USA). The capillary column used for the separation of FAME was 100 m in length with an internal diameter of 0.25 mm and a film thickness of 0.25 μm (CP-Sil 88, Chrompack, Middelburg, Netherlands). Gas chromatography conditions and identification of each FA were as described by Bonanno et al. (2013). Individual FA were expressed as g/100 g total identified FA.

The thrombogenic index (TI) was calculated as proposed by Ulbricht and Southgate (1991) using equation (2):

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

The health-promoting index (HPI) was calculated as proposed by Chen et al. (2004) applying equation (3):

$$HPI = \frac{n-3\ PUFA + n-6\ PUFA + MUFA}{(C12:0+4) \times (C14:0 + C16:0)} \quad (3)$$

2.9. Analysis of volatile organic compounds emitted from salamis

Gas chromatography-mass spectrometry (GC/MS) was used to analyse the volatile organic composition of AVP and salamis after 20 d of ripening. Samples of AVP and salami (5 g) were finely chopped and placed in separate glass vials for headspace solid-phase microextraction (SPME). A SPME fiber (DVB/CAR/PDMS, 50 mm, Supelco) was exposed to the samples while continuously stirring at 60 °C for 15 min. Desorption was carried out for 1 min at 250 °C through a GC splitless injector with a SPME inlet liner. Chromatographic separation was carried out using a DB-624 capillary column (Agilent Technologies, 60 m, 0.25 mm, 1.40 μm). Helium was utilized as the carrier gas with an ionization voltage of 70 eV at a flow rate of 1 mL/min. The oven temperature program consisted of an initial 5-min isothermal period at 40 °C, followed by a linear temperature increase of 5 °C per minute up to 200 °C, which was maintained for 2 min. Mass spectra were recorded in the range of m/z 40–400 amu under full-scan acquisition mode, fitting interface temperature at 230 °C. Single volatile organic compounds were identified by comparing each MS spectrum with the commercial NIST05 library.

2.10. Sensory evaluation of salamis

A panel of 18 trained judges (11 males and 7 females, aged between 18 and 52 years) evaluated the sensory attributes of all salamis. The analysis was conducted following the general ISO 6658 (2017) guidelines in the sensory laboratory of the Department of Agricultural, Food and Forest Sciences (University of Palermo, Italy), which is equipped with single chambers, white light and controlled temperature. For each salami, the panellists evaluated seventeen sensory attributes grouped into aspect, flavor and rheology categories as reported by Gaglio et al. (2016b). The judges scored the level of each sensory descriptor on a 9-point hedonic scale (1 = low intensity; 9 = high intensity) using iPad logged to the Smart Sensory Box software (Smart Sensory Solutions S.r. l., Sassari, Italy).

2.11. Statistical analysis

Statistical analysis of data was performed by SAS 9.2 software (2010). Raw materials (avocado pulp, casing, pork fat and pork meat) were statistically compared for their physicochemical and microbiological parameters using the GLM procedure with the material as unique effect. Physicochemical and microbiological parameters of salami were analysed statistically using the MIXED model procedure where fixed effects were represented by the level of avocado inclusion (AL: 0, 10 and 20% corresponding to CTR, 10-AVP and 20-AVP), the ripening time (RT: 0 and 20 d) and the AL*RT interaction, whereas the batch of salami (2 productions) was included as a random effect. Tukey's test was used to compare the means when the effects were significant ($P < 0.05$).

3. Results and discussion

3.1. Avocado fruit characteristics

Avocado ripening is a desirable feature for growers, allowing them to strategically delay harvesting, especially when market prices are unfavorable. As observed by Obenland et al. (2012), during the postharvest stage, the fruit undergoes ripening due to a gradual reduction in mesocarp moisture content and a corresponding increase in dry matter (Clark et al., 2007; Parodi et al., 2007). Notably, the oil content of the mesocarp steadily rises as the fruit matures (Hofman et al., 2002). A strong correlation exists between increasing oil content and decreasing water content, until the proportions of oil and water stabilize (Ozdemir and Topuz, 2004). Across avocado-producing regions worldwide, oil and dry matter content serve as key indicators to determine the minimum fruit maturity (Parodi, 1996). According to Gamble et al. (2010), fruits with a dry matter value ranging from 20 to 40% are considered ripe and fully ripe, respectively. For the "Hass" cultivar, a minimum dry matter percentage of 22% is required for harvested fruits to be sold to the public (Carvalho et al., 2014).

The avocado fruits used in this study exhibited dry matter percentages ranging from 30.63 ± 0.16 to 31.88 ± 1.40. According to Gamble et al. (2010) and Ozdemir and Topuz (2004), changes in lipid content closely paralleled those in dry matter. Furthermore, Villa-Rodríguez et al. (2011) highlighted that during the ripening of the Hass variety, MUFA increase at the expense of PUFA. Consequently, we can infer that the samples used were at a ripe stage and had a high lipid content. Specifically, in the Hass variety, ripening led to an increase in oleic and linoleic acid levels, accompanied by a decrease in palmitic acid (Ozdemir and Topuz, 2004).

3.2. Microbial evolution during salami productions

Table 3 reports the microbial loads estimated throughout salami productions, from raw materials (including AVP) to CTR, 10-AVP and 20-AVP salamis after 20 d of ripening. None of the analysed samples showed the presence of *L. monocytogenes* and *Salmonella* spp., which are the primary human pathogens commonly isolated from the surface of Hass avocados (Cabrera-Díaz et al., 2022). These bacteria are also responsible for food poisoning outbreaks associate with avocado consumption (CDC, 2021). Despite AVP typically having an initial amount of aerobic bacteria and yeasts around 10^2 CFU/g (Filannino et al., 2020), the samples analysed in this study did not reveal the presence of any of the microbial populations object of investigation. These results clearly indicated adherence to high hygienic standards during AVP production and confirmed the suitability of this product for food applications. The natural pork casing was characterized by presence of TMM, LAB cocci, and rods at approximately 10^4 CFU/g. Similar findings were previously reported by Pisacane et al. (2015) for different types of natural pork casing used in the production of Salame Mantovano.

The microbial communities in both pork fat and meat were predominantly composed of LAB, as evidenced by the levels of TMM, LAB cocci, and LAB rods exceeding 5.0 log CFU/g. This dominance is likely attributed to contamination from the butchery environment (Aquilanti et al., 2016). The levels of all microbial groups investigated in both CTR and AVP salamis from the beginning until the 20th d of ripening did not exhibit any significant differences ($P > 0.05$). Soon after stuffing, TMM, LAB cocci, and LAB rods were above 6.0 log CFU/g for all trials. During the 20-day fermentation period, the levels of these microorganisms increased and reached values of approximately 10^9 CFU/g in all trials, indicating that AVP did not significantly impact the development of LAB. All staphylococci detected throughout the production chains of CTR and AVP salamis belonged to the CNS group. After 20 d of ripening, the levels of these bacteria reached approximately 7 log CFU/g for all trials, consistent with trends commonly observed in matured salamis (Rocchetti et al., 2023). CNS play a crucial role in enhancing the color and flavor of salami during ripening (Khusro and Aarti, 2022). Yeasts, which are commonly involved in salami fermentation (Cocolin et al., 2006), were present from the raw materials (fat and meat) until the end of ripening, reaching levels of approximately 7 log CFU/g. Similar behavior has been observed in traditional salamis from southern Italy by Gardini et al. (2001) and Settanni et al. (2020). Interestingly, after 20 d of ripening, the levels of Enterobacteriaceae family members, particularly *E. coli*, known for causing enteric infections (Poirel et al., 2018),

Table 3
Microbial loads of samples collected during experimental salami productions.

Samples	Bacterial counts						
	TMM	Rod LAB	Coccus LAB	CNS	Yeasts	Enterobacteriaceae	*E. coli*
Raw materials							
Avocado pulp	<2 d	<1 c	<1 c	<2 c	<2 b	<1 c	<2 c
Casing	3.62 c	3.63 b	3.52 b	<2 c	<2 b	<1 c	<2 c
Fat	5.24 b	5.44 a	5.41 a	5.57 a	5.02 a	3.15 b	3.10 b
Pork meat	5.9 a	5.48 a	5.24 a	4.55 b	5.15 a	5.17 a	4.82 a
SEM	0.198	0.202	0.175	0.068	0.206	0.074	0.117
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Salamis at t_0							
CTR	6.60	6.30	6.44	4.94	5.28	3.65	3.41
10-AVP	6.20	6.70	6.42	5.39	5.65	3.44	3.22
20-AVP	6.10	6.20	6.28	4.78	5.00	3.90	3.55
Salamis at 20 d							
CTR	8.44	8.73	8.47	7.31	6.58	<2	<2
10-AVP	8.60	9.06	8.72	7.15	6.59	<2	<2
20-AVP	8.70	8.83	8.50	7.27	6.49	<2	<2
SEM	0.243	0.137	0.174	0.361	0.326	0.196	0.108
p value:							
AL	0.7132	0.0040	0.4015	0.4193	0.3633	0.1722	0.2005
RT	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AL*RT	0.0809	0.4526	0.5937	0.1299	0.5781	0.1722	0.2005

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*; CTR, control salami prepared without avocado pulp (AVP) addition; 10-AVP, experimental salami prepared with the partial replacement of pork fat with AVP; 20-AVP, experimental salami prepared with the total replacement of pork fat with AVP; SEM, standard error of the mean; AL, avocado level; RT, ripening time.
Data within a column followed by different letters are significantly different according to Tukey's test ($P < 0.05$).

decreased below the detection limit (<2 log CFU/g) in both CTR and AVP salamis. This decline is likely attributed to the activity of LAB, which effectively inhibit the growth of undesirable microorganisms (Sydykova et al., 2019).

3.3. Characterization of salamis microbiota by MiSeq illumina

The bacterial composition of AVP and salami samples was investigated using MiSeq Illumina technology. This culture-independent approach is commonly applied to provide a comprehensive overview

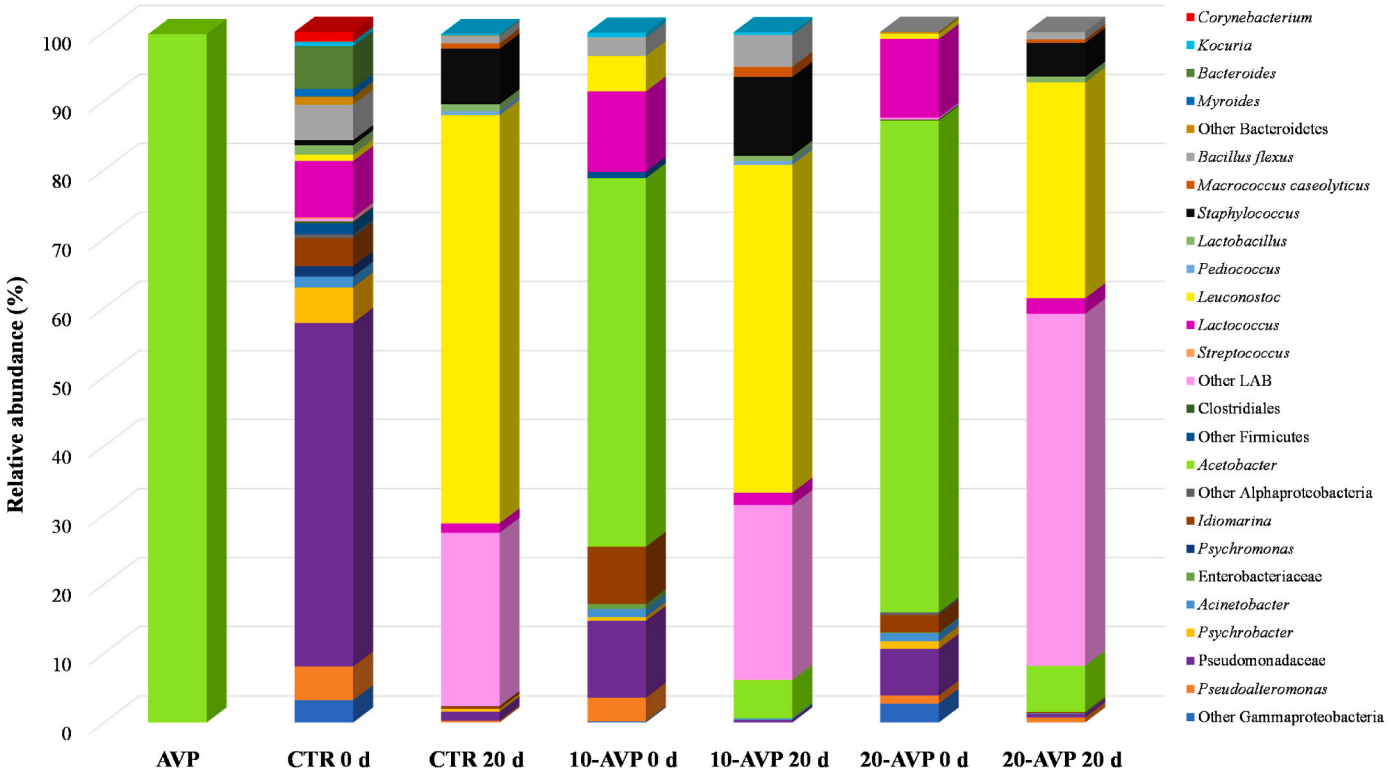

Fig. 1. Relative abundances (%) of bacteria identified by MiSeq Illumina. Abbreviations: AVP, avocado pulp; CTR 0 d, control salami prepared without avocado pulp addition immediately after stuffing; CTR 20 d, control salami prepared without avocado pulp addition after 20 d of ripening; 10-AVP 0 d, experimental salami prepared with the partial replacement of pork fat with AVP immediately after stuffing; 10-AVP 20 d, experimental salami prepared with the partial replacement of pork fat with AVP after 20 d of ripening; 20-AVP 0 d, experimental salami prepared with the total replacement of pork fat with AVP immediately after stuffing; 20-AVP 20 d, experimental salami prepared with the total replacement of pork fat with AVP after 20 d of ripening.

of the microbiota associated with fermented foods (Gaglio et al., 2019). Unlike culture-based techniques, which tend to underestimate subdominant species (Zotta et al., 2021), MiSeq Illumina sequencing allows for a more accurate assessment. The OTUs with a relative abundance (RA) exceeding 0.1%, a threshold indicative of abundant bacterial communities (Logares et al., 2014), are presented in Fig. 1. Twenty-six taxonomic groups were detected primarily at the genus level.

The genus *Acetobacter* was the sole bacterium found in AVP sample (100%). However, in experimental salamis after 20 d of ripening, its presence ranged between 5.54 and 6.68%. Acetobacters exhibit an obligate aerobic metabolism and can efficiently oxidize sugars and alcohols to acetic acid (Sengun and Karabiyikli, 2011). The occurrence of these bacteria in AVP is not unusual, as they are commonly associated with fruits, cereals, herbs, etc. (Sun et al., 2016). On the other hand, Pseudomonadaeae, known for their role in meat spoilage (Mohareb et al., 2015), were detected in both CTR and AVP salamis soon after stuffing. Their RA was ranged from 6.72 to 49.77%. Interestingly, their percentage significantly decreased after 20 d of ripening, consistent with findings from a previous study on Italian dry fermented salamis (Greppi et al., 2015).

The genus *Idiomarina*, known for its halophilic species (Jean et al., 2006), was initially found in both CTR and AVP salami samples at day 0, albeit at low levels. However, their presence was not detected in the salamis after 20 d of fermentation. A similar trend was observed for *Psychrobacter* and *Acinetobacter*, both of which belong to the Moraxellaceae family (Özen and Ussery, 2012). Additionally, *Bacillus flexus*, a species that has obtained the Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA, 2021), was detected in all salami samples at RA between 0.27 and 5.20%.

Different proportions of lactococci, lactobacilli, leuconostocs, pediococci, streptococci, and other LAB were detected among the salami samples. These bacterial groups are commonly associated with Italian salamis (Połka et al., 2015). *Leuconostoc* emerged as the primary LAB group in all salami samples at the end of the ripening, constituting 31.26–59.12% of OTUs. Similar percentages of leuconostocs were observed in another study conducted on fermented dry sausages during the same ripening period in central Serbia (Borovic et al., 2015) and in Central Italy (Belleggia et al., 2020). *Staphylococcus* genus was detected in all salamis at the end of ripening, accounting for 4.90–11.41% of RA. This range aligns with findings reported for Chinese smoked-cured sausages produced without the addition of starter cultures (Wang et al., 2018). Importantly, pathogenic bacteria such as *L. monocytogenes* and *Salmonella* spp. were not detected in any of the samples analysed, corroborating the results obtained through the culture-dependent approach.

3.4. Physicochemical characterization of salamis

Table 4 reports the physicochemical parameters of salamis. Notably, significant changes in DM occurred during the ripening process. The interaction effect on DM highlights how AVP influenced the water content, resulting in higher water levels at day 0 and a more rapid water loss up to day 20. As expected, the fat content of the salamis, expressed on DM basis, decreased as the proportion of avocado used increased. Specifically, CTR salamis contained 46.40% fat, while the reformulated 10-APV salamis had 36.51% fat and 20-AVP salamis had 24.05% fat. These findings align with similar results observed in salamis produced using gelled oils as substitutes for animal fat (Alejandre et al., 2016; Campagnol et al., 2012; Fernández-Diez et al., 2016). The reduction in fat due to avocado inclusion was accompanied by a significant relative increase in other components, such as ash and protein. Furthermore, this fat reduction contributes to the higher compressive strength observed in the salami containing avocado, as fat plays a crucial role in increasing tenderness.

Table 4
Effect of AVP inclusion and ripening time on physicochemical traits and oxidative status of salamis.

	CTR	10-AVP	20-AVP	SEM	0 d			20 d			SEM	*p* value		
					CTR	10-AVP	20-AVP	CTR	10-AVP	20-AVP		AL	RT	AL*RT
Dry matter (DM), %	53.58	51.58	51.35	1.66	40.98 c	35.79 d	30.96 d	66.18 b	67.36 ab	71.74 a	1.95	0.2641	<0.0001	0.0002
Ash, % DM	9.60 c	11.03 b	12.64 a	0.430	9.45	10.79	12.57	9.75	11.27	12.71	0.444	<0.0001	0.0281	0.5467
Protein, % DM	42.36 b	47.61 ab	53.68 a	2.93	42.05	48.08	53.05	42.68	47.15	54.32	2.99	0.0001	0.6658	0.4800
Fat, % DM	46.40 a	36.51 b	24.05 c	3.35	48.46	37.89	24.87	44.34	35.14	23.23	3.64	0.0003	0.1500	0.8359
pH	5.70	5.75	5.80	0.062	5.72	5.76	5.77	5.67	5.74	5.84	0.073	0.1766	0.9415	0.5555
Cross-section area, cm^2	31.00 a	29.60 ab	28.45 b	0.506	33.70	33.00	32.40	28.30	26.20	24.50	0.716	0.0033	<0.0001	0.2255
Water activity, a_w								0.809	0.816	0.771	0.035	0.5372		
Hardness, N/mm^2								0.408	0.437	1.084	0.192	<0.0001		
Lightness L*	51.51 a	51.52 a	44.06 b	2.07	57.02	55.21	52.93	47.10	48.57	36.96	2.54	0.0004	<0.0001	0.0507
Redness a*	9.56 a	2.75 b	0.13 c	1.50	9.92	3.82	−1.23	9.27	1.90	1.19	1.61	<0.0001	0.8459	0.1280
Yellowness b*	5.72 c	11.01 b	16.28 a	1.23	7.22	9.80	15.71	4.52	11.98	16.74	1.55	<0.0001	0.8957	0.2524
Polyphenols, g GAE/kg DM	1.19 c	1.51 b	1.90 a	0.047	1.15 d	1.55 c	2.13 a	1.23 d	1.47 c	1.67 b	0.051	<0.0001	<0.0001	<0.0001
TEAC, mmol/kg DM	27.0 c2	45.19 b	60.30 a	3.51	23.06	41.85	53.15	30.98	48.52	67.46	4.02	<0.0001	0.0005	0.3552
POV, mEq O_2/kg fat	3.55 a	3.31 a	2.93 b	0.215	3.83 a	3.50 abc	3.76 ab	3.28 bc	3.12 c	2.10 d	0.235	0.0008	<0.0001	0.0003
TBARS, mg MDA/kg DM	0.282 a	0.269 a	0.172 b	0.015	0.362 a	0.350 a	0.209 b	0.201 b	0.187 b	0.135 c	0.0152	<0.0001	<0.0001	<0.0001

Results indicate mean values of determinations carried out in duplicate for each of the two independent productions. Abbreviations: CTR, control salami prepared without avocado pulp (AVP) addition; 10-AVP, experimental salami prepared with the partial replacement of pork fat with AVP; 20-AVP, experimental salami prepared with the total replacement of pork fat with AVP; SEM, standard error of the mean; AL, avocado level; RT, ripening time; GAE, gallic acid equivalent; TEAC, Trolox equivalent antioxidant capacity; POV, peroxide value; TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde.
Data within a row followed by different letters are significantly different according to Tukey's test ($P < 0.05$).

The color parameters of the salamis were primarily influenced by the inclusion of avocado rather than the ripening time. Indeed, AVP led to a significant decrease in redness and an increase yellowness, regardless of the ripening time. Both factors also caused significant variations in lightness, which decreased as the avocado level and ripening time increased; notably, this reduction was more pronounced with the higher avocado content.

3.5. Antioxidant properties of salamis

Table 4 shows also the parameters related to the antioxidant status of the salamis. The inclusion of avocado and the ripening time of the salamis had significant effects on polyphenols, which are known for their antioxidant activity. Both the avocado level and ripening time significantly influenced the antioxidant capacity, expressed as TEAC. The observed increasing trends in polyphenols and TEAC with higher avocado levels suggest that the partial and total replacement of pork fat with AVP during salami production contributes to enhancing product quality in terms of antioxidant properties. Specifically, AVP exhibited polyphenol levels equivalent to 4.37 g GAE/kg DM and TEAC levels of 8.86 mmol/kg DM (Table 4). During ripening, the polyphenol levels decreased, but this trend was observed only in salamis containing AVP, explaining the significant interaction between avocado level and ripening time (AL*RT). However, the TEAC values showed an increasing trend over time, regardless of avocado inclusion, suggesting that other components within the salami matrix also contribute to enhancing its overall antioxidant capacity.

Salami is a food product that is particularly susceptible to lipid oxidation (Hur et al., 2007; Sammet et al., 2006; Wójciak and Dolatowski, 2012) due to its high fat content (Wirth, 1991). To mitigate this issue, the addition of antioxidant substances during processing is considered the most effective strategy (Kim et al., 2013). In this study, the pork fat, commonly used in salami production, was replaced with AVP as an alternative approach.

Table 4 presents the results regarding the effect of AVP as a replacement for animal fat on lipid oxidation indexes. The inclusion of AVP had significant impact in limiting both primary (POV) and secondary (TBARS) lipid oxidation. Moreover, this effect was further evident during the ripening process. The significant AL*RT interaction emerged for both POV and TBARS demonstrates that as the salamis ripened, the reduction in lipid oxidation was more pronounced in the samples containing avocado. Indeed, on day 20 of processing, POV and TBARS significantly decreased across all treatments, but the most substantial decrease occurred in the 20-AVP salami. These favourable outcomes can be attributed to the antioxidant properties of avocado since, when used as a substitute for animal fat in salami production, avocado appears to enhance the stability of finished products by effectively protecting them from lipid oxidation.

3.6. Fatty acid composition

Fatty acid analysis was carried out on raw materials (Table 5) and salamis after 20 d of ripening (Table 6). When comparing the fat composition of AVP to that of pork meat and fat, AVP exhibited higher levels of monounsaturated FA (MUFA), primarily due to the presence of oleic acid (OLA, C18:1 c9). Conversely, AVP had lower levels of saturated FA (SFA), mainly consisting of palmitic acid (C16:0) and stearic acid (C18:0). However, no significant differences were observed among the raw materials for linoleic acid (LA, C18:2 n-6) and α-linolenic acid (ALA, C18:3 n-3); consequently, there was no significant difference in the total content of n-3 and n-6 FA, of which LA and ALA serve as the respective precursors.

Typically, the fat content and FA profile of processed products vary based on the ingredients in the mixture. The specific type of salami used significantly affected the levels of SFA, MUFA, the unsatured FA/SFA ratio, as well as HPI and TI. However, the PUFA, mostly represented by LA, did not exhibit significant differences among the various types of salamis, consistent with their content in the raw materials (Alabiso et al., 2021).

In all salami samples, MUFA were the most abundant, followed by SFA and PUFA, as reported by Vargas-Ramella et al. (2020). Across various types of salamis, the dominant FA was OLA, followed by palmitic acid, LA, and stearic acid. This acidic profile aligns with previous findings in similar fermented sausages (Alejandre et al., 2016; Franco et al., 2020; Lorenzo et al., 2016; Utrilla et al., 2014). Notably, in this experiment, the replacement of pork fat with increasing levels of AVP led to a significant reduction in SFA and a gradual increase in MUFA within the fermented sausages. These changes, mainly linked to the amounts of palmitic acid for SFA and OLA for MUFA, reflect the FA composition of either pork or AVP used in their production. Similar results have been observed by other researchers in sausages reformulated with emulsified and gelled oils, which exhibited markedly lower SFA values compared to traditionally produced sausages using exclusive pork fat (Alejandre et al., 2016; Franco et al., 2020; Jiménez-Colmenero et al., 2013; Pintado and Cofrades, 2020).

The replacement of pork fat with increasing levels of AVP in processed salamis, which reduced SFA and increased MUFA, contributed to an improved health quality of salami fat, as reflected by both HPI and TI.

Table 5
Composition, fatty acid (FA) profile (g/100 g identified FA) and health indexes of raw materials used for salami productions.

	Samples			SEM	*p* value
	Avocado pulp	Pork meat	Pork fat		
Dry matter (DM)	44.77 b	27.51 c	94.89 a	2.28	0.0005
Fat, % DM	39.47 b	18.52 b	83.14 a	5.93	0.0100
Polyphenols, g GAE/kg DM	4.37	–	–	–	–
TEAC, mmol/kg DM	8.86	–	–	–	–
Identified FA, % total FA	99.78 a	98.40 b	99.71 a	0.286	0.0699
C14:0	0.05 c	1.62 b	1.96 a	0.023	<0.0001
C16:0	14.90 b	25.35 a	26.48 a	0.409	0.0005
C16:1 c9	7.15 a	3.02 b	3.10 b	0.343	0.0054
C18:0	0.32 b	11.49 a	10.34 a	0.337	0.0003
C18:1 c9, OLA	55.78 a	38.19 b	36.49 b	1.35	0.0036
C18:2 n-6, LA	13.73	11.23	13.96	1.59	0.4901
C18:3 n-3, ALA	0.92	0.59	0.93	0.085	0.1047
Saturated FA, SFA	15.62 b	39.96 a	40.11 a	0.351	<0.0001
Monounsaturated FA	69.72 a	46.22 b	43.93 b	1.52	0.0022
Polyunsaturated FA, PUFA	14.66	13.82	15.95	1.67	0.6946
Unsaturated FA/SFA	5.41 a	1.50 b	1.49 b	0.135	0.0004
∑ n-3	0.92	0.67	1.04	0.083	0.1063
∑ n-6	13.74	13.16	14.92	1.63	0.7596
∑ n-6/∑ n-3	14.90	19.76	14.50	1.54	0.1589
Health Promoting Index (HPI)	5.59 a	1.88 b	1.74 b	0.134	0.0004
Thrombogenic index (TI)	0.34 b	1.21 a	1.19 a	0.014	<0.0001

Abbreviations: OLA, oleic acid; LA, linolenic acid; ALA, α-linolenic acid.
HPI = (n-3 PUFA + n-6 PUFA + MUFA)/(C12:0 + 4 × C14:0 + C16:0) (Chen et al., 2004).
TI = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA) + (0.5 × n-6 PUFA) + (3 × n-3 PUFA) + (n-3/n-6) (Ulbricht and Southgate. 1991).
SEM, standard error of the mean. Data within a row followed by different letters are significantly different according to Tukey's test ($P < 0.05$).

Table 6
The effect of avocado inclusion on fatty acid (FA) profile (g/100 g identified FA) and health indexes of 20 d ripened salamis.

Items	Samples			SEM	*p* value
	CTR	10-AVP	20-AVP		
Identified FA, % total FA	98.80	99.14	99.31	0.264	0.4707
C14:0	1.74 a	1.15 ab	0.88 b	0.110	0.0253
C16:0	26.58 a	22.98 ab	20.43 b	0.766	0.0245
C16:1 c9	3.43	4.42	5.01	0.643	0.3486
C18:0	10.37	7.80	5.67	0.813	0.0591
C18:1 c9, OLA	39.83 b	44.41 ab	47.21 a	1.21	0.0507
C18:2 n-6, LA	10.07	11.60	12.15	1.11	0.4801
C18:3 n-3, ALA	0.57	0.74	0.76	0.071	0.2457
Saturated FA, SFA	40.30 a	33.02 ab	27.99 b	1.66	0.0301
Monounsatured FA	48.21 b	53.89 a	58.37 a	0.912	0.0098
Polyunsatured FA, PUFA	11.49	13.09	13.65	1.18	0.4913
Unsaturated FA/SFA	1.49 b	2.03 ab	2.58 a	0.151	0.0324
$\sum$ n-3	0.62	0.79	0.80	0.078	0.3275
$\sum$ n-6	10.86	12.30	12.84	1.12	0.5131
$\sum$ n-6/$\sum$ n-3	17.40	15.81	15.94	1.03	0.5498
Healt Promoting Index (HPI)	1.78 b	2.43 ab	3.00 a	0.142	0.0205
Trombogenic index (TI)	1.23 a	0.90 ab	0.71 b	0.075	0.0350

Abbreviations: CTR, control salami prepared without avocado pulp (AVP) addition; 10-AVP, experimental salami prepared with the partial replacement of pork fat with AVP; 20-AVP, experimental salami prepared with the total replacement of pork fat with AVP; SEM, standard error of the mean.; OLA, oleic acid; LA, linolenic acid; ALA, α-linolenic acid.
HPI = (n-3 PUFA + n-6 PUFA + MUFA)/(C12:0 + 4 × C14:0 + C16:0) (Chen et al., 2004).
TI = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA) + (0.5 × n-6 PUFA) + (3 × n-3 PUFA) + (n-3/n-6) (Ulbricht and Southgate. 1991).
Data within a row followed by different letters are significantly different according to Tukey's test ($P < 0.05$).

3.7. VOC profiles of salamis

The volatile profile of salamis can result from intricate chemical reactions involving matrix components, including the oxidation of unsaturated FA and the microbiological metabolism of lipids, proteins, and carbohydrates (Bianchi et al., 2007; Bleicher et al., 2022). Table 7 presents the results of the volatile composition analysis of AVP, CTR and AVP salamis after 20 d of ripening. Across all salami samples, volatile compounds belonging to the acid, aldehyde, ketone, alcohol, ester, and terpene classes were identified. Similar volatile profiles have been documented in the literature for various types of dry sausages (Bianchi et al., 2007; Liu et al., 2023; Lorenzo et al., 2012). Specifically, in CTR production, four acid compounds were identified, with acetic acid being the most abundant, followed by butyric acid. The presence of acetic acid is primarily associated with microbial carbohydrate metabolism, while butyric acid is linked to fat oxidation (Liu et al., 2023). Among the aldehyde group, eight compounds were identified in CTR salamis. Notably, these compounds are attributed to lipid oxidation (Meynier et al., 1999).

In CTR salamis, four ketone compounds were also identified, which may originate from lipid oxidation or raw meat, contributing to the dry-sausage aroma, similar to linear aldehydes (Demeyer et al., 2000). Diketones such as 2,3-butanedione and 2,3-octanedione were detected in the salami samples, known for their buttery-creamy flavor (Bianchi et al., 2007). Additionally, seven alcohol compounds were found in the CTR salamis. Their presence can be attributed to the degradation of lipid hydroperoxides (Frankel, 1980; Meynier et al., 1999). Furthermore, three ethyl esters were identified. These esters could arise from the oxidation of alcohols or the reaction between alcohols and acids (Edwards et al., 1999). Remarkably, they contribute fruity notes to the flavor (Demeyer et al., 2000) and are frequently present in fermented meat products (Bianchi et al., 2007; Liu et al., 2023; Lorenzo et al., 2012; Wagner and Franco 2012).

Table 7
Volatile Organic Compounds determined by GC-MS.

VOC	Samples				SEM	*p* value
	AVP	CTR	10-AVP	20-AVP		
Acids						
Acetic acid	n.d b	4.50 a	5.28 a	6.30 a	0.93	0.0001
Butyrric acid	n.d c	2.10 a	1.01 b	0.81 b	0.29	<0.0001
Octanoic acid	n.d c	0.50 b	1.10 a	0.89 a	0.16	<0.0001
Nonanoic acid	n.d b	0.60 a	0.11 b	0.10 b	0.09	<0.0001
Aldehydes						
Propanal	n.d c	2.68 a	0.60 b	0.20 bc	0.40	<0.0001
Butanal	2.75 c	6.20 a	5.94 ab	3.81 bc	0.59	0.003
Pentanal	1.20 c	5.01 a	3.44 ab	2.31 bc	0.56	0.001
Hexanal	2.23 c	12.22 a	6.28 b	4.40 bc	1.43	<0.0001
Heptanal	n.d c	2.04 a	1.19 b	1.09 b	0.28	<0.0001
Octanal	n.d b	0.27 a	0.03 b	0.24 a	0.05	<0.0001
Nonanal	3.13 a	n.d b	0.21 b	n.d b	0.50	<0.0001
Ketones						
2-Pentanone	n.d b	1.65 a	1.71 a	0.38 b	0.29	<0.0001
2,3-Butanedione	n.d b	0.70 a	0.94 a	n.d b	0.16	<0.0001
2-Heptanone	n.d b	0.20 a	0.26 a	0.25 a	0.04	<0.0001
2,3-Octanedione	n.d c	0.14 a	0.08 b	0.15 a	0.02	<0.0001
2-Hexanone	3.68 a	n.d c	0.61 bc	1.27 b	0.52	<0.0001
Alcohols						
2-Butanol	5.65 b	9.77 a	7.76 ab	6.61 ab	0.69	0.021
1-Propanol	n.d c	0.92 ab	0.72 b	1.25 a	0.18	<0.0001
1-Penten-3-ol	n.d c	2.46 a	n.d c	0.82 b	0.38	<0.0001
1-Pentanol	n.d d	3.13 a	1.93 b	1.02 c	0.43	<0.0001
1-Hexanol	n.d c	0.15 a	0.02 b	n.d c	0.02	<0.0001
1-Octen-3-ol	n.d b	1.39 a	0.12 b	0.09 b	0.22	<0.0001
1-Octanol	4.35 a	0.10 b	0.28 b	0.29 b	0.67	<0.0001
2,3-Butanediol	3.17 a	n.d b	0.20 b	0.12 b	0.49	<0.0001
1,3-Butanediol	3.16 a	n.d b	0.22 b	0.25 b	0.49	<0.0001
Esters						
Methyl acetate	n.d b	n.d b	0.29 a	n.d b	0.05	<0.0001
Ethyl acetate	n.d b	1.73 a	1.76 a	1.94 a	0.30	<0.0001
Ethyl propanoate	n.d c	0.18 a	0.17 a	0.09 b	0.03	<0.0001
Ethyl butanoate	n.d c	0.14 b	0.38 a	n.d c	0.06	<0.0001
Butyl ester	2.97 a	n.d b	0.22 b	0.13 b	0.46	<0.0001
Isopropil acetate	3.51 a	n.d b	0.13 b	0.09 b	0.56	<0.0001
Ethyl acetate	3.24 a	n.d c	0.49 c	1.07 b	0.46	<0.0001
Hydrocarbons						
Heptane	n.d c	0.52 b	0.62 b	1.88 a	0.26	<0.0001
Octane	n.d c	1.56 b	2.58 a	1.99 ab	0.37	0.0001
Tridecane	2.91 a	n.d b	n.d b	n.d b	0.47	<0.0001
Dodecane	1.74 a	n.d b	n.d b	n.d b	0.28	<0.0001
2-Octene	4.61 a	n.d b	n.d b	n.d b	0.74	<0.0001
Terpenes						
α-Pinene	2.79 b	6.19 a	8.25 a	9.01 a	0.96	0.001
β-Pinene	3.92 a	1.30 bc	1.90 b	0.60 c	0.47	<0.0001
β-Myrcene	2.29 a	0.1 b	n.d b	n.d b	0.37	<0.0001
α-Thujene	3.37 a	n.d b	n.d b	n.d b	0.54	<0.0001
3-Carene	n.d c	11.52 b	16.40 ab	18.08 a	2.69	<0.0001
Limonene	4.47 c	9.39 bc	14.50 ab	17.40 a	1.93	0.0001
β-Ocimene	3.46 a	n.d b	n.d b	n.d b	0.56	<0.0001
α-Phellandrene	n.d b	6.87 a	7.01 a	9.65 a	1.36	<0.0001
α-Terpinene	1.44 a	0.21 b	1.34 a	2.01 a	0.26	0.0001
α-Cubebene	3.63 a	n.d d	2.24 b	1.02 c	0.51	<0.0001
α-Copaene	4.68 a	n.d b	1.06 b	0.99 b	0.67	<0.0001
α-Bergamotene	3.29 a	n.d b	n.d b	n.d b	0.53	<0.0001
α-Caryophyllene	2.37 a	n.d b	0.01b	0.01 b	0.39	<0.0001
β-Caryophyllene	4.74 a	3.56 a	0.22 b	0.60 b	0.73	<0.0001
Nerolidol	2.66 a	n.d b	n.d b	n.d b	0.44	<0.0001
Humulene	3.95 a	n.d b	0.18 b	0.41b	0.61	<0.0001
Farnesene	4.64 a	n.d b	0.20 b	0.38 b	0.72	<0.0001

Results indicate the mean percentage values of four measurements (carried out in duplicate for two independent productions) and are expressed as relative peak areas (peak area of each compound/total area of the significant peaks) x l00. Abbreviation: Volatile Organic Compounds (VOC); AVP, avocado pulp; CTR, control salami prepared without AVP addition; 10-AVP, experimental salami prepared with the partial replacement of pork fat with AVP; 20-AVP, experimental salami prepared with the total replacement of pork fat with AVP; SEM, standard error of the mean; n.d., not detectable.

Terpenes, the most abundant class of aroma compounds in salamis (Bianchi et al., 2007; Liu et al., 2023), primarily originate from the spices used in salami production, such as black pepper (Guadayol et al., 1997). Specifically, terpenes like pinene, caryophyllene, 3-carene, and limonene contribute to the release of volatile compounds derived from these spices (Chevance and Farmer, 1998; Chevance et al., 2000). Interestingly, when pork fat was replaced with AVP, the relative percentage of terpene compounds increased, while lipid oxidation products (such as aldehydes and alcohols) decreased with the progressive substitution of pork fat. Similar trends have been observed in meat products where ingredients were replaced with flaxseed flour (Dong et al., 2023), as well as in salami products with varying fat levels (Lorenzo et al., 2012).

The reduced presence of lipid oxidation products in salamis can be attributed to the antioxidative activity of the added product (Barthet et al., 2014; Herchi et al., 2015), as well as the inclusion of spices (Aguirrezábal et al., 2000) and molds (Bruna et al., 2001). In salamis produced with the complete replacement of pork fat by AVP, alcohol compounds decreased by 40%, and aldehydes by 58%. This phenomenon can be attributed to both the transition from animal fat to plant-based fat and the inherent antioxidative properties of AVP (Lyu et al., 2023). For instance, hexanal, which forms due to the breakdown of PUFA through peroxidation, exhibited a substantial 64% decrease in content, further emphasizing the antioxidative potential of avocado fruits (Lyu et al., 2023; Rizzo, 2014).

However, more significant reductions were reported in meat products where 80% of the fat was replaced with flaxseed flour, resulting in remarkable decreases of 90% for heptanal (Dong et al., 2023). The slight variation detected in our study is likely attributed to the distinct contributions of compounds present in AVP. Furthermore, the effects of fat replacement are evident in the identification of specific terpenes, such as α-cubebene, α-copaene, α-caryophyllene, humulene, and farnesene, which are exclusively found in AVP salamis. This suggests a minimal aromatic impact on the final product, as avocado fruit lacks predominant aromatic compounds (Ferranti and Hayward, 2022; Galvao et al., 2016; Mahendran et al., 2019).

3.8. Sensory evaluation of salamis

The sensory characteristics of CTR and AVP ripened salamis were meticulously evaluated by a panel of experts during official tasting sessions (Fig. 2). This evaluation is essential before scaling up production to an industrial level and ensuring the commercial success of new food products (Świąder and Marczewska, 2021). The sensory attributes of salamis were significantly influenced by the partial or total replacement of pork fat with AVP. Generally, substiting animal fat with vegetable oils strongly impacts the sensory traits of meat products (Martínez et al., 2023).

In our study, several sensory parameters exhibited notable changes with increasing percentages of pork fat replacement. Specifically, color uniformity and intensity, as well as hardness and chewiness, increased significantly. Conversely, fat/lean connection, fat/lean distribution, elasticity, juiciness, and fattiness showed a decreasing trend directly proportional to pork fat substitution. Interestingly, no significant differences ($P > 0.05$) were observed between CTR and AVP productions regarding attributes such as acid, rancidness, moldiness, bitterness, saltiness, and flavor intensity. These findings align with previous research by Vaca and Pacheco (2015), who evaluated the sensory traits of chicken sausages produced with partial or total replacement of pork backfat with avocado oil.

The overall acceptability, representing the degree of overall sensory satisfaction (Barbaccia et al., 2022), was higher for 10-AVP salamis produced with partial pork fat replacement compared to CTR productions. However, 20-AVP salamis received a lower score than CTR productions, confirming that the total replacement of pork fat with vegetable-based products in fermented salamis compromises consumer

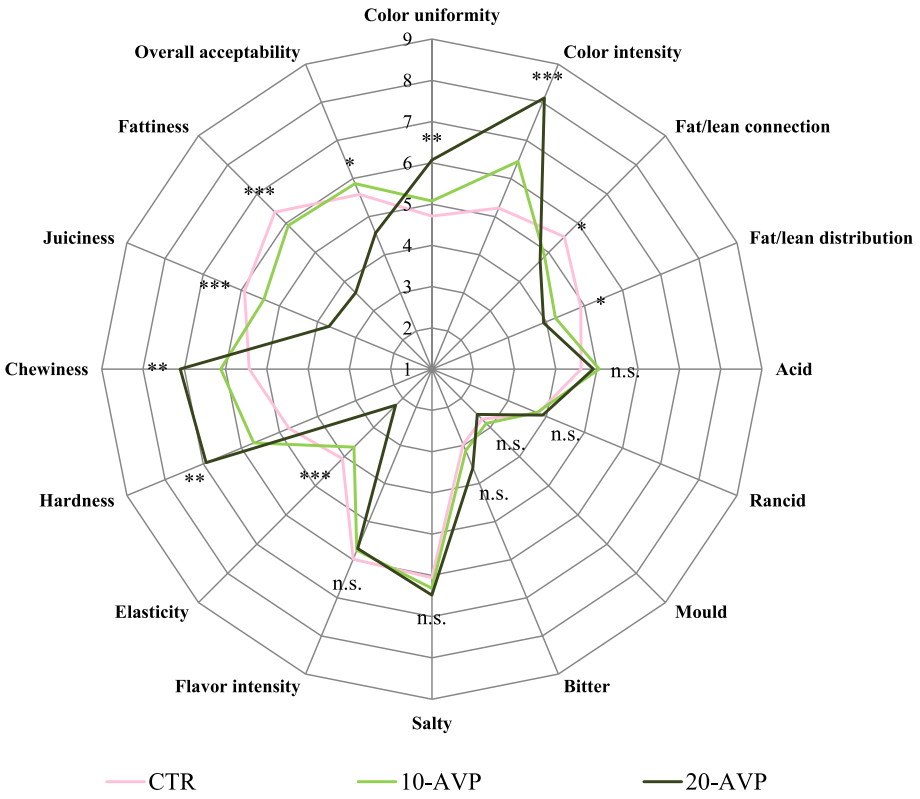

Fig. 2. Radar chart of descriptive sensory analysis of salamis. Abbreviations: CTR, control salami prepared without avocado pulp (AVP) addition; 10-AVP, experimental salami prepared with the partial replacement of pork fat with AVP; 20-AVP, experimental salami prepared with the total replacement of pork fat with AVP. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant ($P > 0.05$).

acceptability. After all, nutritional information alone cannot satisfy consumers when the final product lacks sensory appeal (Marino et al., 2017).

4. Conclusions

The partial and total replacement of pork fat with AVP did not significantly impact the evolution of LAB, CNS, and yeasts during experimental salami productions. However, AVP salamis exhibited distinct characteristics compared to control salamis. Specifically, AVP salamis showed lower fat content, higher protein content, higher values of antioxidant capacity. Furthermore, the substitution of pork fat with AVP resulted in the following changes: MUFA and terpene compounds increased, while SFA, alcohols and aldehydes decreased. Interestingly, sensory analysis revealed that salamis produced with the partial replacement of pork fat with AVP were particularly appreciated by evaluators. These results open new routes to produce low-fat pork salamis.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Giuliana Garofalo: Formal analysis. **Marialetizia Ponte:** Formal analysis. **Gabriele Busetta:** Formal analysis. **Marcella Barbera:** Writing – original draft, Formal analysis, Data curation. **Ilenia Tinebra:** Writing – original draft, Formal analysis, Data curation. **Daniela Piazzese:** Methodology, Data curation. **Elena Franciosi:** Software, Formal analysis, Data curation. **Antonino Di Grigoli:** Software, Methodology. **Vittorio Farina:** Methodology, Funding acquisition. **Adriana Bonanno:** Writing – original draft, Data curation. **Raimondo Gaglio:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology. **Luca Settanni:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that there is no conflict of interest for this research.

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