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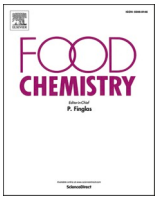

Bacterial-driven lipid remodeling in avocado fermentation via hydroxylation and oxidation routes

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ABSTRACT

We investigated the lipid metabolism of three lactic acid bacteria in fermenting two avocado cultivars. Using Liquid Chromatography-High-Resolution Mass Spectrometry, we tracked the fermentation-induced modifications in native lipid profiles. These modifications unveiled an increase in free fatty acids (FA) but also the generation of their oxidized derivatives associated with 14 different *m/z* ratios. Mono-, di-, and tri-hydroxylated derivatives originating from major unsaturated FA (18:1, 18:2, 18:3), along with oxidized forms of 16:0 and 18:0 FA, were recognized through the interpretation of tandem mass spectrometry data. Tandem mass spectrometry also suggested oxidized derivatives from minor FA (16,1, 16:2). In certain cases, bacterial-driven hydroxylation processes were confirmed on oleic acid in synthetic media. Overall, bacterial-driven hydroxylation and oxidation enriched avocado with potentially bioactive lipids, offering opportunities for nutraceutical and food applications, with minimal undesirable sensory effects specifically affected by microbial species and avocado cultivar.

1. Introduction

Dietary fatty acids (FA) play a pivotal role in human well-being and longevity, establishing them as a key area of study in nutritional science. Although they are commonly associated with animal-derived foods, FA actually constitute a structurally and functionally diverse class of biomolecules that are widely distributed across plant foods. Fruits such as avocados are a notable source of fatty acids, particularly monounsaturated fatty acids, highlighting their importance in modern dietary strategies for health promotion (Nascimento et al., 2025). Recent literature shows a growing and sustained interest in the lipid fraction of avocados, particularly in relation to its lipidomic signature and functional properties (Aras & Rohman, 2025).

Avocado (*Persea americana* Mill.) has experienced remarkable market growth in the last few years. Notably, in the European Union (EU), countries like Italy have experienced significant import growth (Migliore et al., 2017). This increasing demand has prompted investment in expanding avocado cultivation areas, including southern regions of Italy, where the Mediterranean climate offers favorable conditions for its cultivation (Cucuzza et al., 2024; Migliore et al., 2018; Schaffer et al., 2013). Recognized as a "superfood" for its lipid composition, including healthy saturated and unsaturated FA, avocado has been associated with antioxidant, antihypertensive, cardioprotective, and anti-obesity effects (Bhuyan et al., 2019; Dreher et al., 2021; Dreher & Davenport, 2013; Stephen & Radhakrishnan, 2022).

While the benefits of polyunsaturated and conjugated FA have been widely acknowledged, recent data underscore a spectrum of lesser explored yet indispensable FA derivatives. Indeed, FA can traverse various metabolic pathways, leading to the formation of diverse secondary plant metabolites. Specifically, hydroxy FA and oxo FA, with one or more hydroxyl and oxo groups, respectively, located at varied positions in long saturated or unsaturated carbon chains can be generated (Fiorino et al., 2023). In contrast, oxylipins are generated from the oxidation or further transformation of only polyunsaturated FA

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(Nayeem, 2018). These derivatives, characterized by potent functional properties, have garnered significant attention from the pharmaceutical and medical research fields (Kim & Oh, 2013; Nayeem, 2018). Their critical involvement in brain structure, cognitive function, neural disease progression, and various physiological processes, including inflammation, cardiovascular system, and aging, underscores their multifaceted importance (Caligiuri, 2017; Shinto et al., 2022). Notably, their positive impact extends to improving intestinal health, regulating energy metabolism, and mitigating obesity (Kim et al., 2017; Miyamoto et al., 2015; Miyamoto et al., 2019; Yamada et al., 2018).

The synthesis of these compounds occurs in animals, plants, and microorganisms, each employing distinct enzymatic or nonenzymatic free-radical-catalyzed mechanisms, resulting in the production of various FA derivatives. Plant enzymes, including 12-hydroxylase, lipoxygenase, and cytochrome P450 monooxygenase, produce ricinoleic acid and other hydroxy FA. Microbial enzymes, such as hydratase, hydroxylase, and diol synthase, generate distinct mono and di-hydroxy FA (Kim & Oh, 2013). Animals' lipoxygenase transforms arachidonic acid into leukotriene and lipoxin. Notably, microbial hydroxy FA are more regio-specific and diverse than plant and animal hydroxy FA due to the exclusive presence of hydratase and diol synthase in microorganisms (Takeuchi et al., 2016). Consequently, the synergistic interplay between plant components and microbial agents is proposed as an efficient strategy to enhance the generation of value-added FA derivatives. This approach is realized through natural fermentation, which, compared to chemical synthetic routes, offer eco-sustainable transformations with high diversity and specificity and reduced manufacturing costs.

Despite their low lipolytic activity compared to other microbial groups, lactic acid bacteria (LAB) can contribute to the hydrolysis of triacylglycerols (TAG) to produce free fatty acids, glycerol, and intermediates such as monoacylglycerols (MAG) and diacylglycerols (DAG). Lipolysis by LAB and subsequent catabolism in fermented foods, especially those characterized by long fermentation and ripening, contribute to the development of aroma and the release of bioactive derivatives (Balcázar-Zumaeta et al., 2025; Fiorino et al., 2023; Servent et al., 2018). In the context of plant matrix fermentation, research has been limited mainly to the synthesis of short-chain fatty acids and conjugated fatty acids (Annunziata et al., 2020; Fiorino et al., 2023).

LAB have been already reported to convert FA into hydroxy or oxo-derivatives with additional functionalities in fermented sausage, fermented milk, cheeses and sourdough. In recent research, our focus has been on unraveling the potential of LAB to enrich plant matrices with potentially bioactive FA derivatives. In particular, we demonstrated the metabolism of oleic and linoleic acids in avocado by autochthonous *Lactiplantibacillus plantarum*, resulting in elevated levels of mono-, di-, and tri-hydroxy-octadecenoic acids (Filannino et al., 2020). Subsequently, employing advanced processing and identification methods, strains of *Weissella cibaria*, *Leuconostoc mesenteroides*, and *Enterococcus faecalis* emerged as good producers of hydroxy- and epoxy-FA in walnuts substrate, some of which were previously unreported (Fiorino et al., 2023).

These findings motivate a reconsideration of avocado fermentation by employing alternative LAB species to more effectively exploit its lipidic potential. In this study, we have used two different avocado cultivars (Bacon and Hass) as model systems. Employing Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS), we elucidated the alterations in native FA during fermentation. Thereafter, we intended to confirm possible associations between these modifications and the lipid metabolism of our bacteria.

2. Materials and methods

2.1. Chemicals

Perchloric acid ($HClO_4$), acetonitrile (CH_3CN, LC-MS grade), chloroform ($CHCl_3$, HPLC grade), methanol (CH_3OH, LC-MS grade), ammonium acetate ($CH_3CO_2NH_4$) and water (LC-MS grade) were purchased from Sigma Aldrich (Milan, Italy). Fructose, glucose, mannitol, and lactic, acetic, and citric acids were obtained from Sigma Aldrich (Milan, Italy). Oleic acid, linoleic acid, α-linolenic acid, palmitic acid, stearic acid, ricinoleic acid, arachidonic acid, cis-5, 8, 11, 14, 17-eicosapentaenoic acid (EPA), and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) were purchased from Sigma Aldrich (Milan, Italy). The linoleic acid oxylipin mixture, corresponding to an ethanol solution of 9S-hydroxy-10E,12Z-octadecadienoic acid, 13S-hydroxy-9Z,11E-octadecadienoic acid, 9-oxo-10E,12Z-octadecadienoic acid, 13-oxo-9Z,11E-octadecadienoic acid, (±)9,10-dihydroxy-12Z-octadecenoic acid, 12,13-dihydroxy-9Z-octadecenoic acid, (±)9,10-epoxy-12Z-octadecenoic acid, and (±)12(13)epoxy-9Z-octadecenoic acid (each at a concentration of 10 μg/mL), was purchased from Cayman Chemical (Ann Arbor, MI, USA) and adopted as representative for the mixture of oxidized FAs detected in avocado samples.

2.2. Avocado characteristics, microorganisms, and culture conditions

Avocado fruits used in this study belonged to the cultivars Bacon and Hass. Avocado trees were cultivated in orchards located in Sicily (Giarre, Italy) and provided by the farm "Sicilia Avocado". The fruits were harvested in February 2022 and allowed to ripen at room temperature until they reached commercial maturity. Bacterial starter cultures were chosen among the dairy LAB collection of SAAF Department – University of Palermo (Italy). The strains *Lacticaseibacillus casei* FMAC16, *Lacticaseibacillus rhamnosus* TP5, and *Streptococcus macedonicus* PON208 were thawed from −80 °C glycerol stocks. The reactivation of *Lacticaseibacillus* spp. strains occurred in de Man-Rogosa-Sharpe (MRS) at 30 °C for 24 h, while *S. macedonicus* PON208 was reactivated in medium 17 (M17) at 44 °C for 24 h. Both media were purchased from Oxoid (Basingstoke, UK).

2.3. Avocado puree fermentation

The two-step washing procedure (Alfonzo et al., 2018), including the immersion in chlorinated water solution (0.2 %, *v*/v) for 2 min and the subsequent rinse in cold (1–2 °C) water to remove residual chlorine was applied to the fruits before peeling. Avocado puree was obtained by blending fruit pulp with an immersion blender (Moulinex Easychef DD45A1, Milan, Italy) at the highest speed, until reaching a uniform consistence. The avocados from the two cultivars were processed separately and the fermentation was carried out inoculating LAB strains individually. For this purpose, aliquots of 100 g of bulk puree were transferred into 200 mL volume sterile cups (Anicrin, Scorzé, Italy). Before inoculation, overnight (O/N) grown LAB cells were washed through two consecutive steps of centrifugation (5 min at 5000 x*g*) followed by resuspension in Ringer's solution (Sigma-Aldrich, Milan, Italy). Each LAB strain was inoculated at a final cell density in avocado puree of approximately 6.5 Log CFU/g. Avocado (AV) underwent fermentation at 30 °C for *Lacticaseibacillus* spp. strains and at 37 °C for *S. macedonicus*, resulting in samples referred to as Started-AV. AV chemically acidified with lactic acid (final pH of ca. 4 with 5 M lactic acid) (CA-AV) and AV without microbial inoculum (Unstarted-AV) were incubated at 30 °C, representing the two controls. Samples were taken at the beginning of the fermentation (Raw-AV), after 24 h (T24), and 48 h (T48). With the exception of microbiological, pH analysis, and sensory analysis, all samples were freeze-dried (Epsilon 2-6D LSC plus freeze-drier, Martin Christ, Osterode am Harz, Germany) before being analyzed as described below.

2.4. Microbiological and pH analysis

Avocado purees (10 g) were suspended into sterile 0.9 % (w/ v) Ringer's solution and homogenized using a BagMixer® 400 (Interscience, Saint Nom, France) at full speed for 2 min. Total aerobic

mesophilic microorganisms (TAB) were enumerated on plate count agar (PCA, Oxoid) and incubated aerobically for 72 h at 30 °C. Rod-shaped mesophilic LAB were counted on MRS agar, anaerobically incubated at 30 °C for 48 h, while mesophilic cocci LAB were quantified on M17 agar, also anaerobically incubated but at 44 °C for 48 h. MRS and M17 were supplemented with cycloheximide (0.1 %) to inhibit fungal growth. Anaerobic conditions were obtained in jars closed hermetically containing the AnaeroGen AN25 sachets (Oxoid). The values of pH were obtained by direct immersion of the pH-meter BASIC 20+ (Crison Instrument S.A., Barcelona, Spain) probe.

2.5. Sugars and organic acids quantification

In order to determine sugars consumption and organic acid production during fermentation, freeze dried avocado powder (1 g) was extracted with 10 mL of water/perchloric acid (95:5, v:v) using sonication (amplitude 60) in an ice bath for 1 min (2 cycles, 30 s per cycle, with 5-min intervals between cycles) via a macro-probe sonicator (Vibra-Cell sonicator; Sonic and Material Inc., Danbury, CT). The samples were subsequently subjected to stirring conditions at room temperature for 1 h (150 rpm), followed by an overnight incubation at 4 °C, and centrifugation for 10 min at 11,000 x *g*. The resultant water-soluble extracts (WSE) were ultimately subjected to filtration (0.22 μm). Glucose, fructose, lactic acid, and acetic acid were determined by HPLC analysis Ultimate 3000 high-performance liquid chromatography (Thermo Fisher Scientific, Massachusetts, MA, USA) equipped with an Aminex HPX-87H column (ion exclusion, Biorad, Hercules, CA, USA), an UV detector operating at 210 nm, and a Perkin Elmer 200a refractive index detector (IDEX Health & Science, Rohnert Park, CA, USA) (Tlais et al., 2021). Data acquisition and analysis were performed using the Chromeleon™ Data System software (Thermo Fisher Scientific).

2.6. Preparation of lipid standards

Nine standards were used for calibrations based on the same LC-HRMS method applied to detect and quantify the evolution of FA during avocado fermentation. External standards included oleic acid, linoleic acid, α-linolenic acid, palmitic acid, stearic acid, ricinoleic acid, arachidonic acid, and EPA, while DHA was used as internal standard, since it was not detected in avocado samples. Stock solutions of these FA were prepared individually in a $CH_3CN/(CH_3)_2CHOH/H_2O$ (65:30:5, v:v:v) mixture, with the exception of stearic and palmitic acids, that were dissolved in a $CHCl_3/CH_3OH$ (1:1, v:v) mixture due to the high hydrophobicity related to their saturated carbon chains. HPLC-grade solvents were used for the preparation of the standard stock solutions. A combined solution containing all standards was prepared by diluting the respective stock solution in pure CH_3OH (LC-MS grade). The combined standard solution was then used to prepare, by dilution, calibration solutions at decreasing concentrations. The final standard solutions were stored at −20 °C.

The linoleic acid oxylipin mixture purchased from Cayman Chemical and described before was adopted to calibrate the MS response of hydroxylated fatty acids and then evaluate the concentrations of singly or multiply hydroxylated fatty acids in real samples from the corresponding MS responses (vide infra).

2.7. Lipid extraction

The lipidic fraction was extracted from Raw-AV at time 0 h, as well as from Unstarted-, CA- and fermented-AV after 24 and 48 h of incubation at 30 °C (Fiorino et al., 2023). One gram of freeze-dried sample was mixed with 10 mL of HPLC grade chloroform ($CHCl_3$) and vortexed for 2 min. The mixtures were stirred at room temperature for 15 min at 150 rpm and then decanted for 2 h at 4 °C. Following this, 1.5 mL of the supernatants were diluted with pure methanol (1:10, v:v), and subjected to a second decantation phase, overnight at room temperature. Subsequently, 5 mL of supernatant was evaporated under nitrogen in a Multivap 8 (Labtech). The resulting residue was resuspended in 0.5 mL of methanol containing 15 ppm of internal standard (cis-4,7,10,13,16,19-DHA) and filtered through a 0.22 μm filter. The final extracts were stored at −20 °C until analysis was performed.

2.8. LC-HRMS/MS instrumental apparatus and operating conditions

LC-HRMS/MS analyses were performed using a LC-MS platform composed of an Ultimate 3000 RSLCnano system, coupled to a Q-Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The LC-MS system was equipped with Higher Collisional energy Dissociation cell (HCD) and a HESI (Heated Electro Spray Ionization) (Thermo Fisher Scientific, Waltham, MA, USA) interface for LC-HRMS coupling. Before LC-HRMS analysis, the mass spectrometer was periodically calibrated by direct infusion-HESI-HRMS analysis of positive and negative ion calibration solutions provided by the manufacturer. A mass accuracy better than 5 ppm was achieved. For chromatographic separation and MS detection of FA, the LC-MS operating conditions reported in Fiorino et al. (2023) were used with slight modifications. A Supelco Ascentis Express C18 column (150 × 2.1 mm ID, 2.7 μm particle size) (Merck KGaA, Darmstadt, Germany) was used for chromatographic separations of lipid fractions, by applying the following binary gradient program: 0–50 min, linear from 70 to 100 % B; 50–60 min, isocratic at 100 % B; 60–65 min, return to 80 % B, followed by a 20 min equilibration time (solvent A = water +2.5 mM ammonium acetate; solvent B = methanol +2.5 mM ammonium acetate). The flow rate was 0.3 mL min^{-1} and the temperature of the column was set at 31 °C. MS detection following chromatographic separation was performed in negative polarity. The electrospray and ion optic parameters used during LC-MS acquisition were the following: sheath gas flow rate, 35 (arbitrary units); auxiliary gas flow rate, 15 (arbitrary units); spray voltage, −3.50 kV (negative polarity); capillary temperature, 350 °C; S-lens Radio frequency level, 55 arbitrary units. The settings for the Q-Exactive Plus mass spectrometer for the Full MS scan were the following: mass scan range, 150–1500 *m/z*; resolution, 140,000 (FWHM at *m/z* 200); Automatic Gain Control (AGC) Target, $1*10^6$ ions; maximum injection time (IT), 100 ms. The settings for the Q-Exactive Plus mass spectrometer for PRM (Parallel Reaction Monitoring) acquisitions were the following: mass scan range, 150–1500 *m/z*; default charge state, 1; resolution, 140,000 (FWHM at *m/z* 200); AGC Target, $1*10^6$ ions; maximum IT 50 ms; isolation window, 1.0 *m/z* (centred on the calculated *m/z* value of the monoisotopic peak of the precursor ion); stepped normalized collision energy (NCE), 40 eV. The Xcalibur™ v.3.1 software (Thermo Fisher Scientific, Waltham, MA, USA) was used for the control of the Q-Exactive Plus spectrometer and for data elaboration.

2.9. Fatty acid quantification

Chromatographic peaks corresponding to the major FA found in avocados (detected as $[M - H]^-$ ions) were identified by comparing their retention times with those obtained for the corresponding standards. To further confirm the identification, a search was conducted on the LipidMaps database LIPID MAPS (2025), freely accessible on the Internet (https://www.lipidmaps.org). The *m/z* value for the monoisotopic peak of each FA was used as an input for the search, with a mass tolerance of ±0.005 *m/z* units and considering $[M - H]^-$ ions. The peak areas, obtained from the eXtracted Ion Current (XIC) chromatographic traces, i.e., chromatograms created by extracting the ion current from HRMS spectra in an interval centred on the calculated *m/z* value of the monoisotopic peak of the analyte of interest, were used as a measurement of MS response. In particular, the area obtained by integrating the peak detected for a FA in the respective XIC trace was normalized to the peak area obtained for DHA, added to each sample as internal standard at 15 ppm concentration. This procedure aimed at minimizing the effect of eventual fluctuations of the instrumental response on response

precision. Afterwards, quantification of the FA in avocado lipid extracts was performed using external calibrations based on standard FA XIC peak areas normalized to the DHA one.

2.10. Tentative identification of MAG and DAG in LC-HRMS spectra obtained from avocado lipid extracts

To identify other potential lipid classes present in avocado samples, in particular mono- (MAG) or diacyl- (DAG) glycerols, accurate *m/z* values, retrieved from HRMS spectra averaged under chromatographic peaks not associated with the major FA, were used for a search in the LipidMaps database. A mass tolerance of ±0.005 *m/z* units was also applied in this case, but acetate adducts $[M + CH_3COO]^-$ were proposed as the ions potentially generated under negative polarity since acylglycerols are not expected to be ionized efficiently as $[M\text{-}H]^-$ ions. As a result, several *m/z* values were found to correspond to monoisotopic acetate adducts of MAG and DAG species (vide infra).

2.11. Tentative identification and quantification of fatty acids oxidized derivatives by LC-HRMS and MS/MS analysis

Based on the results of previous studies (Fiorino et al., 2023; Losito et al., 2018), oxidized FA, including those designated as oxylipins, were expected to be eluted at low retention times in LC-HRMS chromatograms. In the present case, the LC-MS operating conditions reported in Fiorino et al. (2023) were slightly modified to enhance their separation by increasing their retention times. PRM acquisition was then applied to 14 target precursor ions whose *m/z* ratios potentially corresponded to those of hydroxylated, epoxidized, and carbonylated derivatives of FA, like those previously reported in the literature (Losito et al., 2018). Ion currents related to selected precursor ions *m/z* ratios were extracted from LC-HRMS data sets obtained. In accordance with the elution program modification, peaks were detected in the interval 0 to 10 min instead of the 0 to 5 min interval reported by Fiorino et al. (2023). In a subsequent step, selected monoisotopic ions of oxidized derivatives were fragmented in the high energy collisional dissociation (HCD) cell, according to conditions described before for MS/MS analyses, and all the product ions were then transferred into the Orbitrap mass analyzer to generate a high resolution/accuracy full product ion mass spectrum (MS/MS). The latter was used to get more information about chemical structure through a careful consideration of the *m/z* values of the detected product ions, in order to achieve a possible identification of the analyte (vide infra). The ChemDraw ultra 12.0.2 (Cambridge Soft Corporation, Cambridge, MA, USA) software was used to draw chemical structures of precursor ions and hypothetical product ions based on fragmentation profiles obtained.

Bands observed in extracted ion current chromatograms obtained from LC-HRMS data for specific *m/z* ratios of oxidized FAs were integrated and the resulting areas, normalized to that of the internal standard DHA (at a 15 ppm concentration), were adopted to estimate the respective concentrations in avocado extracts through comparison with the DHA-normalized peak areas obtained for standard mono- and bis-hydroxylated derivatives of linoleic acid (vide infra).

2.12. In vitro fatty acid metabolism mediated by LAB starters

Oleic and palmitic acids were utilized as substrates to investigate the direct impact of LAB starters on their metabolism. *L. casei* FMAC16 and *L. rhamnosus* TP5 were inoculated at a 1 % concentration in MRS broth, while *S. macedonicus* PON208 in M17 broth. Before inoculation, both broths were supplemented with 0.5 mg mL^{-1} of oleic acid or palmitic acid and filtered through a 0.22-μm filter. Incubation was carried out for 48 h at 30 °C for MRS and 44 °C for M17. Controls included broth with oleic acid that was not incubated (Oleic-MRS-Not Incubated), broth with oleic acid that was incubated but lacked microbial starters (Oleic-MRS-Unstarted), and broth that was chemically acidified with lactic acid (final pH of 4) and incubated (Oleic-MRS-CA). The lipidic fraction was extracted from bacterial supernatant and subjected to identification analysis as previously described.

2.13. Sensory analysis

Fermented avocado purees were evaluated for their sensory attributes after 48 h. The evaluator panel included 10 men and 5 women aged between 20 and 46 years old. The judges were recruited among panellists with experience in taste sessions on fruits. To eliminate the effect of avocado color perception on taste evaluation, fermented purees were judged in private tasting booths with uniform lighting. The samples (20 g), kept at 16 °C, were labelled with randomly generated numerical codes and delivered in plastic containers. Sensory evaluations were carried out under blind tasting conditions at the sensory analysis laboratory of SAAF Department following the guidelines of the ISO 13299:2003. Sensory analysis — Methodology — General guidance on establishing a sensory profile. International Organization for Standardization (2003). Thirteen descriptors were chosen among those generally considered to evaluate the sensory characteristics of avocado fruits and purees (Marco et al., 2019; Obenland et al., 2012; Wang & Ajji, 2022). The score for each attribute ranged from 0 to 9 (on an unstructured 9 cm scale) with score = 0, very low and score = 9, very high. The panellists were also asked to score the overall assessment (14th attribute) of the fermented avocado purees based on the scores of the 13 attributes regarding visual, olfactory and taste characteristics.

2.14. Statistical analysis

All analyses were performed in technical duplicates, and the entire experiment was conducted in three independent replicates. Data from microbiological, pH, biochemical, and free FA analysis were subjected to two-way analysis of variance (ANOVA), while other datasets were analyzed using one-way (ANOVA). Data were tested for normality and homogeneity of variances using the Shapiro–Wilk test on residuals and Levene's test, respectively. As the assumptions of normality and variance homogeneity were occasionally violated, the validity of ANOVA results could not always be ensured. Therefore, a Generalized Linear Model (GLM) with an identity link function was applied to evaluate differences among factors, offering a flexible framework capable of accommodating heteroscedasticity in the data. Pairwise comparison of treatment means was achieved by Tukey-adjusted comparison procedure at a P value of <0.05 (R Core Team, 2003; Seixas et al., 2025). The data of free fatty acids and mono, bis, and tris-hydroxy fatty acids in fermented samples after 48 h were subjected to principal component analysis (PCA) after centering and scaling, using OriginPro 2025.

3. Results and discussion

3.1. Microbiological and pH analysis

To the best of our knowledge, little is known about the exploitation of avocado as microbial substrate during fermentation. This study explores the symbiotic interplay between LAB and avocado biochemistry to unravel the mechanisms involved in the enrichment of FA derivatives that may serve as pivotal health or sensory markers.

Lipolysis induced by LAB in cheese, buttermilk, and fermented milks plays a role in the development of flavor and aroma compounds, influencing not just their overall sensory attributes but also the liberation of bioactive FA-derivatives through a combined action of two enzymatic pathways, lipid hydrolysis and lipid saturation (Ares-Yebra et al., 2019). In light of this, three species of LAB namely *L. casei* FMAC16, *L. rhamnosus* TP5, and *S. macedonicus* PON208, isolated previously from dairy products, and well-noted to follow one or both lipolytic enzymatic pathways (Georgalaki et al., 2000; Kishimoto et al., 2003) were used to ferment two distinct cultivars of avocado (Hass and Bacon), each

representing a unique matrix with varying chemical and nutritional profiles. Hass dominates global production, while Bacon, a hybrid variety, ranks third in agricultural production after Hass and Fuerte. Differences in fruit characteristics are often attributed to genetic variation, natural hybridization, and cultivation practices (Vincent et al., 2020). Through plating on different agar media before incubation, both avocado cultivars displayed relatively low microbial populations, with comparable counts of TAB and LAB (Fig. 1A), confirming the generally low LAB load in avocados (Filannino et al., 2020) and supporting our approach of using starters-assisted fermentation. As expected, in terms of LAB rods, *L. casei* FMAC16 and, more efficiently, *L. rhamnosus* TP5 showed a significant increase in cell density compared to the initial counts, while no colony was detectable with *S. macedonicus* PON208 as starter. Unstarted-AV and CA-AV demonstrated a significant ($P < 0.05$) increase in LAB rod counts, with similar values at 24 h in the Bacon cultivar and higher values for CA-AV over Unstarted-AV at 48 h. In the Hass cultivar, the LAB rod counts for CA-AV were lower compared to Unstarted-AV, but consistently higher than the initial counts. Among the started samples, LAB cocci colonies were exclusively observed in PON208-AV and exhibited a significant ($P < 0.05$) increasing trend over time, with the highest counts observed after 48 h. The cell density of LAB cocci also increased during incubation of CA-AV and Unstarted-AV,

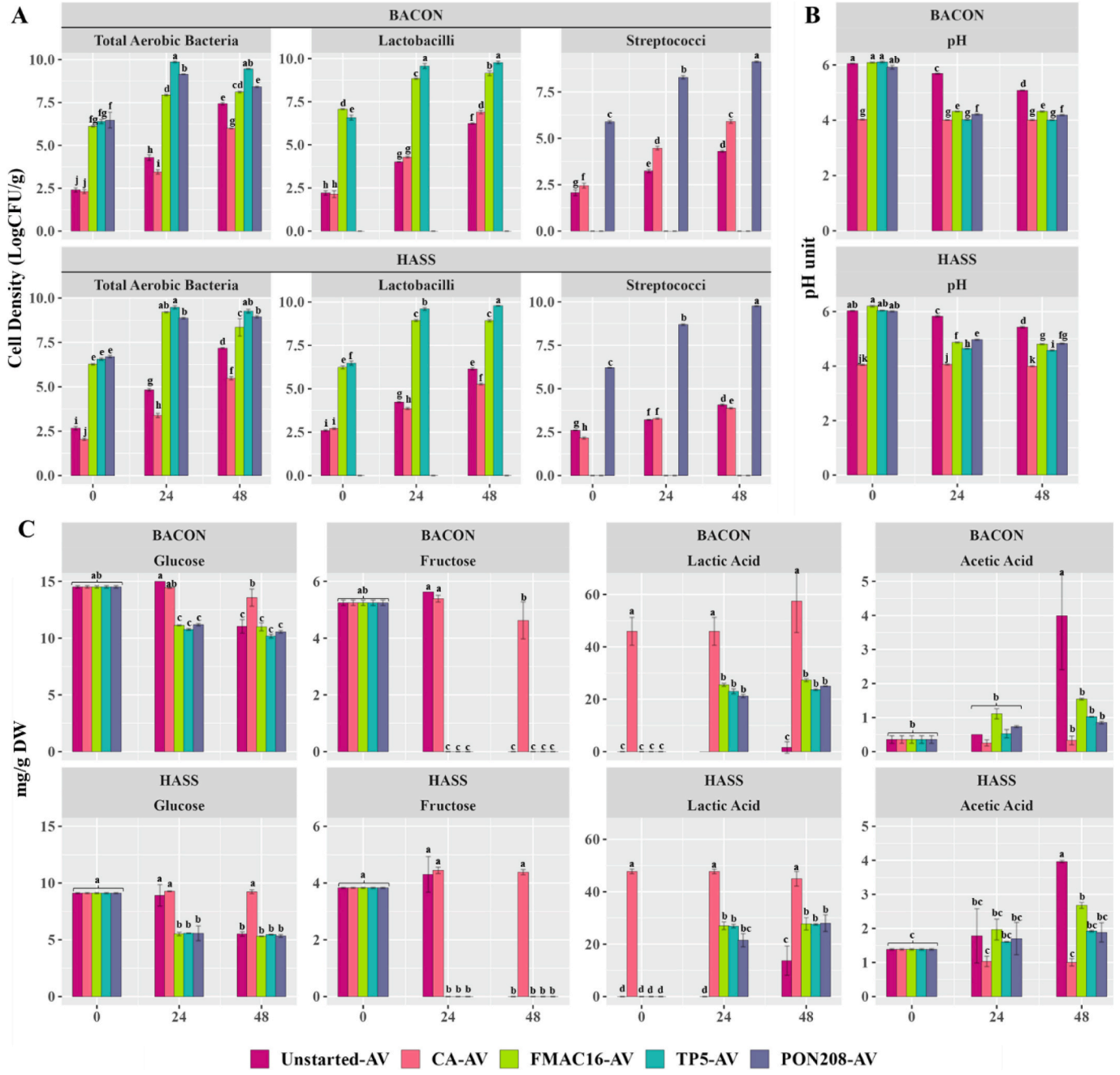

Fig. 1. Microbial dynamics and biochemical parameters during 48 h fermentation of two avocado cultivars (Bacon and Hass). Panel A shows the cell density of total aerobic bacteria, lactobacilli, and streptococci (Log CFU/g). Panel B illustrates the pH variation. Panel C reports sugar consumption and the production of organic acids (mg/g DW). Avocado was fermented with *Lacticaseibacillus casei* FMAC16 (FMAC16-AV) and *Lacticaseibacillus rhamnosus* TP5 (TP5-AV) at 30 °C, and *Streptococcus macedonicus* PON208 (PON208-AV) at 37 °C. Controls included: (i) AV without microbial inoculum (Unstarted-AV) and (ii) chemically acidified AV without microbial inoculum (CA-AV), both incubated at 30 °C. Error bars represent standard deviations ($n = 3$) and data points labelled by different superscript letters indicate significantly differing ($P < 0.05$) average values.

especially in the Bacon cultivar, where chemical acidification seemed to favor their growth.

Although the initial pH values for Bacon and Hass cultivars were comparable (ca. 6.00 pH unit), a noteworthy distinction emerged during the fermentation process, with the Bacon cultivar demonstrating a pronounced acidification effect (Fig. 1B). After 48 h, Unstarted-AV showed a significant ($P < 0.05$) pH reduction trend over time, with pH levels reaching 5.08 in the Bacon cultivar and 5.43 in the Hass cultivar. LAB starters achieved the highest significant ($P < 0.05$) pH reduction within 24 h in the Bacon cultivar, maintaining stability over the subsequent 24 h, whereas 48 h was required to reach the lowest pH in the Hass cultivar. *L. rhamnosus* TP5 yielded the lowest pH values in both the Bacon and Hass cultivars. CA-AV, regardless of the cultivar, displayed no statistically significant ($P > 0.05$) fluctuations in pH during the fermentation, maintaining a steady pH level of ca. 4.00.

3.2. Biochemical characterization

Glucose and fructose were identified as the primary sugars in both cultivars, with Bacon exhibiting a higher sugar concentration (14.5 ± 0.1 compared to 9.1 ± 0.0 mg/g DW for glucose and 5.2 ± 0.1 compared to 3.8 ± 0.0 mg/g DW for fructose) (Fig. 1C). Throughout the fermentation, the similar sugar depletion rationalized the elevated and comparable release of organic acids in both cultivars but did not justify the differences in their acidification capacities. All starters sharply reduced the glucose concentrations after 24 h, maintaining their stability in the subsequent 24-h period, while completely metabolizing the fructose content within the initial 24 h. In contrast, Unstarted-AV characterized by underexplored endogenous microbiota, displayed significant ($P < 0.05$) reductions in sugar concentration only during the second day of fermentation, reaching similar sugar residues as the started samples. CA-AV did not experience significant ($P > 0.05$) alterations in sugar concentration during the fermentation process in both cultivars. Within the first 24 h, all LAB showed a substantial increase in lactic acid levels, which remained unchanged until the end of fermentation. While the levels of lactic acid showed non-significant changes ($P > 0.05$) in CA-AV, Unstarted-AV followed a similar trend only in the Bacon cultivar. However, in the Hass cultivar, Unstarted-AV showed a significant increase in the last 24 h of fermentation, albeit remaining consistently lower than those in the started samples. Acetic acid levels increased significantly ($P < 0.05$) only in the Unstarted-AV samples during the last 24 h of fermentation in both cultivars, which suggested microbiota with enhanced acetic acid production or the ability to convert lactic acid to acetic acid (Filannino et al., 2020; Quatravaux et al., 2006). Other samples did not exhibit significant ($P > 0.05$) variations in their fermentation profiles, with the exception of FMAC16-AV for the Hass cultivar, where appreciable levels of acetic acid were recorded, although they remained lower than acetic acid observed in the Unstarted-AV samples.

3.3. Quantitative LC-HRMS analysis of free FA

Generally, adaptation of LAB is mediated by the downregulation of central metabolism genes with subsequent upregulation of transport and metabolism of alternative substrates like lipids (Filannino et al., 2018). Aiming to evaluate the effects of fermentations on free FA, their profile was analyzed through LC-HRMS. Five saturated or unsaturated FA were identified in lipid extracts through the comparison with external standards, i.e., palmitic, stearic, oleic, linoleic, and α-linolenic acids. Among FA also available as standards, EPA, ricinoleic, and arachidonic acids were present in negligible amounts and were therefore not included in the quantitative determinations. Conversely, XIC traces obtained for the respective $[M\text{-}H]^-$ ions suggested the presence of minor unsaturated free FA with side chain compositions 16:1, 16:2, 18:1, 18:2 and 18:3. Specifically, the retention time of the additional peak detected for the 18:3 composition (along with that of α-linolenic acid) was consistent with the occurrence of γ-linolenic acid, a well-known positional isomer of α-linolenic acid. Notably, minor FA were not quantified, yet some structures hypothesized for oxidized FA, based on the respective MS/MS spectra, suggested that they could be involved in oxidation processes too (vide infra).

As for major free FA, in Raw-AV ($t = 0$ h) the observed FA were ranked in decreasing order as oleic, palmitic, linoleic, stearic, and α-linolenic acids (USDA, 2018). While the Bacon and Hass cultivars showed a comparable overall FA profile, Hass cultivar demonstrated higher concentrations of each FA and consequently higher fat content (Fig. 2) (Nasri et al., 2023). Most of these are essential FA for humans and need to be obtained through diet, such as linolenic acid (omega-3) and linoleic acid (omega-6). They have been shown to reduce the risk of cardiovascular disease, have beneficial effects on metabolic syndrome, and are associated with lower LDL-cholesterol levels (Nagatake & Kunisawa, 2019; Stark et al., 2008). During the incubation period, variations in the concentrations of free FA were evident in the samples. For linoleic and stearic acids, the differences in average values discerned over the course of incubation, were not statistically significant ($P > 0.05$), due to the high variability affecting replicates. For α-linolenic acid, distinct trends were observed between the two cultivars. In the Hass cultivar, no significant differences ($P > 0.05$) were found during the fermentation, due to the high variability. In the Bacon cultivar, α-linolenic acid exhibited a noticeable increase after 24 h in all started-AV and CA-AV, exceeding the increase observed in Unstarted-AV. Over the next 24 h, α-linolenic acid continued to rise, attaining the highest values in FMAC16-AV and TP5-AV (90 ± 2 and 93 ± 3 μg g^{-1} DW, respectively), and showing the lowest levels in Unstarted-AV (55 ± 3 μg g^{-1} DW). Oleic acid levels increased consistently in most samples from both cultivars. In the Bacon cultivar, all fermented samples, excluding CA-AV, significantly increased oleic acid levels after 24 h, with a continuous rise until the end of fermentation. After 48 h, all samples showed significantly higher values than the initial levels, with TP5-AV having the highest concentration and CA-AV the lowest. In the Hass cultivar, after 24 h, only Unstarted-AV and PNO208-AV showed significantly ($P < 0.05$) higher values, maintaining this trend after an additional 24 h, along with FMAC16-AV. Palmitic acid behaved similarly to oleic acid, especially in the Hass cultivar. In the Bacon cultivar, despite the rising trend, the only significant difference ($P < 0.05$) among the samples emerged after 48 h, with TP5-AV and FMAC16-AV recording the highest concentration and CA-AV the lowest. The increasing trend of most free FA (oleic, palmitic, and linolenic acids) during avocado fermentation was not surprising. As documented in the literature, TAG are the primary lipid present in avocados (Qin & Zhong, 2016). The catalytic action of lipases (triacylglycerol hydrolases) and esterases within LAB results in the hydrolysis of carboxyl ester bonds in triglycerides, releasing intermediates like di- and monoacylglycerols, and free FA and glycerol. The observed variations in hydrolysis were not only linked to LAB species traits (Fiorino et al., 2023) but were also cultivar dependent. Indeed, a significant increase in linolenic acid was exclusively observed in all Bacon cultivar samples, albeit to varying degrees. The chemically acidified samples of the same cultivar showed an enhanced release of linolenic, oleic, and palmitic acids despite lower microbial activity and unchanged sugar profile. This led to speculation that distinct levels of endogenous lipase activity may exist among different avocado cultivars or be enhanced by the starters or pH variations occurring during fermentation. The acidification of the substrate can alter the conformational state and catalytic efficiency of lipases, as well as affecting their accessibility to lipid substrates. While the lipase activity of avocado seeds is well established, this activity in avocado pulp has yet to be confirmed (Chase, 1921; Kupnik et al., 2023).

3.4. Trend of MAG and DAG

To further elucidate the changes in free FA concentrations and to acquire a deeper understanding of LAB lipid metabolism, we identified

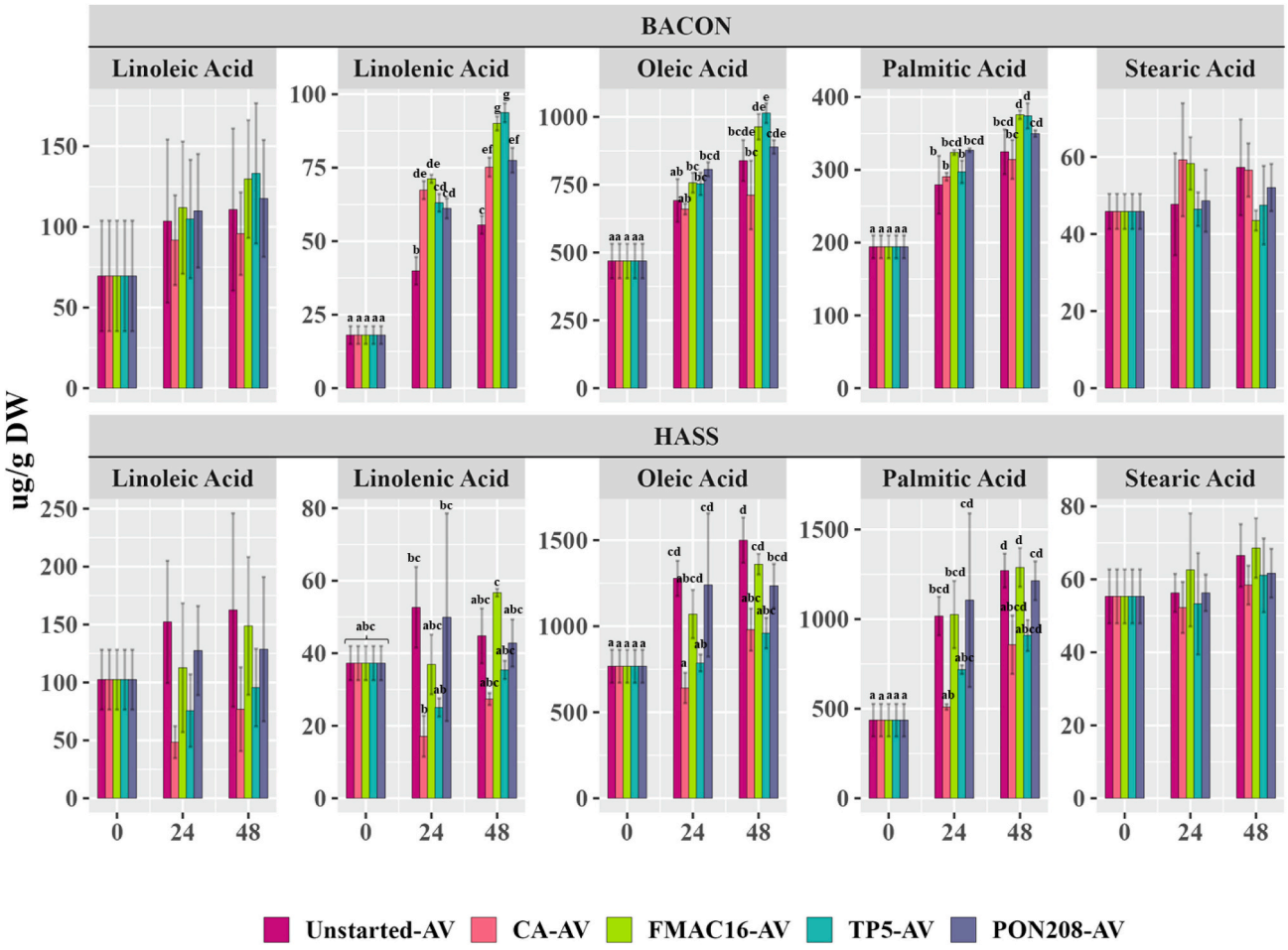

Fig. 2. Quantification of free fatty acids (μg g^{-1} DW) through HPLC-HRMS. Determinations were performed during 48 h fermentation of two avocado cultivars (Bacon and Hass). Avocado was fermented with *Lacticaseibacillus casei* FMAC16 (FMAC16-AV) and *Lacticaseibacillus rhamnosus* TP5 (TP5-AV) at 30 °C, and *Streptococcus macedonicus* PON208 (PON208-AV) at 37 °C. Controls included: (i) AV without microbial inoculum (Unstarted-AV), and (ii) chemically acidified AV without microbial inoculum (CA-AV), both incubated at 30 °C. Error bars represent standard deviations ($n = 3$) and data points labelled by different superscript letters indicate significantly differing ($P < 0.05$) average values.

lipolysis by-products of TAG, including DAG and MAG building upon the groundwork of Fiorino et al. (2023). Twelve distinct *m/z* values were assigned to acetate adducts ($[M + CH_3COO]^-$) of putative MAG (4 compounds) and DAG (8 compounds), by setting a mass tolerance of +/− 0.005 *m/z*. As inferred from Fig. 3, *m/z* values of MAG ions were consistent with the presence of 16:0, 18:0, 18:2 and 18:3 chains, i.e., with chain compositions corresponding to those of free FA detected in the same samples. Actually, the response for MAG(18:2) and MAG(18:3) was much lower in all samples and, apparently, MAG(18:1) was not detected, thus suggesting that MAG with a residual unsaturated chain were minor species in the class. As for DAGs, the *m/z* ratios indicated the presence of 34 or 36 carbon atoms on the two side chains, thus indirectly confirming the expected occurrence of mixed combinations of side chains with 16 and 18 carbon atoms (but not of two chains with 16 carbon atoms). Interestingly, given a certain total number of carbon atoms on side chains, DAG including a higher number of C=C bonds (34:3, 36:5 and 36:6) exhibited a lower response compared to less unsaturated ones. The MS response of the cited MAG and DAG was monitored in Raw, Unstarted-AV, CA-AV, and fermented samples after 48 h to delineate eventual variations of their levels during fermentation. FMAC16-fermented sample, marked by elevated free FA content in Bacon avocados, served as a pertinent model to state the boosted enzymatic hydrolysis of TAG. Summarized outcomes, presented in Fig. 3, highlight significant ($P < 0.05$) increases in MAG (18:0), MAG (18:2), and DAG (36:2) responses in Bacon avocados fermented with *L. casei* FMAC16 compared to controls.

3.5. Identification of oxidized FA derivatives by LC-MS/MS analysis

The increasing trend of free FA during fermentation does not rule out the possibility that some of them underwent metabolic conversions into FA derivatives. This became evident upon a more in-depth analysis of the fourteen *m/z* values retrieved from high resolution MS spectra averaged under peaks eluting in the first 10 min in avocado lipid extracts chromatograms. These *m/z* values were compatible with exact *m/z* of $[M-H]^-$ ions of oxidized derivatives of FA previously detected in avocado samples, namely those including 16 or 18 carbon atoms and 0, 1, 2 or 3 C=C bonds (see the first column of Table S1 in the Supplementary Material). In a subsequent step, the occurrence of chromatographic band/peaks actually related to the selected *m/z* ratios was carefully checked through ion current extraction based on extremely narrow *m/z* intervals, centered on each of the exact *m/z* ratios referring to the major isotopologues (M + 0 ions) of the $[M-H]^-$ ions of putative oxidized FA. As evidenced, for example, in the bottom panel of Fig. S1, the extraction of ion current in a *m/z* interval having a 0.0084 units width and centered on the exact *m/z* value 293.2122, related to mono-hydroxylated 18:3 FA (18:3; 1), enabled the retrieval of a complex chromatographic band eluting between 2.6 and 4.4 min, potentially resulting from the close elution of several isomers of that oxidized FA. The importance of high mass accuracy/resolution for the correct recognition of oxidized FA

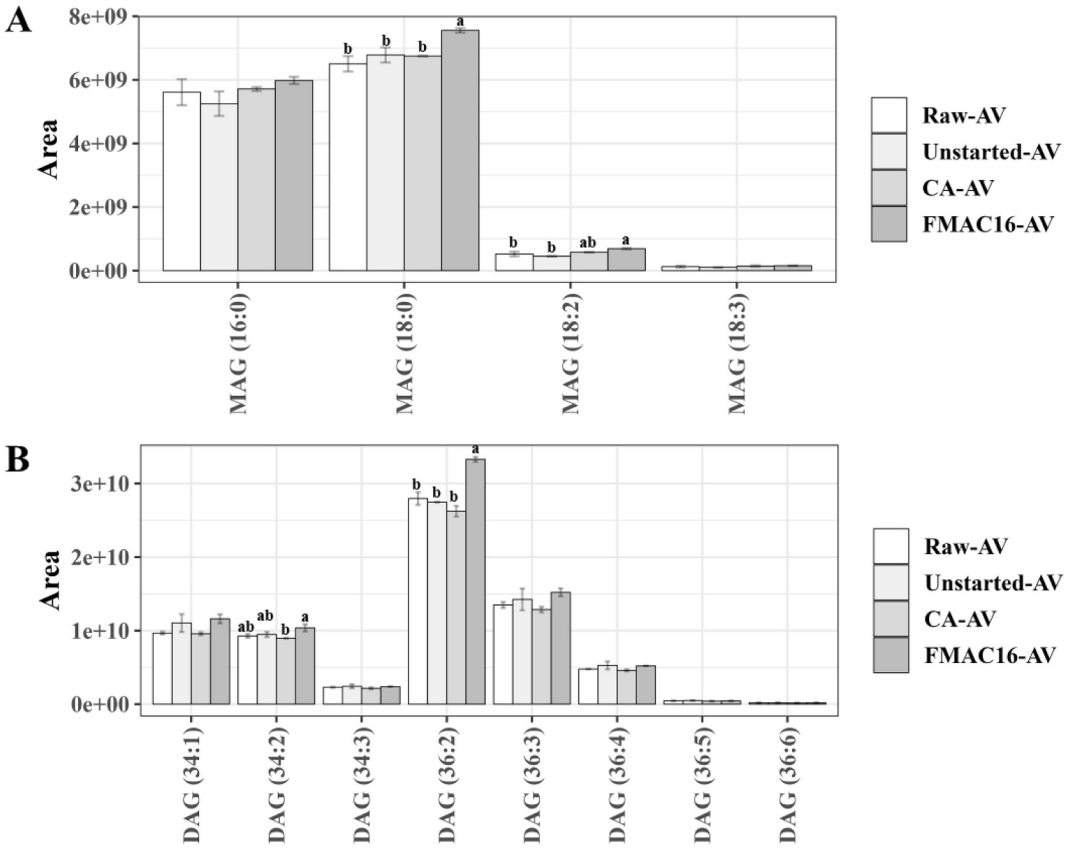

Fig. 3. Peak areas from eXtracted Ion Current (XIC) chromatograms of monoacylglycerols (MAG) (panel A) and diacylglycerols (DAG) (panel B) in Bacon avocado cultivar. Chromatograms were obtained after HPLC-HRMS analysis of lipid extracts from raw avocado (Raw-AV), and from: (i) avocado without microbial inoculum (Unstarted-AV), (ii) avocado without microbial inoculum and chemically acidified with lactic acid (CA-AV), and (iii) avocado fermented with *Lacticaseibacillus casei* FMAC16 (FMAC16-AV), which were incubated for 48 h at 30 °C. Error bars represent standard deviations (n = 3) and different superscript letters indicate significantly differing ($P < 0.05$) average values.

species is emphasized by the fact that if a much wider *m/z* interval had been adopted for ion current extraction, like the 1 *m/z* unit wide one (293.0000–294.0000) adopted in the top panel of Fig. S1, an additional peak would have been observed in the XIC trace at 2.63 min. As evidenced in the central panel of Fig. S1, the ion current extraction on an appropriate, narrow *m/z* interval indicated the additional peak to be related to an interfering species, detected at *m/z* 293.1757.

MS/MS acquisitions were subsequently performed on precursor ions having the *m/z* ratios recognized for oxidized FA and the information acquired for the respective product ions was employed to infer the putative structure of oxidized derivatives. As a result, fifty-seven isomeric species with different hydroxylation or epoxidation sites and/or double bond positions were hypothesized. Among them, the structures of species related to major free FA, i.e., palmitic, oleic, linoleic, α-linolenic, and stearic ones, are reported in Fig. 4. The structures of oxidized derivatives likely generated from minor free FA, i.e., those with side chain compositions 16:1, 16:2, and FA with compositions 18:1, 18:2 and 18:3 representing positional isomers of oleic, linoleic and linolenic acids, respectively, are shown in Fig. S2.

It is worth noting that the procedure adopted to hypothesize structures for oxidized FAs started by considering chemical oxidation previously proposed for unsaturated FAs (Köckritz & Martin, 2008). In particular, single or multiple hydroxylations, hydroperoxylations and carbonylations of allylic or bis-allylic carbon atoms, and also hydroxylations of carbon atoms involved in a C═C bond (with consequent displacement and change to *trans* geometry for the latter) were hypothesized, along with the epoxidation of C═C bonds. In a subsequent step, other possible oxidation sites, not related to C=C bonds, were hypothesized both for unsaturated and for saturated FA, considering the occurrence of enzymatic oxidation. Each proposed structure was first checked for consistency between the calculated *m/z* ratio of its $[M\text{-}H]^-$ ion and one of the experimentally found *m/z* ratios. Afterwards, *m/z* ratios of peaks detected in the corresponding MS/MS spectrum were compared with the calculated values referred to product ions expected for the proposed structure, based on side chain fragmentation processes typically triggered by the presence of one or more O-containing groups linked to the chain (Fiorino et al., 2023; Losito et al., 2018). An example of the procedure is schematically represented in Fig. S3 for the $[M\text{-}H]^-$ ion whose *m/z* ratio was consistent with the exact value (293.2122) expected for mono-hydroxylated α-linolenic acid. As apparent from the XIC-MS trace referred to that value, already discussed before and shown also in the bottom panel of Fig. S1, several partially co-eluting and perfectly isobaric (i.e., having an identical molecular mass) species were present, leading to a complex chromatographic band. HRMS/MS spectra acquired along this band were then carefully examined and XIC-MS/MS traces were generated by extracting ion currents for the *m/z* ratios of all major peak signals detected in those spectra, corresponding to specific fragments. Three examples of such traces are reported in Fig. S3, showing that, in some cases, a product ion with the same *m/z* ratio could be obtained upon fragmentation of different species, since either multiple peaks or quite broad bands (reasonably arising from the co-elution of at least two peaks) were found in some XIC-MS/MS traces.

As evidenced in the bottom part of Fig. S3, the *m/z* ratios of product ions were fundamental to recognize their putative structure, after considering general fragmentation pathways observed for oxidized FA, and then infer the structural features of each oxidized derivative. An identity was finally assigned to the latter only if one or more diagnostic fragments, found in the MS/MS spectrum, were coherent with that

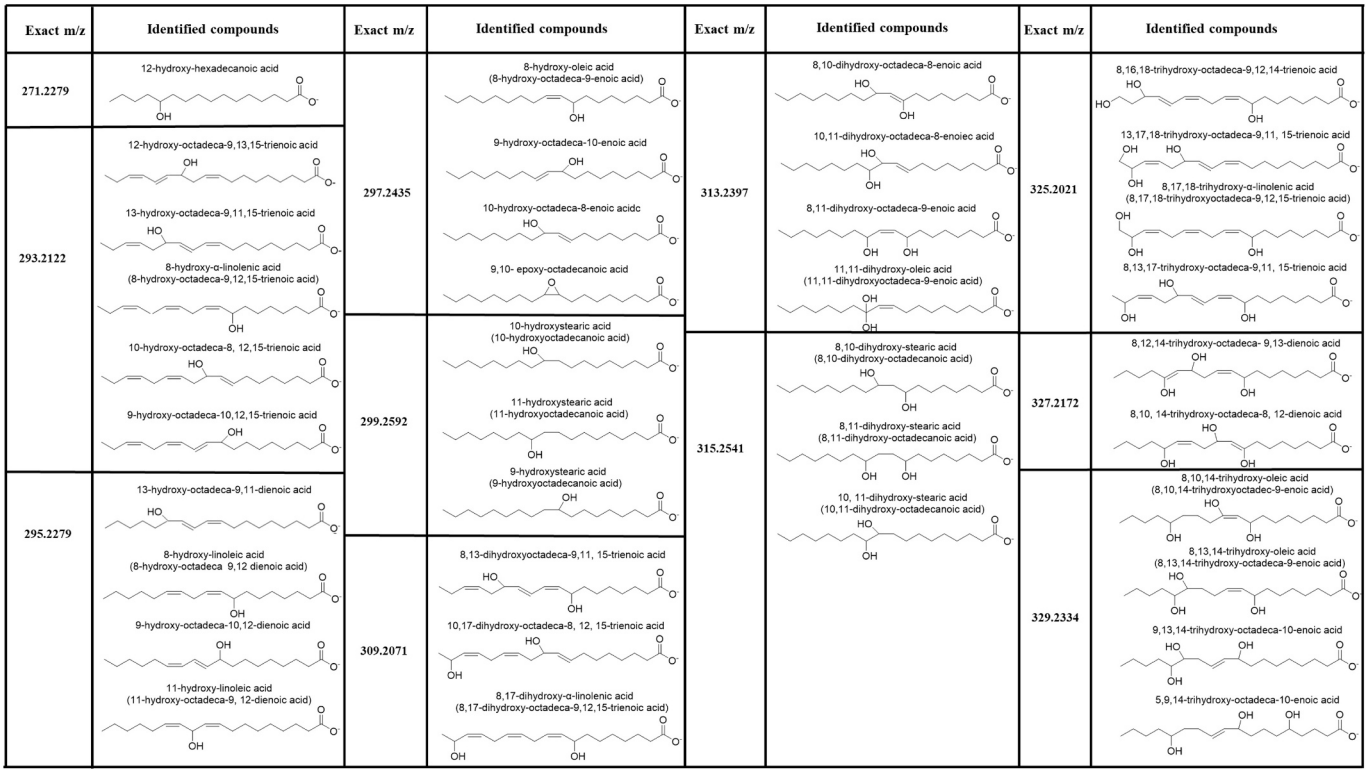

Fig. 4. Chemical structures hypothesized for precursor ions of hydroxylated/epoxidized fatty acids detected in lipid extracts of avocado samples and generated from major free fatty acids (i.e., palmitic, oleic, linoleic, α-linolenic, and stearic ones).

identity. Interestingly, a proton transfer from an OH group towards the deprotonated COOH group was found to occur in some cases during fragmentation of hydroxylated FA, leading to enolate-like anions (like the one shown in Fig. S3 for the *m/z* value 137.0972) which were stable enough to be transferred safely from the HCD cell towards the Orbitrap mass analyzer of the mass spectrometer. This gas-phase process, along with other chain breakages close to oxygenated functionalities, generated several interesting product ions from the precursor ions of oxidized derivatives, helping in finding the position of OH groups (or, in one case, of an epoxydic group) along the acyl chain. For the sake of completeness, the entire set of 12 XIC-MS/MS traces obtained for product ions referring to the precursor ions with exact *m/z* 293.2122, which ultimately enabled the recognition of eight different hydroxylated 18:3 isomers in avocado samples, is reported in Fig. S4. The list of those isomers and that of the related product ions is included in the comprehensive summary, provided in Table S2, for the *m/z* ratios (exact values) of precursor ions and of diagnostic product ions employed for the structural assignment of the 57 oxidized derivatives recognized in this study, along with the names of those derivatives and the retention time information. Notably, the retention time for a specific compound was found, in this case, after aligning the peaks detected in the XIC-MS/MS chromatograms referring to diagnostic product ions for that compound.

As described in Tables S1 and S2 and Figs. 4 and S2, several isomeric forms emerged for most of the fourteen selected *m/z* ratios, with single or multiple hydroxylations prevailing, whereas epoxidation was inferred in the case of oleic acid and no MS/MS-based evidence for the presence of C═O or OOH groups linked to side chains was obtained. Notably, as mentioned before, some of the oxidized derivatives seemed to be related to FA occurring as minor species in avocado samples, like γ-linolenic acid, 11Z-octadecaenoic acid (a positional isomer of oleic acid, corresponding to the *cis* isomer of vaccenic acid), 6Z,9Z-octadecadienoic acid (a positional isomer of linoleic acid), and different isomers of 16:1 and 16:2 FA. Hydroxylated derivatives of saturated FA (palmitic and stearic ones) were also recognized, suggesting the involvement of specific enzymatic oxidation processes. The frequent occurrence of multiple species, sometimes even perfectly co-eluting, for each *m/z* ratio, clearly indicated by Figs. 4 and S2 and by Table S2, was indirectly confirmed by the detection of complex chromatographic bands in the XIC-MS traces referred to specific *m/z* ratios, suggesting the overlap of single species peaks.

3.6. Trend of FA derivatives in avocado lipid extracts

Due to the lack of chromatographic resolution between peaks related to single isomeric forms, complex band resulting from their overlap were clearly visible in the XIC traces referred to each of the *m/z* ratios of oxidized FA. A further example of these bands, namely those obtained for mono-hydroxylated derivatives of fatty acids with sum compositions 18:3, 18:2, 18:1 and 18:0, respectively, is reported in Fig. S5. Bands were progressively displaced towards higher retention times at the decrease of the number of C═C bonds along the C18 side chain, which is consistent with the reduced interaction with the C18 stationary phase observed when unsaturations are present along the side chain of FA species. Nonetheless, a partial co-elution between bands related to hydroxylated FA differing by just one C═C bond was sometimes observed. It is thus worth noting that the risk of Type II isotopic interference, e.g., the presence of a contribution from the M + 2 isotopologue of the $[M\text{-}H]^-$ ion for FA 18:3;1 in the MS response due to the M + 0 isotopologue of the $[M\text{-}H]^-$ ion for FA 18:2;1, in their co-elution interval (3–4.5 min, see Fig. S5), could be avoided by a careful choice of *m/z* intervals adopted for ion current extraction. Indeed, the interval adopted for ion current extraction for the M + 0 isotopologue of the $[M\text{-}H]^-$ ion of FA 18:2;1 (295.2240–295.2314) did not include the *m/z* ratio expected for the M + 2 isotopologue of the $[M\text{-}H]^-$ ion for FA 18:3;1 (295.2190). The same occurred for all the couples of oxidized FA differing just for one C═C bond detected in avocado samples and partially co-eluting from the C18 column.

Bands like those shown in Fig. S5 could thus be reliably integrated

and the resulting area was employed as the cumulative MS response of the corresponding group of isomeric forms, correlated to their overall concentration. In fact, starting from the assumption that the ionization yields for isomeric oxidized FA, i.e., for compounds differing just for the position of oxygenated groups and/or C=C bonds, are comparable, since the negative ionization site is always the COOH group, the cumulative MS response found for a specific *m/z* ratio detected in HRMS spectra can be considered proportional to the total concentration of species corresponding to that ratio. Notably, the similarity of ionization yields could be ascertained during this study also for oxidized FA differing for the number of oxygenated functionalities or even for the nature of the latter. Indeed, comparable XIC peak areas were obtained when standard mono-hydroxylated 18:2 FA and bis-hydroxylated 18:1 FA were analyzed at the same concentration with the adopted RPLC-ESI-HRMS method. A similar finding was also obtained when comparing standard mono-hydroxylated 18:2 FA and mono-epoxidated 18:1 FA and even for standard mono-hydroxylated 18:2 and oxo-18:2 FA. These results indicate that ionization yields for the oxidized species detected in avocado samples can be considered comparable despite differences in the number and type of oxygenated groups and in the number of carbon atoms on the side chain. Based on these premises, concentration of oxidized FA in avocado samples could be estimated by considering calibration lines obtained for standard mono-hydroxylated 18:2 and bis-hydroxylated 18:1 species, which were considered, respectively, as a reference for the quantification of mono-hydroxylated/mono-epoxidized and for bis−/tris-hydroxylated FA derivatives recognized in the samples. As for non-oxidized FA, responses adopted for quantification corresponded to XIC peak areas normalized for the peak area of DHA, added at a 15 ppm concentration to each sample.

The resulting concentration values were subsequently used to follow the trends of production of oxidized FA in all avocado samples. As higher changes in concentrations were observed towards the end of fermentation, exclusive consideration was given to treated samples subjected to a 48-h incubation in contrast to the raw avocados, thus only data referred to that treatment time are reported, along with those referred to raw avocados, in Fig. 5.

For mono-hydroxylated 16:0 FA, FMAC16-AV demonstrated a significantly ($P < 0.05$) higher concentration ($P < 0.05$) compared to all other samples in Bacon cultivar. The TP5-AV strain in the Hass cultivar also produced higher levels of mono-hydroxylated 16:0 FA than the controls and Raw-AV. An increasing trend was noted in the concentrations of mono-hydroxylated 16:1 and 16:2 species for both cultivars compared to Raw-AV, without major differences observed between the incubated samples. In the case of mono-hydroxylated FA with 18 carbon atoms, FMAC16-AV and TP5-AV exhibited a dramatic increase ($P < 0.05$) in concentration for 18:0 species across all samples, regardless of

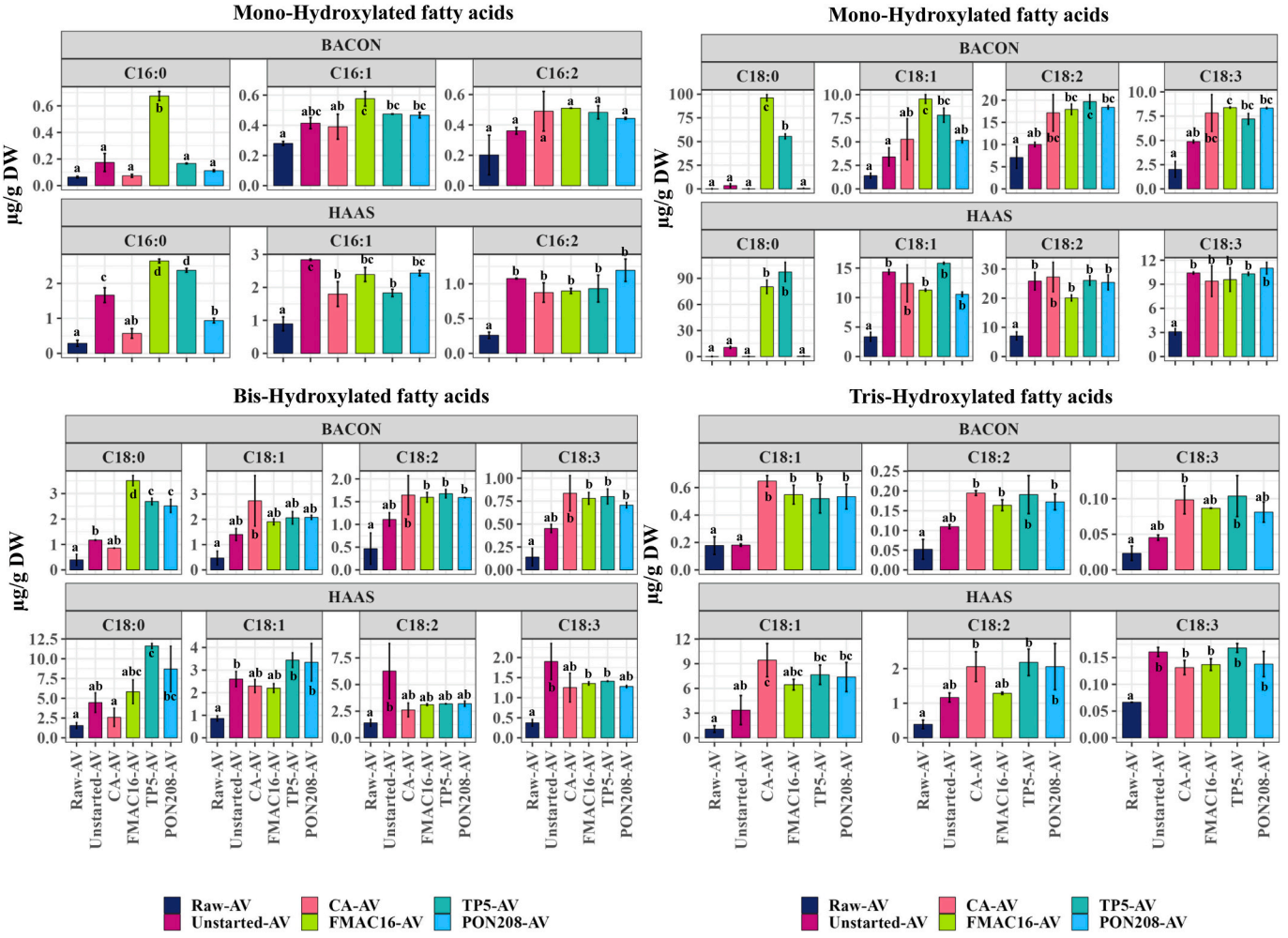

Fig. 5. Concentrations (mg/g DW) of mono, bis, and tris-hydroxy fatty acids in Bacon and Hass avocado cultivars. Values were estimated after the HPLC-HRMS analysis of lipid extracts from raw avocado (Raw-AV), and from: (i) avocado without microbial inoculum (Unstarted-AV), (ii) avocado without microbial inoculum and chemically acidified with lactic acid (CA-AV), and (iii) avocado fermented with three strains of lactic acid bacteria, which were incubated for 48 h. Avocado was fermented with *Lacticaseibacillus casei* FMAC16 (FMAC16-AV) or *Lacticaseibacillus rhamnosus* TP5 (TP5-AV) at 30 °C, or *Streptococcus macedonicus* PON208 (PON208-AV) at 37 °C. Error bars represent standard deviations (n = 3) and different superscript letters indicate significantly differing ($P < 0.05$) average values.

the cultivars. For these two strains, the final concentration of mono-hydroxylated 18:0 FA ranged between 50 and 100 μg g^{-1} DW, thus exceeding the residual concentration of the corresponding precursor in the same samples (see data for stearic acid reported in Fig. 2). This finding represents the most pronounced change observed in hydroxylated FA profiles. Overall, the levels of mono-hydroxylated 18:1, 18:2 and 18:3 species had an increasing trend for both cultivars compared to Raw-AV, but FMAC16-AV emerged for the higher ($P < 0.05$) level of mono-hydroxylated 18:1 in Bacon cultivar with respect to the other incubated samples.

In the context of dihydroxy 18:x FA, there was an overall increasing trend compared to Raw-AV, with few significant differences between the incubated samples. For the 18:0 ones, all started samples of the Bacon cultivar showed higher MS responses than the controls, with the highest level being found in FMAC16-AV. Only TP5-AV showed a prevailing concentration for dihydroxy-18.0 FA in the Hass cultivar ($P < 0.05$).

For 18:2 species only the Unstarted-AV was found to be prevalent in the Hass cultivar. Compared to the Raw-AV, the CA-AV and started samples demonstrated similar or increased values of tri-hydroxylated fatty acids (C18:1, C18:2 and C18:3) for both the Bacon and Hass cultivars. No differences were observed between the CA-AV and the started samples.

To evaluate the evolution of FA derivatives in avocado samples using also a multivariate approach, the corresponding concentration values obtained after 48 h were autoscaled and used as input variables for PCA. Each cultivar was considered separately, with each replicate sample treated as an individual object for PCA processing. The resulting bi-plots are reported in Fig. S6. In Bacon cultivar, the Unstarted-AV and CA-AV samples were clearly separated from those fermented with LAB strains, despite the remarkable variability associated with the CA-AV group. As previously mentioned, the presence of mono- and bis-hydroxylated 18:0 FA, likely arising from enzymatic processes, was particularly pronounced in the FMAC16-AV and TP5-AV samples. This feature distinctly differentiated them from both the Unstarted-AV and CA-AV samples.

In Hass cultivar, the higher incidence of mono- and bis-hydroxylated 18:0 FA pushed TP5-AV samples towards the upper left quadrant of the biplot. The increase in these oxidized species in FMAC16-AV samples, also observed in Fig. 5, was masked by the remarkable concentration of linolenic acid detected after 48 h of fermentation (Fig. 2). As evidenced in the Hass biplot (Fig. S6), linolenic acid had the most relevant negative loading on PC2, shifting FMAC16-AV samples in the lower right quadrant of the PCA score plot.

Literature states that enzymatic hydroxylation and chemical oxidation of saturated and unsaturated FA have the capacity to produce a diverse array of oxidized FA species (Kim & Oh, 2013). In our study, enzymatic hydroxylation was likely more effective in generating saturated hydroxy FA, whilst the generation of unsaturated mono, bis, and tri-hydroxy FA in fermented samples closely mirrored those resulting from chemical oxidations in CA-AV. The exception occurred with the mono-hydroxylated forms of the 18:1 FA (mainly corresponding to oleic acid), specifically related to ions at *m/z* 297.2435, which showed a notable increase due to enzymatic activity from *L. casei* FMAC16. Generally, bacterial production of hydroxy unsaturated FA requires specific, regioselective hydroxylation enzymes, limited by expression levels and turnover rates (Takeuchi et al., 2016). The mono-hydroxy derivative of oleic acid has various potential applications in food industry and synthesis of fine chemicals and pharmaceuticals (due to its similarity with ricinoleic acid), prompting much effort to develop methods for its high-yield production (Bódalo et al., 2005). Despite the statistically insignificant differences observed in other unsaturated hydroxy FA compared to chemically acidified samples, the role of enzymes during fermentation cannot be excluded, leading to a substantial increase in mono hydroxylated 18:2 and 18:3 FA compared to raw samples. Some of the isomeric compounds associated with ions having *m/z* 295.2279 and 293.2122 were identified as 9-HODE and 13-HODE, and 13-HOTE and 9-HOTE, respectively. The bacterial diol synthase, a fusion protein consisting of lipoxygenase and allene oxide synthase, is able to convert linoleic acid to mono-hydroxy FA such as 9-hydroxy-10,12-octadecadienoic acid and 13-hydroxy-9,11(Z,E)-octadecadienoic acid (Spiteller & Spiteller, 1997). While 9-HODE exhibits pro-inflammatory properties and plays a critical role in human skin photoaging (Tsuchida & Sakiyama, 2023), 13-HODE demonstrates predominantly protective effects against inflammation and atherosclerosis (Szczuko et al., 2020). The same enzyme metabolized linolenic acid to 9-HOTE and 13-HOTE derivatives with high functionalities. 9-HOTE regulated plant stress responses during pathogen defense (Vellosillo et al., 2007), while (13*S*)-HOTE showed promise in preventing TNBS-induced acute colitis in rats by suppressing specific inflammatory markers. This hints at the therapeutic potential of (13*S*)-HOTE-derived compounds for treating active inflammatory bowel diseases (Avila-Román et al., 2014).

Multiple isomeric forms of mono-hydroxystearic acid and di-hydroxystearic acid were hypothesized for ions with exact *m/z* values of 299.2592 and 315.2541, respectively. Hydroxystearic acids, notably 12-hydroxystearic acid sourced from castor oil, have been extensively researched due to their economic significance. Recent attention has shifted towards less-explored positional isomers, driven by their specific biological activities. Notably, 9-hydroxystearic acid (9-HSA) demonstrated significant inhibitory effects against various human tumor cell lines, functioning as an inhibitor of histone deacetylase HDAC1 (Calonghi et al., 2022) while 10-hydroxystearic (10-HSA) acid proved to reduce skin age spots and visible pores (Schütz et al., 2019). FA hydratase, which is unique to microorganisms including *L. casei and* L. *rhamnosus* (Serra et al., 2020), is responsible for oxidizing unsaturated C=C bonds, aiding in the detoxification of environmental toxins. Specifically, oleate hydratases within this enzyme family can catalyze the hydroxylation of oleic acid to produce 10-HSA (Serra et al., 2020). Conversely, research on the capability of LAB to convert specific FA into di-hydroxystearic acid is not well-documented, although most starters showed upward trends in these derivatives in both cultivars compared to the controls. Existing literature indicates that microbial diol synthases can synthesize dihydroxy FA (Kim & Oh, 2013). For instance, 8,11-linoleate diol synthase from *Penicillium chrysogenum* has been shown to convert oleic acid into 8,11-dihydroxy-9-octadecenoic acid, which can subsequently be hydrogenated to yield 8,11-dihydroxystearic acid (Shin et al., 2022). The regioselectivity exhibited by diol synthase is crucial in determining the positioning of hydroxyl groups along the stearic acid chain (Shin et al., 2022). The distinct levels of fatty acid derivatives found in Hass and Bacon avocados can be attributed to cultivar-specific fermentation dynamics. For example, differences in acidification patterns are known to influence lipid metabolism in LAB (Hadinia et al., 2022; Wu et al., 2023).

3.7. Trend of FA derivatives in synthetic growth models

One of the identified isomers for mono-hydroxylated 18:1 FA, 10-hydroxy-8-octadecenoic acid, is an intermediate in the conversion of oleic acid to 7,10-dihydroxy-8-octadecenoic acid by certain strains of *Pseudomonas* spp., *Bacillus*, spp., and fungi (Bódalo et al., 2005; Hou et al., 1991). While this pathway is uncommon in LAB, its occurrence by *L. casei* was validated by examining the lipolytic activity of starters on oleic acid in synthetic media. In MRS supplemented with oleic acid, a significant depletion of the latter occurred upon fermentation with two of the three bacterial species under study compared to controls, with a 73 % decrease observed for *L. casei* FMAC16 and a 45 % decrease for *L. rhamnosus* TP5 (Fig. 6). This depletion was concomitant with the significant release of mono-, bis- and tris-hydroxy 18:1 FA during fermentation by *L. casei* FMAC16, and of bis-hydroxy (compared to all controls) and tris-hydroxy (compared to raw and unstarted samples) 18:1 FA when *L. rhamnosus* TP5 was involved (Fig. 6). The level of oleic acid remained almost unchanged in the unstarted sample and in chemically acidified broth, as well as in the M17 broth inoculated with *S. macedonicus* PON208 (thus data were not reported in Fig. 6 for this

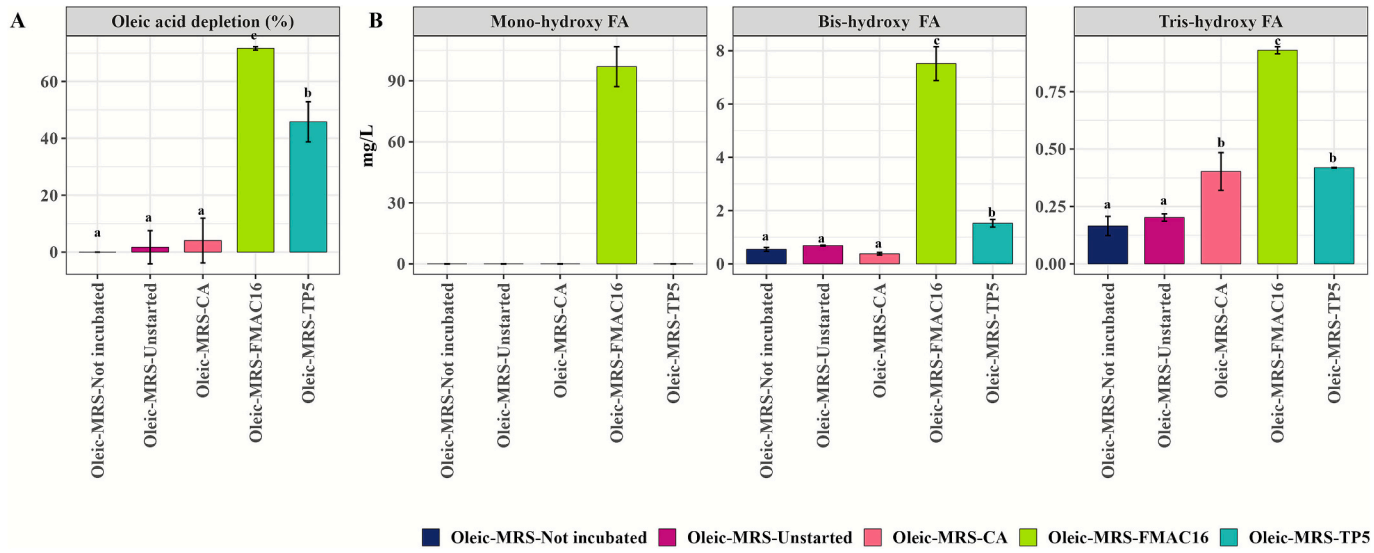

Fig. 6. Results of the HPLC-HRMS analysis of MRS broths supplemented with 0.5 mg mL^{-1} of oleic acid. The broth was incubated with *Lacticaseibacillus casei* FMAC16 (Oleic-MRS-FMAC16) or *Lacticaseibacillus rhamnosus* TP5 (Oleic-MRS-TP5) at 30 °C for 48 h. Panel A shows the percentual depletion observed for oleic acid. Panel B reports the concentrations estimated for mono, bis, and tris-hydroxy 18:1 fatty acids. Controls included broth with oleic acid that was not incubated (Oleic-MRS-Not Incubated), broth with oleic acid that was incubated but lacked microbial starters (Oleic-MRS-Unstarted), and broth that was chemically acidified and incubated without microbial starters (Oleic-MRS-CA). Bars with different superscript letters represent average values that differ significantly ($P < 0.05$).

sample). Interestingly, small amounts of bis- and tris-hydroxylated 18:1 acid were found also in controls, thus suggesting the occurrence of chemical oxidation of oleic acid, in accordance with the results observed for all FA in avocado control samples.

The hydroxylation of palmitic acid was more pronounced by *L. casei* FMAC16 in the two avocado cultivars and by *L. rhamnosus* and endogenous microbiota only in the Hass cultivar. However, such metabolism was not confirmed in synthetic media. Typically, cytochrome P450 enzymes in the cytochrome P450 (CYP)153 family convert palmitic acid into three hydroxylated products, with hydroxylation occurring at C-11, C-12, and C-13 (Bhattarai et al., 2013). Among these, only one isomer, associated with ions having *m/z* 271.2297, was detected, indicating the absence of carbon double bonds and the presence of a hydroxyl group at C-12. Literature review reveals that lactobacilli and species of the genera *Streptococcus*, *Listeria*, *Geobacillus*, *Lactococcus*, and *Leuconostoc* lack P450 enzymes (Padayachee et al., 2020). Conversely, plants possess numerous cytochrome P450 enzymes that are influenced by their lifestyles (Bezirtzoglou, 2012; Padayachee et al., 2020). This led to the hypothesis that fermentation-associated microorganisms may affect these plant cytochromes, inducing the release of such derivatives, particularly emphasizing the role of *L. casei*. Supporting this, literature indicates that *L. casei* impacts the expression and activity of cytochromes P450 and the CYP mRNA levels in the intestine and liver of male rats (Matuskova et al., 2011). Although the specific functional role of hydroxy palmitic acid remains inadequately characterized, existing knowledge about palmitic acid derivatives indicates their significant regulatory involvement in cancer tumor resistance mechanisms (Wang et al., 2023).

3.8. Sensory analysis

Sensory descriptors for visual, olfactory, and taste characteristics were used to assess the impact of FA hydrolysis and oxidation during fermentation on avocado cultivars (Fig. S7). Differences were notable depending on the starter cultures and avocado cultivars. In the Bacon cultivar, fermented samples generally exhibited higher color scores than Raw-AV, while in the Hass cultivar, Raw-AV had intermediate scores between PON208-AV (highest) and FMAC16-AV/ TP5-AV (lower). Odor intensity was highest in the Bacon Raw-AV and lowest in FMAC16-AV and TP5-AV, while TP5-AV exhibited the highest intensity in Hass. FMAC16-AV in Bacon had significantly ($P < 0.05$) lower unpleasant odor scores compared to Raw-AV, whereas, in the case of Hass, TP5-AV had the highest unpleasant odor scores. Raw-AV samples in both cultivars had lower acidity compared to fermented samples, with varying acidity rankings among starters. For bitterness, PON208-AV scored highest in Bacon, while in Hass, TP5-AV and FMAC16-AV scored highest compared to Raw-AV. PON208-AV had the highest unpleasant aroma score, and FMAC16-AV the lowest in Bacon, whereas TP5-AV, and, to a lesser extent, FMAC16-AV, had the highest scores in Hass. Overall, in Bacon, FMAC16-AV showed similar acceptability to Raw-AV, while in Hass, Raw-AV and PON208-AV were the most acceptable. Lipolysis and lipid oxidation are known to substantially contribute to the characteristic color, odor, and aroma of fermented foods (Feng et al., 2014). However, hydrolytic and oxidative rancidity can lead to the formation of unpalatable and toxic compounds, making them nutritionally undesirable, as seen in olive oil (Morales & Przybylski, 2013). In our study, incubation of avocado (lipid-rich matrix) was expected to lead to oxidative processes, rancidity, and unpleasant aromas/flavors. Contrary to this expectation, particularly in the Bacon cultivar, incubation with FMAC16 was associated with the lowest levels of undesirable odor and aroma, potentially linked to specific FA derivatives released. Conversely, in Hass cultivar, the *L. rhamnosus* TP5 starter exhibited the highest degree of unpleasant sensory characteristics, despite a similar FA profile. This discrepancy may be attributed to lipolysis producing volatile compounds, such as aldehydes, ketones, and alcohols, which are also responsible for the adverse sensory effects (Feng et al., 2014).

4. Conclusions

This study highlighted the potential of starter-assisted fermentation in modifying the lipid profile of avocados, unlocking bioactive FA derivatives with functional and health benefits. This enrichment was shaped by both starter cultures and distinct biochemical profiles of the avocado cultivars. Both cultivars revealed comparable starter adaptation, though acidification capacities varied between starters and cultivars, despite similar organic acid production trends. Free FA analysis indicated Hass as a richer source of FA, while the enzymatic activity in Bacon led to a greater release of free FA, particularly by *L. rhamnosus*

TP5. A total of 57 potential oxidized FA, related to 14 groups of isomeric species and including mono-, di-, and tri-hydroxylated species and one epoxidized FA were further identified. This set of potentially bioactive compounds was generated mainly by major unsaturated FA detected in avocado samples, like oleic, linoleic and α-linolenic ones, but also oxidized derivatives of saturated FA, like palmitic and stearic ones, were recognized. Accordingly, the increase of mono-hydroxylated 18:0 FA induced by *L. casei* FMAC16 had the most significant impact on the hydroxylated FA profile, alongside other less abundant derivatives. Surprisingly, *L. casei* FMAC16 also increased mono-hydroxy 18:1 FA in Bacon avocado. Overall, fermentation enriched the portfolio of bioactive lipid compounds of avocado, offering potential for nutraceutical applications, with minimal undesirable sensory effects influenced by specific species and cultivar employed. Despite the limitations in quantifying each FA derivative, our study provided valuable insights into the evolution of the fermentation process and laid the groundwork for future research aimed at expanding the profile of fermented avocado bioactivities and validating its functional activity through scaled-up fermentation and in vivo studies.

CRediT authorship contribution statement

Ali Zein Alabiden Tlais: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ilario Losito:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Pasquale Filannino:** Writing – review & editing, Methodology, Conceptualization. **Elisabetta Trossolo:** Formal analysis. **Stefano Tonini:** Formal analysis. **Giuliana Garofalo:** Investigation. **Vittorio Farina:** Methodology, Investigation, Formal analysis. **Luca Settanni:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Marco Gobbetti:** Project administration, Funding acquisition. **Raffaella Di Cagno:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Ethical statement

Appropriate protocols to protect the rights and privacy of all participants were implemented during the sensory analysis, ensuring that no coercion was used to participate, that participants were able to withdraw from the study at any time, that study requirements and risks were fully disclosed, that written consent was obtained from participants, and that participants' data were not disclosed without their knowledge. The research did not involve vulnerable populations. Personal data were processed as outlined in the specific data processing information provided, in compliance with EU Regulation 2016/679 and Legislative Decree No. 196 of June 30, 2003.

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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.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.147546.

Data availability

Data will be made available on request.

References

Alfonzo, A., Gaglio, R., Miceli, A., Francesca, N., Di Gerlando, R., Moschetti, G., & Settanni, L. (2018). Shelf life evaluation of fresh-cut red chicory subjected to different minimal processes. *Food Microbiology, 73*, 298–304. https://doi.org/10.1016/j.fm.2018.02.008

Annunziata, G., Arnone, A., Ciampaglia, R., Tenore, G. C., & Novellino, E. (2020). Fermentation of foods and beverages as a tool for increasing availability of bioactive compounds. Focus on short-chain fatty acids. *Foods, 9*(8), 999. https://doi.org/10.3390/foods9080999

Aras, A. K., & Rohman, A. (2025). Global research trends on avocado oil and its fatty acid composition: A bibliometric review. *Journal of Food and Drug Analysis, 33*(3), 179. https://doi.org/10.38212/2224-6614.3548

Ares-Yebra, A., Garabal, J. I., Carballo, J., & Centeno, J. A. (2019). Formation of conjugated linoleic acid by a lactobacillus plantarum strain isolated from an artisanal cheese: Evaluation in miniature cheeses. *International Dairy Journal, 90*, 98–103. https://doi.org/10.1016/j.idairyj.2018.11.007

Avila-Román, J., Talero, E., Alcaide, A., de Los Reyes, C., Zubía, E., García-Mauriño, S., & Motilva, V. (2014). Preventive effect of the microalga *Chlamydomonas debaryana* on the acute phase of experimental colitis in rats. *British Journal of Nutrition, 112*(7), 1055–1064. https://doi.org/10.1017/S0007114514001895

Balcázar-Zumaeta, C. R., Maicelo-Quintana, J. L., Chagas Junior, G. C., Ferreira, N. R., Pinheiro, W. B. D. S., Santos, A. S., … Castro-Alayo, E. M. (2025). Physicochemical exploration of cocoa butter during spontaneous fermentation: A comparative study across three Latin American countries. *Fermentation, 11*(9), 507. https://doi.org/10.3390/fermentation11090507

Bezirtzoglou, E. E. V. (2012). Intestinal cytochromes P450 regulating the intestinal microbiota and its probiotic profile. *Microbial Ecology in Health and Disease, 23*(1), Article 18370. https://doi.org/10.3402/mehd.v23i0.18370

Bhattarai, S., Liou, K., & Oh, T. J. (2013). Hydroxylation of long chain fatty acids by CYP147F1, a new cytochrome P450 subfamily protein from *Streptomyces peucetius*. *Archives of Biochemistry and Biophysics, 539*(1), 63–69. https://doi.org/10.1016/j.abb.2013.09.008

Bhuyan, D. J., Alsherbiny, M. A., Perera, S., Low, M., Basu, A., Devi, O. A., … Papoutsis, K. (2019). The odyssey of bioactive compounds in avocado (*Persea americana*) and their health benefits. *Antioxidants, 8*(10), 426. https://doi.org/10.3390/antiox8100426

Bódalo, A., Bastida, J., Máximo, M. F., Hidalgo, A. M., & Murcia, M. D. (2005). Production of (E) 10-hydroxy-8-octadecenoic acid with lyophilized microbial cells. *Amer. J. Biochem. Biotechnol, 1*(1), 1–4.

Caligiuri, P. M. (2017). Selecting expatriates for personality characteristics: A moderating effect of personality on the relationship between host national contact and cross-cultural adjustment. In *International human resource management* (pp. 33–52). Routledge.

Calonghi, N., Boga, C., Nitti, P., Telese, D., Bordoni, S., Farruggia, G., Asaro, F., Telese, D., Bordoni, S., Grandi, M., Zalambani, C., & Micheletti, G. (2022). Effects of regioisomerism on the antiproliferative activity of hydroxystearic acids on human cancer cell lines. *Molecules, 27*(8), 2396. https://doi.org/10.3390/molecules27082396

Chase, E. M. (1921). Some notes on the enzymes of the avocado. *Calif. Avocado Assoc. Ann. Report, 7*, 52.

Cucuzza, G., Giuffrida, L., Cerro, M., & De Salvo, M. (2024). Assessing the market value of a pioneering fruit orchard when comparison data are missing: The remote segments approach. *Journal of Agriculture and Food Research, 18*, Article 101481. https://doi.org/10.1016/j.jafr.2024.101481

Dreher, M. L., Cheng, F. W., & Ford, N. A. (2021). A comprehensive review of Hass avocado clinical trials, observational studies, and biological mechanisms. *Nutrients, 13*(12), 4376. https://doi.org/10.3390/nu13124376

Dreher, M. L., & Davenport, A. J. (2013). Hass avocado composition and potential health effects. *Critical Reviews in Food Science and Nutrition, 53*(7), 738–750. https://doi.org/10.1080/10408398.2011.556759

Feng, Y., Chen, Z., Liu, N., Zhao, H., Cui, C., & Zhao, M. (2014). Changes in fatty acid composition and lipid profile during koji fermentation and their relationships with soy sauce flavour. *Food Chemistry, 158*, 438–444. https://doi.org/10.1016/j.foodchem.2014.02.147

Filannino, P., De Angelis, M., Di Cagno, R., Gozzi, G., Riciputi, Y., & Gobbetti, M. (2018). How *lactobacillus plantarum* shapes its transcriptome in response to contrasting habitats. *Environmental Microbiology, 20*(10), 3700–3716. https://doi.org/10.1111/1462-2920.14372

Filannino, P., Tlais, A. Z., Morozova, K., Cavoski, I., Scampicchio, M., Gobbetti, M., & Di Cagno, R. (2020). Lactic acid fermentation enriches the profile of biogenic fatty acid derivatives of avocado fruit (*Persea americana* mill.). *Food Chemistry, 317*, Article 126384. https://doi.org/10.1016/j.foodchem.2020.126384

Fiorino, G. M., Tlais, A. Z. A., Losito, I., Filannino, P., Gobbetti, M., & Di Cagno, R. (2023). Triacylglycerols hydrolysis and hydroxy-and epoxy-fatty acids release during lactic fermentation of plant matrices: An extensive study showing inter-and intra-species capabilities of lactic acid bacteria. *Food Chemistry, 412*, Article 135552. https://doi.org/10.1016/j.foodchem.2023.135552

Georgalaki, M. D., Sarantinopoulos, P., Ferreira, E. S., De Vuyst, L., Kalantzopoulos, G., & Tsakalidou, E. (2000). Biochemical properties of *Streptococcus macedonicus* strains isolated from Greek kasseri cheese. *Journal of Applied Microbiology, 88*(5), 817–825. https://doi.org/10.1046/j.1365-2672.2000.01055.x

Hadinia, N., Dovom, M. R. E., & Yavarmanesh, M. (2022). The effect of fermentation conditions (temperature, salt concentration, and pH) with lactobacillus strains for producing short chain fatty acids. *Lwt, 165*, Article 113709. https://doi.org/10.1016/j.lwt.2022.113709

Hou, C. T., Bagby, M. O., Plattner, R. D., & Koritala, S. (1991). A novel compound, 7, 10-dihydroxy-8 (E)-octadecenoic acid from oleic acid by bioconversion. *Journal of the American Oil Chemists Society, 68*, 99–101.

ISO 13299:2003. Sensory analysis — Methodology — General guidance on establishing a sensory profile. International Organization for Standardization.(2003).

Kim, K. R., & Oh, D. K. (2013). Production of hydroxy fatty acids by microbial fatty acid-hydroxylation enzymes. *Biotechnology Advances, 31*(8), 1473–1485. https://doi.org/10.1016/j.biotechadv.2013.07.004

Kim, M., Furuzono, T., Yamakuni, K., Li, Y., Kim, Y. I., Takahashi, H., & Goto, T. (2017). 10-oxo-12 (Z)-octadecenoic acid, a linoleic acid metabolite produced by gut lactic acid bacteria, enhances energy metabolism by activation of TRPV1. *The FASEB Journal, 31*(11), 5036–5048. https://doi.org/10.1096/fj.201700151R

Kishimoto, N., Yamamoto, I., Toraishi, K., Yoshioka, S., Saito, K., Masuda, H., & Fujita, T. (2003). Two distinct pathways for the formation of hydroxy FA from linoleic acid by lactic acid bacteria. *Lipids, 38*(12), 1269–1274. https://doi.org/10.1007/s11745-003-1188-4

Köckritz, A., & Martin, A. (2008). Oxidation of unsaturated fatty acid derivatives and vegetable oils. *European Journal of Lipid Science and Technology, 110*(9), 812–824. https://doi.org/10.1002/ejlt.200800042

Kupnik, K., Primožič, M., Kokol, V., Knez, Ž., & Leitgeb, M. (2023). Enzymatic, antioxidant, and antimicrobial activities of bioactive compounds from avocado (*Persea americana* L.) seeds. *Plants, 12*(5), 1201. https://doi.org/10.3390/plants12051201

LIPID MAPS. (2025). *LIPID MAPS®: A free, open access lipidomics resource*. https://www.lipidmaps.org.

Losito, I., Facchini, L., Valentini, A., Cataldi, T. R., & Palmisano, F. (2018). Fatty acidomics: Evaluation of the effects of thermal treatments on commercial mussels through an extended characterization of their free fatty acids by liquid chromatography–Fourier transform mass spectrometry. *Food Chemistry, 255*, 309–322. https://doi.org/10.1016/j.foodchem.2018.02.073

Marco, S. C., Adrien, S., Isabelle, M., Manuel, V. O., & Dominique, P. (2019). Flash vacuum-expansion process: Effect on the sensory, color and texture attributes of avocado (*Persea americana*) puree. *Plant Foods for Human Nutrition, 74*, 370–375. https://doi.org/10.1007/s11130-019-00749-3

Matuskova, Z., Siller, M., Tunkova, A., Anzenbacherova, E., Zacharova, A., Tlaskalova-Hogenova, H., & Anzenbacher, P. (2011). Effects of *lactobacillus casei* on the expression and the activity of cytochromes P 450 and on the CYP mRNA level in the intestine and the liver of male rats. *Neuroendocrinology Letters, 32*(1), 8–14.

Migliore, G., Farina, V., Guccione, G. D., & Schifani, G. (2018). Quality determinants of avocado fruit consumption in Italy. Implications for small farms. *Calitatea, 19*(163), 148–153.

Migliore, G., Farina, V., Tinervia, S., Matranga, G., & Schifani, G. (2017). Consumer interest towards tropical fruit: Factors affecting avocado fruit consumption in Italy. *Agricultural and Food Economics, 5*(1), 24. https://doi.org/10.1186/s40100-017-0095-8

Miyamoto, J., Igarashi, M., Watanabe, K., Karaki, S. I., Mukouyama, H., Kishino, S., & Kimura, I. (2019). Gut microbiota confers host resistance to obesity by metabolizing dietary polyunsaturated fatty acids. *Nature Communications, 10*(1), 4007. https://doi.org/10.1038/s41467-019-11978-0

Miyamoto, J., Mizukure, T., Park, S. B., Kishino, S., Kimura, I., Hirano, K., & Tanabe, S. (2015). A gut microbial metabolite of linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, ameliorates intestinal epithelial barrier impairment partially via GPR40-MEK-ERK pathway. *Journal of Biological Chemistry, 290*(5), 2902–2918. https://doi.org/10.1074/jbc.M114.610733

Morales, M. T., & Przybylski, R. (2013). Olive oil oxidation. In *Handbook of olive oil: Analysis and properties* (pp. 479–522). Boston, MA: Springer US.

Nagatake, T., & Kunisawa, J. (2019). Emerging roles of metabolites of ω3 and ω6 essential fatty acids in the control of intestinal inflammation. *International Immunology, 31*(9), 569–577. https://doi.org/10.1093/intimm/dxy086

Nascimento, A. P. S., Duarte, M. E. M., Rocha, A. P. T., & Barros, A. N. (2025). Bioactive compounds, technological advances, and sustainable applications of avocado (*Persea americana* mill.): A Critical Review. *Foods, 14*(15), 2746. https://doi.org/10.3390/foods14152746

Nasri, C., Halabi, Y., Hajib, A., Choukri, H., Harhar, H., Lee, L. H., & Tabyaoui, M. (2023). Proximate composition, lipid and elemental profiling of eight varieties of avocado (*Persea americana*). *Scientific Reports, 13*(1), 22767. htt://doi.org/10.1038/s41598-023-50119-y.

Nayeem, M. A. (2018). Role of oxylipins in cardiovascular diseases. *Acta Pharmacologica Sinica, 39*(7), 1142–1154. https://doir.org/10.1038/aps.2018.24.

Obenland, D., Collin, S., Sievert, J., Negm, F., & Arpaia, M. L. (2012). Influence of maturity and ripening on aroma volatiles and flavor in 'Hass' avocado. *Postharvest Biology and Technology, 71*, 41–50. https://doi.org/10.1016/j.postharvbio.2012.03.006

Padayachee, T., Nzuza, N., Chen, W., Nelson, D. R., & Syed, K. (2020). Impact of lifestyle on cytochrome P450 monooxygenase repertoire is clearly evident in the bacterial phylum Firmicutes. *Scientific Reports, 10*(1), 13982. https://doi.org/10.1038/s41598-020-70686-8

Qin, X., & Zhong, J. (2016). A review of extraction techniques for avocado oil. *Journal of Oleo Science, 65*(11), 881–888. https://doi.org/10.5650/jos.ess16063

Quatravaux, S., Remize, F., Bryckaert, E., Colavizza, D., & Guzzo, J. (2006). Examination of lactobacillus plantarum lactate metabolism side effects in relation to the modulation of aeration parameters. *Journal of Applied Microbiology, 101*(4), 903–912. https://doi.org/10.1111/j.1365-2672.2006.02955.x

R Core Team. (2003). *R: A language and environment for statistical computing* (Version 1.6.2). R Foundation for Statistical Computing. https://www.r-project.org/.

Schaffer, B., Wolstenholme, B. N., & Whiley, A. W. (Eds.). (2013). *The avocado: Botany, production and uses*. CABI.

Schütz, R., Rawlings, A. V., Wandeler, E., Jackson, E., Trevisan, S., Monneuse, J. M., & Imfeld, D. (2019). Bio-derived hydroxystearic acid ameliorates skin age spots and conspicuous pores. *International Journal of Cosmetic Science, 41*(3), 240–256. https://doi.org/10.1111/ics.12529

Seixas, N. L., Paula, V. B., Dias, T., Dias, L. G., & Estevinho, L. M. (2025). The effect of incorporating fermented elderberries (*Sambucus nigra*) into bread: Quality, shelf life, and biological enhancement. *Foods, 14*(5), 724. https://doi.org/10.3390/foods14050724

Serra, S., De Simeis, D., Castagna, A., & Valentino, M. (2020). The fatty-acid hydratase activity of the most common probiotic microorganisms. *Catalysts, 10*(2), 154. https://doi.org/10.3390/catal10020154

Servent, A., Boulanger, R., Davrieux, F., Pinot, M. N., Tardan, E., Forestier-Chiron, N., & Hue, C. (2018). Assessment of cocoa (*Theobroma cacao* L.) butter content and composition throughout fermentations. *Food Research International, 107*, 675–682. https://doi.org/10.1016/j.foodres.2018.02.070

Shin, K. C., Seo, M. J., Kang, S. H., & Oh, D. K. (2022). Production of 8, 11-dihydroxy fatty acids from oleic and palmitoleic acids by *Escherichia coli* cells expressing variant 6, 8-linoleate diol synthases from Penicillium oxalicum. *Biotechnology Progress, 38*(5), Article e3267. https://doi.org/10.1002/btpr.3267

Shinto, L. H., Raber, J., Mishra, A., Roese, N., & Silbert, L. C. (2022). A review of oxylipins in Alzheimer's disease and related dementias (ADRD): Potential therapeutic targets for the modulation of vascular tone and inflammation. *Metabolites, 12*(9), 826. https://doi.org/10.3390/metabo12090826

Spiteller, P., & Spiteller, G. (1997). 9-Hydroxy-10, 12-octadecadienoic acid (9-HODE) and 13-hydroxy-9, 11-octadecadienoic acid (13-HODE): Excellent markers for lipid peroxidation. *Chemistry and Physics of Lipids, 89*(2), 131–139. https://doi.org/10.1016/S0009-3084(97)00070-4

Stark, A. H., Crawford, M. A., & Reifen, R. (2008). Update on alpha-linolenic acid. *Nutrition Reviews, 66*(6), 326–332. https://doi.org/10.1111/j.1753-4887.2008.00040.x

Stephen, J., & Radhakrishnan, M. (2022). Avocado (*Persea americana* mill.) fruit: Nutritional value, handling and processing techniques, and health benefits. *Journal of Food Processing and Preservation, 46*(12), Article e17207. https://doi.org/10.1111/jfpp.17207

Szczuko, M., Kikut, J., Komorniak, N., Bilicki, J., Celewicz, Z., & Ziętek, M. (2020). The role of arachidonic and linoleic acid derivatives in pathological pregnancies and the human reproduction process. *International Journal of Molecular Sciences, 21*(24), 9628. https://doi.org/10.3390/ijms21249628

Takeuchi, M., Kishino, S., Park, S. B., Hirata, A., Kitamura, N., Saika, A., & Ogawa, J. (2016). Efficient enzymatic production of hydroxy fatty acids by linoleic acid Δ9 hydratase from *lactobacillus plantar*um AKU 1009a. *Journal of Applied Microbiology, 120*(5), 1282–1288. https://doi.org/10.1111/jam.13088

Tlais, A. Z., Da Ros, A., Filannino, P., Vincentini, O., Gobbetti, M., & Di Cagno, R. (2021). Biotechnological re-cycling of apple by-products: A reservoir model to produce a dietary supplement fortified with biogenic phenolic compounds. *Food Chemistry, 336*, Article 127616. https://doi.org/10.1016/j.foodchem.2020.127616

Tsuchida, K., & Sakiyama, N. (2023). 9-Hydroxyoctadecadienoic acid plays a crucial role in human skin photoaging. *Biochemical and Biophysical Research Communications, 679*, 75–81. https://doi.org/10.1016/j.bbrc.2023.08.044

USDA. (2018). *FoodData Central: Avocados, raw, all commercial varieties*. US Department of Agriculture, Agricultural Research Service.

Vellosillo, T., Martínez, M., Lopez, M. A., Vicente, J., Cascon, T., Dolan, L., & Castresana, C. (2007). Oxylipins produced by the 9-lipoxygenase pathway in Arabidopsis regulate lateral root development and defense responses through a specific signaling cascade. *The Plant Cell, 19*(3), 831–846. https://doi.org/10.1105/tpc.106.046052

Vincent, C., Mesa, T., & Munne-Bosch, S. (2020). Identification of a new variety of avocados (Persea americana mill. CV. Bacon) with high vitamin E and impact of cold storage on tocochromanols composition. *Antioxidants, 9*(5), 403. https://doi.org/10.3390/antiox9050403

Wang, C., & Ajji, A. (2022). Development of a novel ethylene scavenger made up of pumice and potassium permanganate and its effect on preservation quality of avocados. *Journal of Food Engineering, 330*, Article 111101. https://doi.org/10.1016/j.jfoodeng.2022.111101

Wang, X., Zhang, C., & Bao, N. (2023). Molecular mechanism of palmitic acid and its derivatives in tumor progression. *Frontiers in Oncology, 13*, Article 1224125. https://doi.org/10.3389/fonc.2023.1224125

Wu, L., Wei, W., Chen, Z., Shi, X., Wang, D., Chen, X., & Ni, B. J. (2023). Medium chain fatty acids production from anaerobic fermentation of food wastes: The role of fermentation pH in metabolic pathways. *Chemical Engineering Journal, 472*, Article 144824. https://doi.org/10.1016/j.cej.2023.144824

Yamada, M., Takahashi, N., Matsuda, Y., Sato, K., Yokoji, M., Sulijaya, B., & Yamazaki, K. (2018). A bacterial metabolite ameliorates periodontal pathogen-induced gingival epithelial barrier disruption via GPR40 signaling. *Scientific Reports, 8*(1), 9008. https://doi.org/10.1038/s41598-018-27408-y