



# Integrating explainable artificial intelligence (AI) to evaluate fermented-roasted avocado seed powder as a natural antioxidant in beef patties

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## ABSTRACT

This study evaluates fermented–roasted avocado seed powder (ASP) as a sustainable antioxidant for beef patties and integrates explainable artificial intelligence (AI) to provide mechanistic insights into lipid oxidation control. ASP was produced through controlled fermentation with *Lactobacillus plantarum* followed by roasting and incorporated into beef patties (0.95 g/kg). Its performance was compared with butylated hydroxytoluene (BHT) and untreated controls during 10-day refrigerated storage at 4 °C. ASP exhibited the highest initial reducing antioxidant capacity (0.283 mg ascorbic acid equivalents (AAE)/g) measured by cupric reducing antioxidant capacity (CUPRAC) and maintained significantly greater activity than BHT and control throughout storage ( $p < 0.05$ ). Thiobarbituric acid reactive substances (TBARS) analysis showed ASP reduced lipid oxidation by 21% relative to control, approaching BHT efficacy. ASP also improved colour stability and preserved key fatty acids, notably oleic acid. Volatile profiling revealed distinct antioxidant behaviour, with ASP generating Maillard-derived compounds rather than relying on synthetic additives. Shapley Additive Explanations (SHAP) analysis, applied to an extreme gradient boosting model (XGBoost), identified  $\gamma$ -linolenic acid, oleic acid, pentanal, and 2-heptanone as major predictors of oxidative stability, highlighting ASP's broad-spectrum protective effect. These findings demonstrate that ASP not only valorises avocado seed waste but also offers a viable alternative to synthetic antioxidants, supporting sustainability goals. The integration of explainable AI with multivariate analysis provides new understanding of lipid oxidation pathways and antioxidant performance in meat systems.

## 1. Introduction

Beef is a major global source of animal protein, valued for its high biological quality and essential micronutrients like iron, zinc, selenium, and B-group vitamins, which support immune function, cognitive health, and metabolism (Nascimento et al., 2024). Regular inclusion in a balanced diet helps prevent nutrient deficiencies and promotes long-term health (Hawley et al., 2022). Processed forms like patties remain dietary staples worldwide. However, beef's high lipid content makes it prone to oxidative degradation, reducing nutritional quality and consumer acceptability (Lu et al., 2022). Lipid oxidation, a chain reaction affecting flavour, colour, texture, and shelf life, accelerates in comminuted products such as patties due to tissue disruption, which exposes

lipids to oxygen and pro-oxidants, forming aldehydes, ketones, and other compounds that cause rancid flavours and off odours.

Lipid oxidation is a major challenge in meat science, particularly in comminuted products like beef patties, where tissue disruption exposes lipids to oxygen and pro-oxidant factors (Tatijaborwormtham et al., 2022). This oxidative process leads to undesirable changes in flavour, colour, texture, and nutritional value, ultimately reducing shelf life and marketability (Oliveira et al., 2025). Synthetic antioxidants such as butylated hydroxytoluene (BHT) have traditionally been used to retard lipid oxidation effectively. However, increasing regulatory restrictions and consumer demand for clean-label products have prompted a shift towards natural alternatives (Bampidis et al., 2022).

Avocado seeds, typically discarded as waste, are an underutilised

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source of phenolic compounds with strong antioxidant potential, comprising 13–18% of fruit biomass (Ejiofor et al., 2018). Fermented and roasted seeds contain up to 2100 mg GAE/100 g and exhibit potent antioxidant activity (Zhao et al., 2022). Metabolite profiling shows enhanced bioactivity, including cytotoxic effects against Hep G2, MDA-MB-231, and MCF-7 cancer cell lines (Zhao et al., 2023), surpassing conventional sources like berry pomace (Tarasevičienė et al., 2022). Tarasevičienė et al. reported that 1% raspberry pomace significantly reduced lipid oxidation in beef patties, suggesting avocado seeds may offer similar or superior efficacy. Likewise, *Nigella sativa* seed extract lowered TBARS in pork to 0.9 mg of malondialdehyde per kilogram of meat (mg MDA/kg meat) versus 1.3 mg MDA/kg meat in controls, comparable to BHT (0.8 mg MDA/kg meat) after nine days (Chauhan et al. (2018)). Pumpkin seed extract also outperformed BHA in sheep mincemeat (Gadekar et al. (2025)), while *Bersama abyssinica* seed extract showed an  $IC_{50}$  of 0.55  $\mu\text{g/mL}$ , surpassing ascorbic acid ( $IC_{50} = 1.10 \mu\text{g/mL}$ ) (Alemu et al. (2024)). These findings highlight the potential of plant-based antioxidants for meat preservation and warrant exploration of whole processed powders like fermented–roasted ASP.

Natural antioxidants are increasingly studied for meat preservation, yet the use of whole, processed plant powders remains limited. Fermentation and roasting enhance phenolic release and antioxidant activity, but their combined application in avocado seed powder (ASP) for lipid oxidation control in beef patties is unexplored. Valorising avocado seeds support circular economy and sustainability. This study aims to evaluate the antioxidant efficacy of fermented–roasted ASP in inhibiting lipid oxidation in beef patties during refrigerated storage, while examining its effects on fatty acid profiles, volatile compound dynamics, and reducing antioxidant capacity compared to BHT.

Artificial intelligence (AI) and machine learning (ML) have become transformative tools in food science, enabling accurate prediction of quality attributes, optimisation of formulations, and modelling of complex biochemical interactions. However, many ML models function as “black boxes,” providing predictions without revealing the underlying reasoning, which limits scientific interpretability and practical adoption (Gunning et al., 2019; Samek et al., 2017). Explainable Artificial Intelligence (XAI) addresses this challenge by offering interpretable insights into model behaviour, allowing researchers to identify feature contributions and uncover causal relationships. Among XAI approaches, SHapley Additive exPlanations (SHAP) has gained prominence for its strong theoretical foundation in cooperative game theory and its ability to quantify the influence of individual input variables on prediction outcomes (Lundberg et al., 2017). This interpretability is particularly valuable in food systems, where oxidative stability, flavour development, and shelf-life depend on multifactorial interactions among lipids, phenolics, and volatiles.

Recent studies have demonstrated the utility of SHAP and other XAI techniques in food science, including predicting antioxidant activity from phenolic profiles, modelling lipid oxidation pathways, and identifying key drivers of sensory quality (Hashem et al., 2025; Zhao et al., 2025). By integrating XAI into food research, scientists can move beyond empirical correlations towards mechanistic understanding, supporting precision food preservation and sustainable ingredient innovation. Building on this foundation, the present study applies SHAP to elucidate predictors of lipid oxidation and mechanistic differences between avocado seed phenolics (ASP) and the synthetic antioxidant BHT, an approach validated for interpreting complex biochemical datasets (Lu et al., 2025; Yi et al., 2025). Clean-label antioxidants such as rosemary and green tea extracts have shown comparable efficacy to synthetic additives (Inguglia et al., 2023), reinforcing the relevance of this work. By valorising avocado seeds, a major underutilised by-product, this work contributes to sustainable food systems through waste reduction and the development of functional clean-label preservatives, while

SHAP analysis identifies bioactive compounds and volatile markers most closely associated with oxidative stability, providing a scientific basis for targeted application of natural antioxidants in meat products.

## 2. Materials and methods

### 2.1. Sample preparation

#### 2.1.1. Fermentation process

Prior to fermentation, ground avocado seed particles (approximately 0.5–1 mm in size) were prepared for inoculation (Olivado Ltd., Kerikeri, New Zealand). A 1 mL stock culture of *Lactobacillus plantarum* (Fonterra Research Centre, Palmerston North, New Zealand), with a bacterial suspension of approximately  $9.0 \times 10^8$  CFU/mL (equivalent to a 3.0 McFarland standard), was stored in 20% glycerol at  $-80^\circ\text{C}$ . The purity of the culture was verified by Gram staining.

A 1 mL aliquot of the pure stock culture was transferred into 100 mL MRS broth and incubated anaerobically at  $35^\circ\text{C}$  for 24 h. The activated culture (1% v/v) was then used to inoculate avocado seed particles, which were fermented in a  $\text{CO}_2$  incubator (Thermo Fisher Scientific, USA) at  $37 \pm 2^\circ\text{C}$  for five days, based on previous findings (Zhao et al., 2022), reporting maximum *Lactobacillus plantarum* viability at this duration. Two sample types were prepared for analysis: non-fermented (Day 0) and fermented (Day 5) to enable direct comparison.

#### 2.1.2. Drying and roasting

Fermented avocado seed particles were dehydrated at  $60^\circ\text{C}$  for 48 h, then wrapped in foil and roasted at  $127^\circ\text{C}$  for 25 min (Magellano Digital oven), conditions optimised for antioxidant and anticancer activity (Zhao et al., 2022), which demonstrated maximum antioxidant and anticancer activity under this treatment. Two sample types were analysed: non-fermented (Day 0, dried and roasted immediately after inoculation) and fermented (Day 5, after five days of fermentation).

### 2.2. Extraction and analysis of amygdalin using LC-MS.

The extraction method was adapted from Zhao et al. (2025). Briefly, 2 g of powdered sample was mixed with 50 mL of ethanol (100%) and boiled for 100 min. After cooling, the extract was filtered and ethanol removed using a rotary evaporator at  $35^\circ\text{C}$  and 7 mbar. To precipitate amygdalin, 10 mL of diethyl ether was added to the dried residue and vortexed for 1 min at ambient temperature. The precipitate was dissolved in 5 mL of Milli-Q water, and 1.5 mL was centrifuged at 10,000 rpm and  $22^\circ\text{C}$  for 10 min. The supernatant was filtered through a 0.45  $\mu\text{m}$  PTFE syringe filter into an autosampler vial for LC-MS analysis.

LC-MS analysis followed the protocol described by Permal et al. (2023). Quantification used a commercial amygdalin standard (99.12% purity) to generate a calibration curve (0.3125–40 mg/L;  $R^2 = 0.9993$ ). Analyses were performed on an Agilent 1260 Infinity LC system coupled to a 6420 triple quadrupole MS with ESI source (Agilent Technologies, USA). Separation employed a Phenomenex Kinetex Evo C18 column (2.1  $\times$  150 mm, 1.7  $\mu\text{m}$ ). Mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Gradient: 95:5 (A:B) initially; B increased to 12% (1–2 min), 20% (2–6 min), 90% (6–8 min, held for 3 min), then returned to 5% at 13 min. Injection volume was 3  $\mu\text{L}$ ; total run time 23 min. MS conditions: capillary voltage 4 kV, corona current 4  $\mu\text{A}$ , drying gas ( $\text{N}_2$ ) at  $300^\circ\text{C}$  and 10 L/min, nebuliser pressure 30 psi. Positive ion mode with MRM was used. Transitions:  $[\text{M} + \text{H}]^+ m/z$  456  $\rightarrow$  323 (quantifier) and 221 (qualifier), fragmentor voltage 220 V, collision energies 3 eV and 8 eV. Relative ion abundance was 17%, within the 20% acceptance criterion.

Matrix effects (ME) were assessed using the method described by Panuwet et al. (2016), The ME was 98.4%, indicating 1.6% ion

suppression, which is acceptable under FDA guidelines (80–120%). Method validation showed high accuracy (97.5–102.3%), confirming negligible matrix effect. Chromatographic repeatability ( $n = 5$ ), residual standard deviation (RSD), limits of detection (LOD) and quantification (LOQ) were calculated following [Permal et al. \(2020\)](#).

### 2.3. Persin extraction and LC-MS analysis

The persin extraction method was adapted from [Oelrichs et al. \(1995\)](#). Five grams of freeze-dried avocado seed powder were extracted with chloroform for 18 h using a Soxhlet apparatus. The solvent was removed under reduced pressure at 40 °C and 450 mbar, and the residue was re-dissolved in ethyl acetate and diluted with methanol to 20 µg/mL. Persin quantification was performed by LC-MS using a calibration curve (0.125–2 µg/mL;  $R^2 = 0.9986$ ) prepared from isolated persin.

Analysis was carried out on an Agilent 1260 Infinity LC system coupled to a 6420 triple quadrupole MS with ESI source. Separation used an Agilent Poroshell 120 EC-C18 column (2.1 × 50 mm, 2.7 µm). The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Gradient: 60:40 (A:B) from 0 to 1 min; B increased to 65% and held for 3 min; 90% from 4 to 5 min; then returned to 40% at 6 min. Injection volume was 1 µL; total run time 11.5 min.

### 2.4. Beef patty preparation

Cubed boneless beef chuck was sourced from a local butcher (The Meat Room, Auckland, New Zealand) was ground and divided into three groups: Control (1000 g beef), BHT-treated (0.1 g BHT/kg), and ASP-treated (0.95 g fermented-roasted ASP/kg, equivalent to 100 mg BHT/kg based on cupric reducing antioxidant capacity (CUPRAC) assay calibration,  $R^2 = 0.9999$ ). Patties (25 g) were shaped, packed in PVC film-covered polypropylene containers, and stored at 4 °C for 10 days with daily analyses. For each analysis day, three replicate patties per treatment group were prepared. All remaining samples were vacuum-sealed and stored at -20 °C.

Total viable counts (TVC) were determined according to ISO 4833-1:2013. Briefly, 1 g of each patty sample was homogenised with 9 mL of sterile peptone water (0.1% w/v) in a test tube for 2 min. Then serially make a tenfold dilution in 0.1% peptone from 10 to 1 to 10<sup>-7</sup>. Take 0.1 mL solution from each dilution and spread plate on Plate Count Agar (PCA) and incubated at 35 °C for 36 h. Colonies were counted and expressed as log<sub>10</sub> CFU/g. Analyses were performed in triplicate for each treatment and storage day (0, 3, 6, 9, 10 days).

### 2.5. Measurement of pH

pH was determined using ISO 2917:1999 (modified by [Kitundu et al. \(2021\)](#)). Briefly, 1 g of patty was homogenised with 9 mL deionised water (Ultra-Turrax, Jankee & Kundel) and measured with a calibrated Eutech 700 pH meter (Thermo Fisher) using pH 4.0 and 7.0 buffers. Analyses were performed in triplicate.

### 2.6. Colour measurement

The surface colour of beef patties was measured using a NIX colour sensor (Nix Sensor Ltd., Canada). Before each measurement session, the instrument was calibrated using the white and matte black reference tiles. Colour readings were performed under standard conditions with illuminant D50 and 2° standard observer. For each treatment group, six readings per patty were taken across three patties per treatment, and five independent replicates were used for beef patties. Colour values

were expressed as CIE L\* (lightness), a\* (red-green), and b\* (yellow-blue) coordinates.

### 2.7. Cupric reducing antioxidant capacity (CUPRAC) analysis

The CUPRAC antioxidant analysis was performed according to the method outlined by [Zhao et al. \(2022\)](#). The assay was conducted at 450 nm using ascorbic acid standards, and results were expressed as mg of ascorbic acid equivalents per gram. While the starting material's phenolic content is typically characterized in Gallic Acid Equivalents (GAE), AAE was selected for this assay to specifically quantify the electron-donating antioxidant capacity relevant to meat preservation. To determine the appropriate dosage for beef patties, a separate standard curve was generated using BHT, establishing that 0.95 g of fermented-roasted ASP possesses antioxidant capacity equivalent to 100 mg of BHT. All samples were analysed in triplicate.

The CUPRAC assay was selected for three key reasons. First, it quantifies both hydrophilic and lipophilic antioxidants, making it suitable for complex natural extracts such as fermented-roasted avocado seed powder (ASP) ([Apak et al., 2004](#); [Apak et al., 2008](#)). Second, CUPRAC operates at near-neutral pH (~7.0), which better reflects physiological and food system conditions compared to FRAP, which functions at strongly acidic pH (~3.6) and can suppress the activity of lipophilic antioxidants ([Apak et al., 2004](#); [Prior et al., 2005](#)). Third, CUPRAC uses a single-electron transfer mechanism in aqueous-alcoholic media, avoiding solubility issues and steric hindrance that often affect radical-based assays such as DPPH ([Apak et al., 2007](#); [Prior et al., 2005](#)). While complementary assays like DPPH or FRAP could provide additional mechanistic insights, CUPRAC offers a robust and physiologically relevant measure of total reducing capacity, which is particularly important for lipid-rich food systems such as beef patties. In addition, previous studies have demonstrated that fermented and roasted avocado seed powder exhibits significant antioxidant activity, primarily attributed to its enriched phenolic profile and Maillard reaction products ([Zhao et al., 2022](#); [Zhao et al., 2023](#)). These findings support the use of CUPRAC, which effectively measures both hydrophilic and lipophilic antioxidants present in ASP.

It should be noted that while the CUPRAC assay provides a comprehensive measure of total reducing capacity under near-physiological conditions, it does not directly quantify other antioxidant mechanisms such as radical scavenging via hydrogen atom transfer (e.g., as assessed by DPPH) or metal chelation, which may also contribute to lipid oxidation inhibition in meat systems. Therefore, the CUPRAC values are interpreted here as a robust indicator of the overall reducing antioxidant potential of ASP.

### 2.8. Measurement of lipid oxidation

Lipid oxidation in beef patties was quantified by measuring malondialdehyde (MDA) using a modified thiobarbituric acid reactive substances (TBARS) assay ([Ahn et al., 1998](#)). Briefly, 3 g of minced meat was homogenised with 9 mL of ice-cold Milli-Q water at 14,000 ×g for 30 s, followed by centrifugation at 5000 ×g for 5 min at 4 °C. To prevent further oxidation, 1 mL of the supernatant was mixed with 50 µL of 7.2% butylated hydroxytoluene (BHT). Subsequently, 2 mL of TBA-TCA reagent (15% trichloroacetic acid and 20 mM thiobarbituric acid) was added, vortexed, and incubated at 90 °C for 15 min to develop the characteristic pink chromogen. After cooling on ice, samples were centrifuged at 10,000 ×g for 5 min at 4 °C, and absorbance of the clear supernatant was measured at 531 nm against a blank (1 mL Milli-Q water +2 mL TBA-TCA reagent). A standard curve was prepared using tetraethoxypropane (TEP), which hydrolyses to MDA under acidic

conditions, with working solutions ( $0\text{--}20 \times 10^{-6}$  M) processed identically to samples. MDA concentrations were calculated from the standard curve as shown below:

$$\text{TBARS (mg MDA/kg meat)} = \frac{\left[ C2 \left( \frac{\text{mol}}{\text{L}} \right) \times \text{Molar mass} \left( \frac{\text{g}}{\text{mol}} \right) \times V1 (\text{L}) \times \text{*dilution factor} \times 10^3 \right] \text{mg}}{\left[ \text{Sample weight (g)} \times 10^{-3} \right] \text{kg}}$$

C2 = MDA concentration (M) calculated from standard curve = concentration in the homogenate.

\*Dilution factor: applied when additional dilution was involved, otherwise use 1.

Final TBARS values, expressed as milligrams of malondialdehyde per kilogram of meat (mg MDA/kg meat), accounted for dilution factors and subsampling ratios.

## 2.9. Determination of fatty acids

Total fatty acid content was determined using a transesterification method coupled with gas chromatography–mass spectrometry (GC–MS) (Lepage et al., 1986). Approximately 20 mg of dried sample (or 100  $\mu\text{L}$  of wet sample/blank) was mixed with 20  $\mu\text{L}$  of surrogate solution (tridecanoic acid) and 2 mL of extraction solution (methanol and internal standard ISTD A in a 1:1 ratio). Acetyl chloride (200  $\mu\text{L}$ ) was added dropwise under nitrogen to prevent oxidation. The mixture was vortexed, sealed with Teflon-lined caps, and incubated at 100  $^{\circ}\text{C}$  for 1 h to complete transesterification.

The protocol incorporated three internal standards for analytical accuracy: ISTD A (128  $\mu\text{M}$  nonadecanoic acid in toluene) for recovery calculations, ISTD B (200  $\mu\text{g}/\text{mL}$  biphenyl in toluene) for diluted samples, and ISTD C (400  $\mu\text{g}/\text{mL}$  methyl nonadecanoate, C19:0 methyl ester) for undiluted samples. Prior to extraction, 20  $\mu\text{L}$  of a surrogate solution containing 400  $\mu\text{g}/\text{mL}$  tridecanoic acid was added to all samples to monitor transesterification efficiency. After cooling, 4 mL of 6% potassium carbonate solution was added to neutralise the reaction, followed by centrifugation (2500 rpm, 5 min) to separate phases. The organic layer was collected, adjusted to 1 mL with toluene, and diluted as required ( $10\times$  or  $100\times$ ) based on calibration range.

GC–MS analysis was performed using an Agilent 6890 N system equipped with a DB-FastFAME capillary column (20 m  $\times$  0.18 mm  $\times$  0.2  $\mu\text{m}$ ) and a 5973 mass spectrometer. The oven programme was: 85  $^{\circ}\text{C}$  (0.74 min), ramp to 175  $^{\circ}\text{C}$  at 43.84  $^{\circ}\text{C}/\text{min}$ , then to 185  $^{\circ}\text{C}$  at 6.75  $^{\circ}\text{C}/\text{min}$  (hold 3 min), followed by a final ramp to 280  $^{\circ}\text{C}$  at 40  $^{\circ}\text{C}/\text{min}$  (hold 2 min). Selective ion monitoring (SIM) mode was used for quantification, with specific quantifier/qualifier ions assigned to each fatty acid methyl ester (FAME) (e.g.,  $m/z$  74 for saturated fatty acids). Methyl nonadecanoate (ISTD C) and biphenyl (ISTD B) served as internal standards for undiluted and diluted samples, respectively. Total fatty acid concentrations were calculated using calibration curves generated from CRM47885 (certified reference material) FAME standards.

## 2.10. Volatile compounds analysis

Volatile compounds were analysed by solid-phase microextraction – gas chromatography – mass spectrometry (SPME–GC–MS) (Ma et al., 2016) to semi-quantitatively compare profiles across treatments and storage days (0, 1, 3, 5, 7, 9, 10). For each batch, 1 g of sample was sealed in 10 mL amber vials and extracted using a CAR/DVB/Carboxen fibre (Supelco) after incubation at 50  $^{\circ}\text{C}$  for 30 min and 15 min extraction. Desorption occurred in an Agilent 7890 A GC with 5977 A

MSD (electron ionization (EI) mode). Separation used DB-FATWAX UI and DB-5MS UI columns (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ) with helium (1.1 mL/min) in splitless mode. Oven programme: 40  $^{\circ}\text{C}$  (2 min), ramp 8  $^{\circ}\text{C}/\text{min}$  to 240  $^{\circ}\text{C}$ , hold 3 min. MSD settings: transfer line 250  $^{\circ}\text{C}$ , ion source

250  $^{\circ}\text{C}$ , quadrupole 150  $^{\circ}\text{C}$ ; scan range 38–450 Da. Compounds were identified via Kovats indices and NIST14 library.

## 2.11. Statistical analysis

Statistical analyses were performed using XLSTAT 2024 (Lumivero, Colorado, USA) and Python (version 3.11) (Python Software Foundation, USA). Data from three independent production batches, each representing a separate preparation run of beef patties, were analysed using a mixed-effects ANOVA model. The model included two fixed factors: treatment (control, BHT-treated, ASP-treated) and storage time (Days 0–10), along with their interaction. Sample was included as a random factor to account for variability. Post hoc comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test to reduce false positives across multiple comparisons, following a significant F-test, with differences considered significant at  $p < 0.05$ . Results are expressed as mean  $\pm$  standard deviation (SD) based on triplicate measurements per batch. ANOVA outputs included F-values and  $p$ -values for main effects and interactions. Multiple Factor Analysis (MFA) was performed on fatty acids, antioxidant activity (CUPRAC), and TBARS values datasets to explore clustering trends among treatments over time.

To interpret complex multivariate relationships between fatty acids, volatile compounds and lipid oxidation markers, SHAP (Shapley Additive Explanations) analysis was applied using Python with the SHAP library (<https://github.com/shap/shap>). A XGBoost was trained to predict TBARS values, and SHAP values were computed to quantify the contribution of individual features (Zhao et al., 2025). This approach enabled identification of key predictors and their directional influence on oxidative stability.

## 3. Results and discussion

### 3.1. Amygdalin and persin content in ASP

This study focused on analysing amygdalin and persin in avocado seed products due to their potential toxicity and food safety implications. Unlike many other plant-based foods, avocado seeds contain persin, a toxin known to cause adverse effects in livestock, raising concerns about its safety for human consumption (Butt et al., 2006). In addition, amygdalin, a cyanogenic glycoside present in certain seeds, can release toxic hydrogen cyanide upon degradation (Bolarinwa et al., 2014).

#### 3.1.1. Amygdalin content

Amygdalin was detected in the fermented and roasted ASP at a concentration of  $7.7 \times 10^{-5}$  mg/kg (equivalent to  $4.54 \times 10^{-6}$  mg/kg HCN). This level is substantially below the EU regulatory limit of 20 mg/kg HCN (approximately 338.8 mg/kg amygdalin) (European, 2022). Furthermore, considering the recommended incorporation rate of 0.95 g/kg in beef patties, the estimated daily exposure to cyanide for a 60 kg adult consuming 300 g of the product would be approximately  $2.15 \times 10^{-11}$  mg/kg body weight. This value is over nine orders of magnitude

lower than the Acceptable Daily Intake (ADI) of 0.05 mg/kg body weight established by the FAO/WHO, confirming that the residual cyanogenic compounds pose no health risk to consumers.

### 3.1.2. Persin content

The persin standard used for quantifying persin concentration was extracted from avocado leaves following a modified procedure described by Permal et al. (2023). The fermented and roasted ASP contained  $0.83 \pm 0.12$  mg/g of persin. This level is slightly higher than the amounts typically found in avocado flesh, which ranges from 0.018 to 0.47 mg/g on a dry weight basis (Rodríguez-López et al., 2015), but significantly lower than the  $1.71 \pm 0.38$  mg/g ( $p < 0.001$ ) detected in air-dried avocado seeds at Day 0. Given that persin remains stable at 130 °C (Permal et al., 2023), the observed ~54% reduction after fermentation likely reflects interactions with other compounds rather than thermal degradation. Toxicological benchmarks from animal studies indicate clear safety limits: Oelrichs et al. (1995) reported necrosis of mammary gland epithelium in mice at 60–100 mg/kg, myocardial damage and hydrothorax above 100 mg/kg, and lethality at 200 mg/kg. Since persin levels in fermented-roasted ASP were far below these thresholds, the powder is considered safe for human consumption.

## 3.2. Beef patties colour and pH analysis

### 3.2.1. Colour analysis

The colour changes in beef patties treated with different additives during refrigerated storage are summarised in the supplementary section 1.1.1 (Table S1). During refrigerated storage, both BHT and ASP treatments improved colour stability compared with the control, particularly in preserving redness ( $a^*$ ) and yellowness ( $b^*$ ). ASP-treated patties maintained slightly higher lightness ( $L^*$ ) values, while BHT showed more consistent retention of  $a^*$  and  $b^*$  values. All samples remained above the consumer-acceptable lightness threshold ( $L^* > 39$ ), indicating that both additives effectively preserved visual quality throughout storage.

### 3.2.2. pH changes

The pH values of beef patties from three treatment groups (Control, BHT, and ASP) showed a significant pH decline ( $p < 0.0001$ ) over a 10-day refrigerated storage period, as shown in the supplementary section 1.1.2 (Table S2). ASP exhibited the greatest pH reduction due to fermentation-derived acids. These trends align with previous findings linking acidification to microbial activity, antioxidant effects, and lactic acid contribution from ASP.

### 3.2.3. Microbiological stability during storage

In addition to lipid oxidation, fermented-roasted ASP conferred measurable microbiological benefits during refrigerated storage. Over 10 days, ASP-treated patties exhibited lower TVC, reaching ~4.85 log<sub>10</sub> CFU/g by Day 3 and stabilising at ~5.8 log<sub>10</sub> CFU/g by Day 10, whereas control and BHT patties remained higher at ~6.0–6.4 log<sub>10</sub> CFU/g (Fig. S1; Supplementary Table S3). In fresh beef, consumer-relevant spoilage likelihood increases sharply as aerobic counts approach ~6–7 log<sub>10</sub> CFU/g, with multiple studies associating ~7 log<sub>10</sub> CFU/g with practical spoilage thresholds and rejection (Frink et al., 2025; Holman et al., 2021). The lower TVC trajectory in ASP patties coincided with a pH decline to ~5.30 by Day 10, consistent with the antimicrobial action of fermentation-derived organic acids (e.g., lactic, acetic), which reduce extracellular pH, destabilise microbial membranes, and potentiate other inhibitory metabolites (bacteriocins, H<sub>2</sub>O<sub>2</sub>, diacetyl) produced by lactic acid bacteria (LAB) (Banicod et al., 2025; Kaveh et al., 2023; Sivamaruthi et al., 2025). These mechanisms are well-documented in meat systems where LAB-based cultures can suppress spoilage organisms and complement antioxidant functions, thereby extending shelf-life without adverse sensory impact when properly dosed (Hernández Figueroa et al., 2024; Hernández-Aquino et al., 2019). Taken together, the ASP-

associated reduction in TVC below levels commonly linked to consumer spoilage judgements, along with acidification and oxidative stability, supports our shelf-life extension claim. Nevertheless, future work can incorporate growth-curve modelling and targeted spoilage community profiling (e.g., *Pseudomonas* spp.) to further elucidate antimicrobial contributions of ASP under varied packaging and temperature regimes (Niu et al., 2025; Yang et al., 2023).

## 3.3. Reducing antioxidant capacity (CUPRAC)

The reducing antioxidant capacity, as determined by the CUPRAC assay, is summarised in the supplementary section 1.1.3 (Table S4). Results showed significant differences among treatments during 10-day storage ( $p < 0.0001$ ). ASP-treated patties exhibited the highest reducing antioxidant capacity throughout, starting at 0.283 mg AAE/g on Day 0 and remaining at 0.191 mg AAE/g by Day 10, compared with BHT (0.143 → 0.063 mg AAE/g) and control (0.112 → 0.044 mg AAE/g). Although activity declined over time in all groups, ASP consistently outperformed BHT and control, confirming its potential as an effective natural antioxidant for meat preservation.

## 3.4. Lipid oxidation of beef patties during refrigerated storage

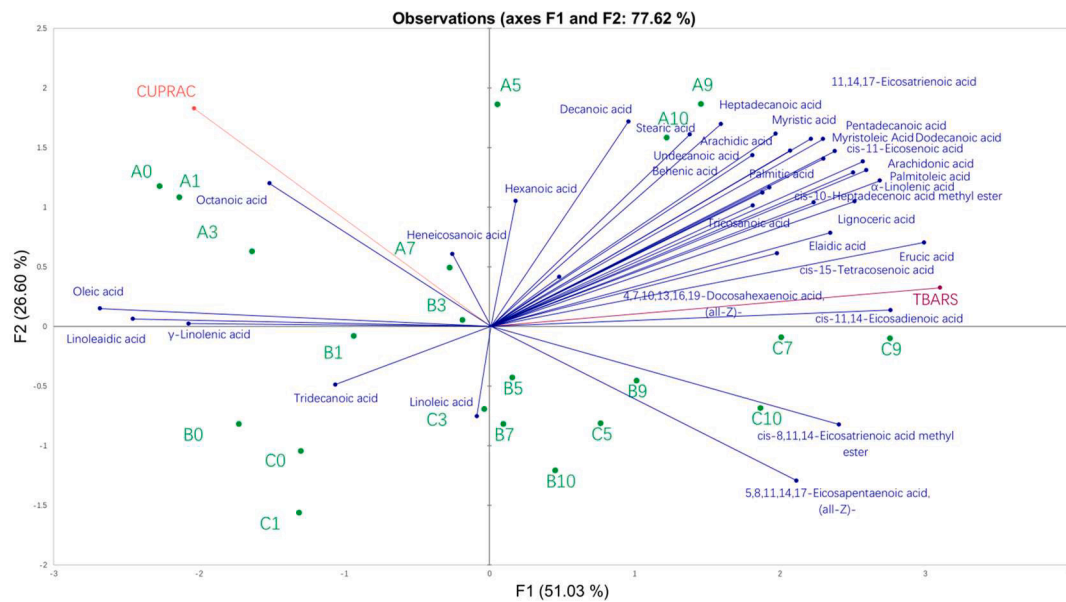
Results showing inhibition of lipid oxidation by ASP and BHT are summarised in the supplementary section 1.1.4 (Fig. S2). TBARS analysis showed that both BHT and ASP significantly inhibited lipid oxidation compared with the control, keeping values below the 2 mg MDA/kg meat sensory threshold. After 10 days, TBARS reached 2.147 mg MDA/kg meat in the control, 1.402 mg MDA/kg meat in BHT, and 1.807 mg MDA/kg meat in ASP, indicating a 21% reduction by ASP. Although BHT was more effective, ASP provided substantial oxidative stability and represents a promising natural alternative for clean-label meat preservation.

## 3.5. Changes in fatty acids of beef patties during refrigerated storage

The mixed-effects ANOVA shown in supplementary section (Table S5) demonstrated significance of storage day for octanoic acid ( $p = 0.001$ ), undecanoic acid ( $p = 0.005$ ), dodecanoic acid ( $p = 0.003$ ), oleic acid ( $p < 0.0001$ ), and gamma linolenic acid ( $p = 0.002$ ). Treatment effects were significant for octanoic acid ( $p = 0.040$ ) and oleic acid ( $p = 0.045$ ), while all other fatty acids exhibited no significant treatment differences ( $p \geq 0.107$ ).

Across all treatments, medium chain fatty acids showed clear time-related trends. Octanoic acid decreased progressively during storage, with concentrations at Day 10 significantly lower than Day 0 ( $p = 0.001$ ). Undecanoic acid and dodecanoic acid also declined significantly over time ( $p = 0.005$  and  $p = 0.003$ ), indicating susceptibility to oxidative or hydrolytic changes during refrigerated storage. The dominant monounsaturated fatty acid, oleic acid, showed a significant reduction with storage ( $p < 0.0001$ ), falling sharply after Day 3 and remaining at substantially lower levels by Day 10 compared with Day 0. Gamma linolenic acid followed a similar pattern, with concentrations significantly lower at late storage than early storage ( $p = 0.002$ ). In contrast, linoleic acid and DHA remained relatively stable throughout the 10-day period, with no significant day-to-day differences.

The observed decrease in medium-chain saturated fatty acids (SFAs; e.g., C8:0, C10:0, C12:0) during refrigerated storage is most plausibly attributed to lipolytic hydrolysis of triacylglycerols rather than direct oxidative loss. Post-mortem and storage conditions allow endogenous and, to a lesser extent, microbial lipases to hydrolyse neutral lipids, liberating free fatty acids (FFAs) (Tatijaborwormtham et al., 2022; Wood et al., 2008). Once released, medium-chain FFAs are more volatile and mobile than their esterified counterparts and can be lost through volatilisation or undergo secondary transformations, resulting in a measurable decline in their quantified concentrations in the fat fraction (Wood



**Fig. 1.** Multiple Factor Analysis (MFA) biplot showing fatty acids, antioxidant activity (CUPRAC), TBARS values, and beef patty samples across the storage period. The first two dimensions (F1 and F2) together explain 77.62% of the total variance. \*A: ASP-treated; B: BHT-treated; C: Control. Storage days: 0, 1, 3, 5, 7, 9, 10.

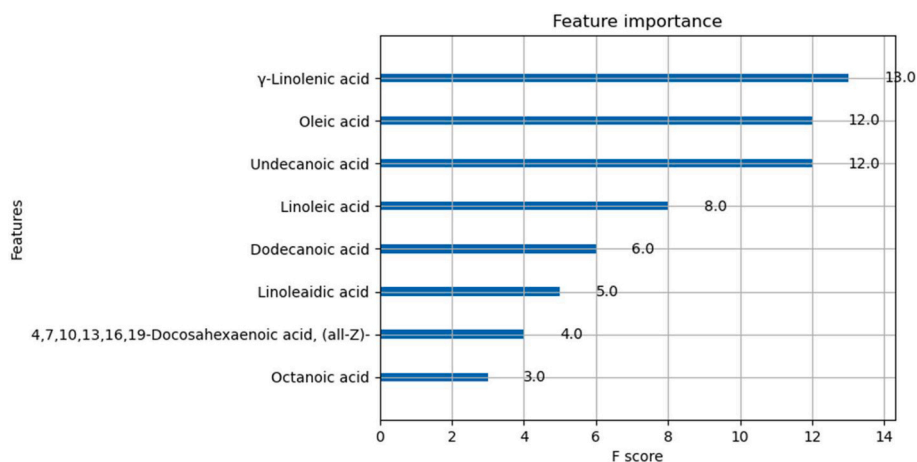
et al., 2008). This hydrolysis-driven pathway is mechanistically distinct from radical-mediated lipid oxidation, which predominantly targets polyunsaturated fatty acids (PUFAs) and is typically evidenced by increases in TBARS and the formation of lipid-derived aldehydes and ketones (Eleftheriadou et al., 2010; Wood et al., 2008).

Treatment effects were evident for octanoic acid and oleic acid. ASP-treated samples retained significantly higher concentrations of octanoic acid compared with BHT and control across several time points ( $p = 0.040$ ). Similarly, ASP maintained higher oleic acid levels than BHT and, at some late-storage points, control ( $p = 0.047$ ), despite the overall downward trend across all treatments. These findings indicate that ASP provided modest but statistically significant protection against fatty acid degradation during storage.

These results confirm that storage time is the primary factor influencing fatty acid composition, with significant reductions in medium chain fatty acids and oleic acid as storage progresses, while ASP treatment confers measurable retention of octanoic and oleic acids compared with BHT and control.

**3.5.1. Multiple factor analysis (MFA) of fatty acid profiles, antioxidant capacity, and lipid oxidation during refrigerated storage**

MFA was applied to the integrated dataset comprising individual fatty acid proportions, CUPRAC antioxidant capacity, and TBARS values across the 10-day refrigerated storage period at 4 °C. The first two dimensions explained 77.62% of the total variance (F1: 51.03%; F2: 26.59%) (Fig. 1). F1 accounted for most of the variance and reflected the universal progression of lipid oxidation observed across all treatments. Samples from days 0 and 1 (A0, A1, B0, B1, C0, C1) clustered tightly on the negative side of F1 and were strongly associated with high proportions of polyunsaturated fatty acids (PUFAs), including linoleic (C18:2n-6),  $\gamma$ -linolenic, arachidonic (C20:4n-6), and longer-chain omega-3 fatty acids, together with elevated CUPRAC antioxidant capacity. Conversely, late-storage samples, particularly the control group at days 7–10 (C7, C9, C10), were located at the extreme positive end of F1 and were closely correlated with elevated TBARS values and increased relative abundance of saturated (e.g., palmitic, stearic) and monounsaturated (e.g., oleic) fatty acids, indicative of extensive PUFA degradation and secondary lipid oxidation. This temporal gradient along F1 was corroborated by significant storage-time effects (two-way ANOVA, Table S5) on most fatty acids ( $P < 0.05$ ), including octanoic (P



**Fig. 2.** Feature importance score of fatty acids for TBARS.

= 0.001), undecanoic ( $P = 0.005$ ), dodecanoic ( $P = 0.003$ ), and oleic acids ( $P < 0.0001$ ). F2 explained treatment-specific variation orthogonal to the general oxidation process. Samples treated with ASP (group A) were positioned in the positive F2 region throughout storage, with A0–A10 forming a clear upward trajectory. This separation was driven principally by the sustained retention of very-long-chain omega-3 PUFAs, most notably 4,7,10,13,16,19-docosahexaenoic acid (DHA, all-Z-), and, to a lesser extent, by decanoic and heptadecanoic acids and residual CUPRAC antioxidant capacity. In contrast, late-storage control samples (C5–C10) and, to a lesser degree, BHT-treated samples (B7–B10) were displaced towards the negative F2 region, reflecting greater depletion of these highly unsaturated fatty acids and reduced residual antioxidant activity.

Although all treatments progressed along the oxidation gradient represented by F1, only the ASP-containing samples retained a fatty acid profile high in nutritionally important long-chain omega-3 PUFAs and maintained higher antioxidant capacity throughout the storage period. BHT afforded intermediate protection, whereas the control showed the most rapid deterioration in both dimensions. The pronounced segregation of ASP-treated samples along F2 demonstrates a distinct and superior protective effect of the ASP compared with synthetic BHT, particularly in preventing oxidation of the most susceptible and biologically valuable PUFAs.

### 3.5.2. SHAP analysis of fatty acid contributions to TBARS prediction

To complement conventional statistical analysis, SHAP was applied to quantify the contribution and directional effect of individual fatty acids on lipid oxidation (TBARS), providing insight into complex interactions between fatty acids and oxidative stability. Fig. 2 shows XGBoost feature importance rankings based on F-score, indicating  $\gamma$ -linolenic (C18:3), oleic (C18:1), and linoleic (C18:2) acids as the most influential predictors of TBARS. Other contributors included erucic

(C22:1) and palmitoleic (C16:1), while saturated fatty acids (octanoic, undecanoic, dodecanoic), despite significant day effects in ANOVA, had negligible practical impact due to low concentrations ( $<0.2 \mu\text{g}/\text{mg}$ ; Table S5) and inherent stability. These rankings suggest antioxidant strategies should prioritise preserving highly unsaturated fatty acids, which are most susceptible to oxidation during storage.

A gradient boosting model (Fig. 3) was trained to predict TBARS, and SHAP values identified fatty acids with the strongest protective effect (negative SHAP values):  $\gamma$ -linolenic, undecanoic, oleic, dodecanoic, and octanoic acids, all showing significant changes in ANOVA (Table S5).  $\gamma$ -Linolenic acid declined rapidly and was undetectable by Day 10, confirming its high susceptibility (Kovalík et al., 2018). On Day 5, BHT-treated patties retained  $\sim 3\times$  more  $\gamma$ -linolenic acid than ASP, while controls retained only 0.96% of BHT levels ( $p < 0.001$ ). This early preservation of  $\gamma$ -linolenic acid by the synthetic antioxidant BHT is consistent with the observation that effective antioxidants can prevent the oxidative loss of labile PUFAs, as previously reported for  $\beta$ -carotene-enriched chicken meat (Marcinčák et al., 2018). Oleic acid, the third-ranked protective fatty acid, showed significant storage-time ( $p < 0.0001$ ) and Treatment  $\times$  Day interaction ( $p < 0.0001$ ; Table S5). By Day 10, ASP-treated patties retained 14.15  $\mu\text{g}/\text{mg}$  oleic acid versus 7.68 and 9.69  $\mu\text{g}/\text{mg}$  for control and BHT. This good retention by ASP supports earlier evidence that effective antioxidant systems stabilise monounsaturated fatty acids during refrigerated storage (Ansorena et al., 2004; Santos et al., 2021).

SHAP dependence plots (Fig. 4) further confirmed these effects. For  $\gamma$ -linolenic acid (Fig. 4A), higher concentrations consistently lowered predicted TBARS (SHAP  $\approx -0.25$ ), matching its rapid decline ( $p = 0.002$ ; Table S5) and ASP's superior retention. Oleic acid (Fig. 4B) showed an almost linear protective effect, with SHAP  $\approx -0.12$  at high levels, consistent with ASP maintaining near-fresh proportions. Linoleic acid (Fig. 4C) exhibited threshold behaviour: SHAP values dropped to  $-0.05$  at 1.5–3.0 units but levelled off beyond, explaining why ANOVA found no time effect ( $p = 0.282$ ) despite its protective role in the ML model.

Fig. 4B shows an almost linear relationship: higher oleic acid content consistently lowered predicted TBARS, with SHAP values reaching about  $-0.12$  at the upper range. This aligns with the significant Treatment  $\times$  Day interaction for oleic acid in ANOVA ( $p = 0.045$ ; Table S5). ASP was the only treatment that maintained oleic acid levels close to fresh-meat proportions, limiting the relative increase that occurs as other unsaturated fatty acids degrade. This retention supports previous findings that effective antioxidant systems stabilise MUFAs during refrigerated storage (Ansorena et al., 2004; Santos et al., 2021).

Fig. 4C presents linoleic acid (C18:2n-6). Although ANOVA showed no overall time effect ( $p = 0.610$ ), the SHAP plot reveals a clear benefit at lower concentrations ( $\approx 1.5$ – $3.0$  units), where SHAP values drop to around  $-0.05$ . Beyond this range, the effect plateaus and points scatter near zero. This threshold behaviour explains why conventional linear tests detected no significant change, yet the machine-learning model identified a protective role for linoleic acid within typical concentration ranges.

## 3.6. Volatile compounds analysis in beef patties during storage

### 3.6.1. ANOVA results of volatile compounds across storage time and treatments

The mixed-effects ANOVA (Table S6) revealed significant time-dependent changes in most volatile compounds, with treatment effects modulating the magnitude and timing of these shifts. Among the ketones, 2-nonanone exhibited a pronounced increase during storage ( $p < 0.0001$ ), reaching significantly higher concentrations at Day 9 compared with Day 0 in ASP-treated samples, before declining slightly at Day 10. In contrast, both BHT-treated and control samples showed progressive accumulation, with Day 10 levels significantly higher than early storage ( $p < 0.0001$ ). Similarly, 2-heptanone increased over time ( $p = 0.019$ ),

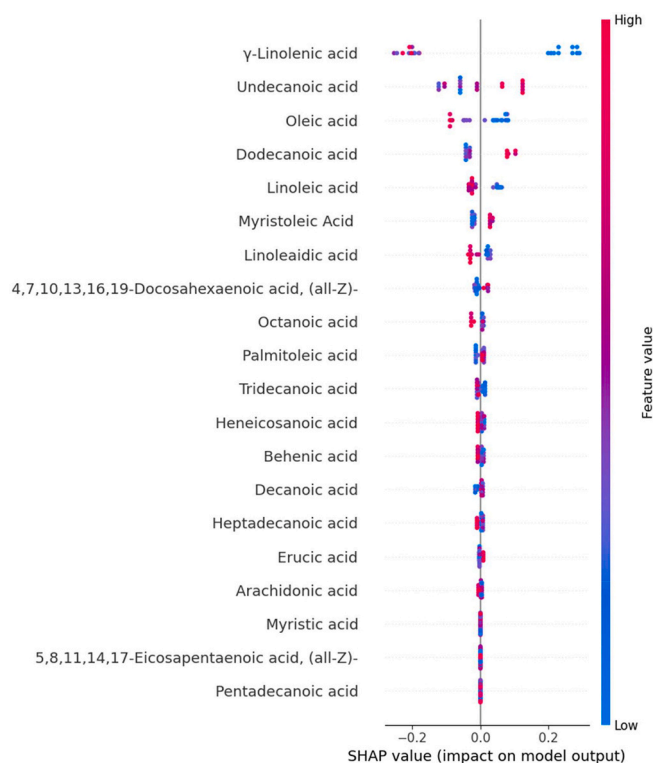
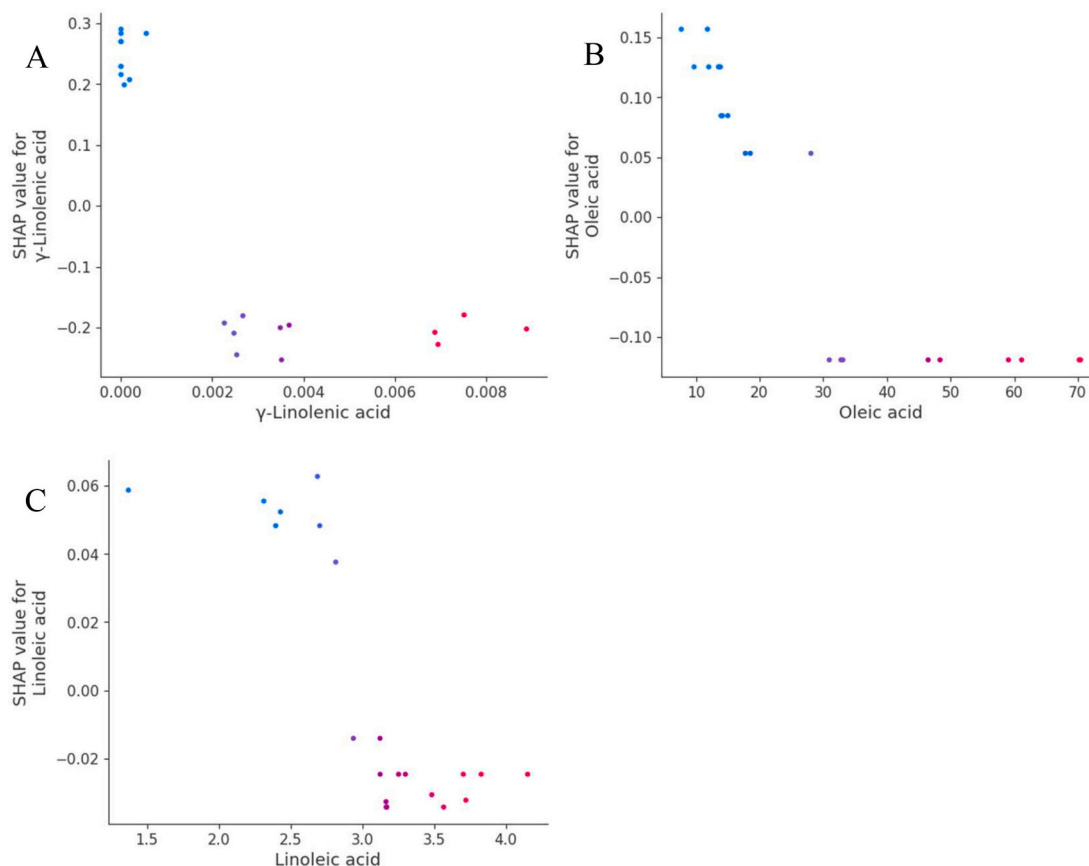


Fig. 3. Magnitude and direction of the effects of fatty acids on the TBARS indicated by SHAP values. In each instance, the given explanation is represented by a single dot on each polyphenol column. The y-coordinate of the dot indicates the direction and magnitude of the effect of its volatile compounds' concentration on the SHAP value.



**Fig. 4.** SHAP-dependence plots displaying the top candidates for key fatty acids influencing TBARS. The vertical axis is the effect of each instance on the predicted value. The horizontal axis is the FAME results of fatty acids. Colour of the dots indicates the concentration of the strongest interacting fatty acid compounds.

although pairwise differences were less distinct, indicating a general upward trend across all treatments. 2-pentanone displayed a mid-storage minimum, with concentrations at Day 5–7 significantly lower than Day 0 and Day 10 in ASP and BHT groups ( $p < 0.0001$ ), while control samples followed a comparable pattern.

For aldehydes, pentanal showed a significant overall effect ( $p = 0.007$ ), with concentrations generally higher at mid- to late-storage across treatments, although most pairwise contrasts were not significant, suggesting a gradual time-related elevation rather than abrupt shifts. In contrast, 2-propenal did not vary significantly with storage ( $p = 0.451$ ).

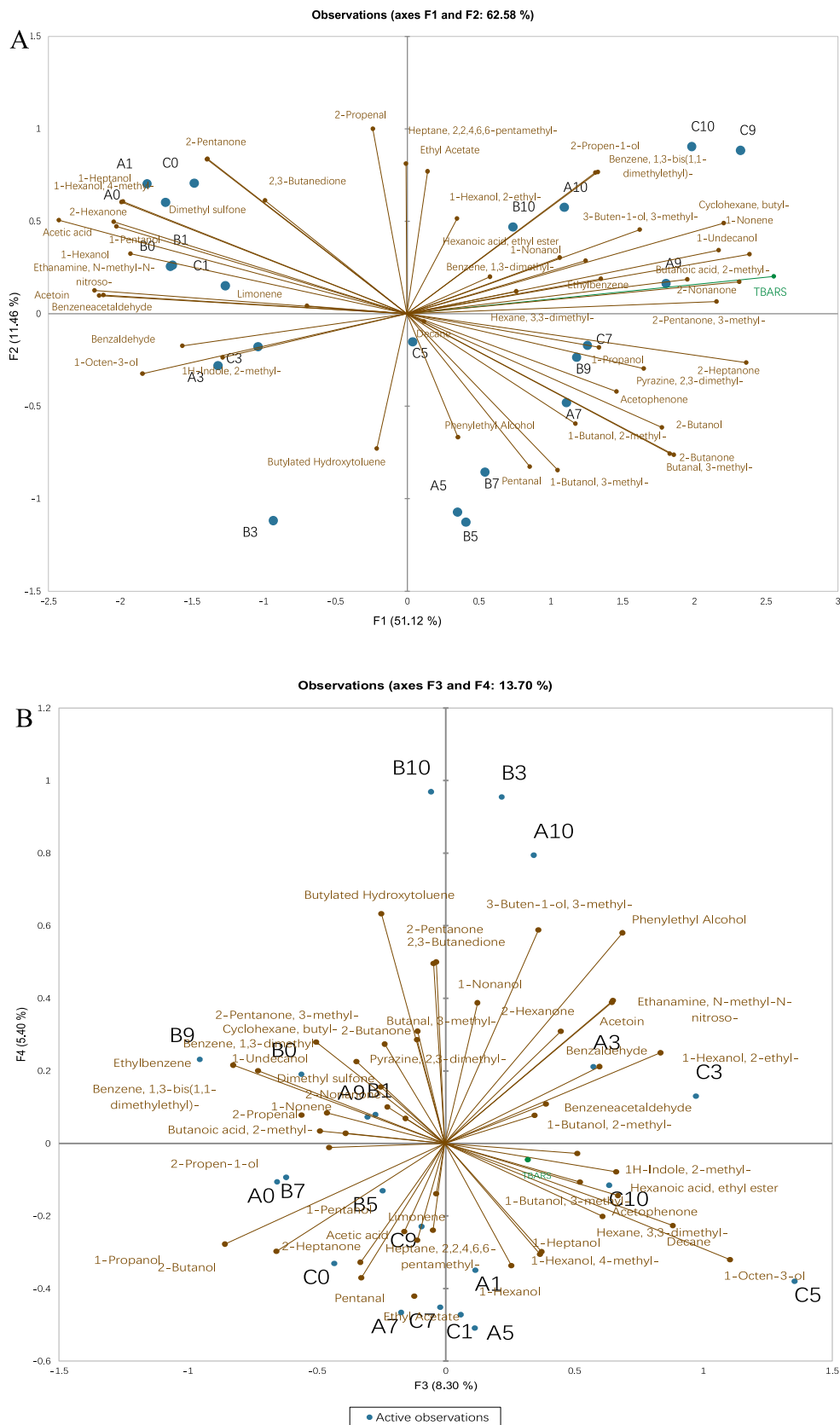
Late-stage lipid oxidation markers exhibited strong time effects. Decane concentrations were significantly higher in control samples at Day 5 compared with all other day  $\times$  treatment combinations ( $p < 0.0001$ ), whereas ASP and BHT maintained significantly lower levels throughout storage. Cyclohexane, butyl- also increased sharply over time ( $p < 0.0001$ ), with late-storage values (Day 9–10) in BHT and control significantly higher than early days, while ASP remained comparatively lower.

Alcohols demonstrated distinct time-related trends. 3-methyl-3-buten-1-ol, identified as a protective feature in the SHAP model, increased significantly across storage in all treatments ( $p = 0.0002$ ), with Day 10 concentrations markedly higher than Day 0–1 in ASP, BHT, and control samples. 1-propanol and 1-nonanol also increased significantly over time ( $p = 0.0001$  and  $p < 0.0001$ , respectively), with late-storage levels exceeding early storage in ASP and BHT groups. Conversely, 1-octen-3-ol showed no significant variation ( $p = 0.811$ ). Finally, 1H-indole, 2-methyl- exhibited an inverse pattern, being significantly higher in early storage (Day 1) than late storage (Day 10) across all treatments ( $p < 0.0001$ ), indicating a decline as refrigeration progressed.

### 3.6.2. Multiple factor analysis (MFA) of volatile compounds

The first two dimensions of the MFA (Fig. 5) explained 62.58% of the total variance and effectively differentiated the beef patties based on their lipid oxidation state. MFA was applied to 62 volatiles detected in control, BHT-, and ASP-treated patties over 10 days of refrigerated storage. The first two dimensions explained 62.58% of variance (F1: 51.12%; F2: 11.46%), while four dimensions accounted for 76.28%. F1 captured lipid oxidation intensity (Fig. 5A): fresh samples (days 0–1) clustered negatively, dominated by alcohols (1-hexanol, 1-heptanol, 1-pentanol), 2-hexanone, acetic acid, and acetoin. As storage progressed, samples shifted positively along F1, where aldehydes (nonanal, decanal), hydrocarbons (1-nonene, 1-undecene), and aromatics (ethylbenzene, p-xylene) prevailed. TBARS loaded strongly on positive F1, confirming oxidation. Control samples advanced fastest, reaching extreme positive scores by days 9–10 (C9, C10), indicating severe oxidation and rancid notes. BHT slowed this progression, while ASP-treated samples (A10) remained near the fresh cluster, showing effective suppression of oxidation volatiles. F2 explained minor functional-group differences but no treatment-specific effects.

F3 and F4 (13.70% variance; Fig. 5B) revealed two deterioration pathways. Negative F3 scores reflected lipid autoxidation markers (pentanal, hexanal, heptanal, 1-octen-3-ol), typical of rancid flavours, and were associated with late control and BHT samples. Positive F3 scores indicated microbial/fermentative metabolism (acetoin, 3-methylbut-3-en-1-ol, 2-phenylethanol), seen in ASP samples from day 3 onward, with A10 highest. Thus, while control and BHT patties deteriorated mainly via lipid oxidation, ASP-treated patties developed mild buttery and floral notes, sensorially less objectionable than rancid flavours.



**Fig. 5.** Multiple Factor Analysis (MFA) biplot of the volatile compounds and beef patty samples throughout storage. (A) The first two dimensions (F1 and F2), explaining the highest proportion of data variance. (B) The third and fourth dimensions (F3 and F4), representing subsequent sources of variation. \*A: ASP-treated; B: BHT-treated; C: Control. Storage days: 0, 1, 3, 5, 7, 9, 10.

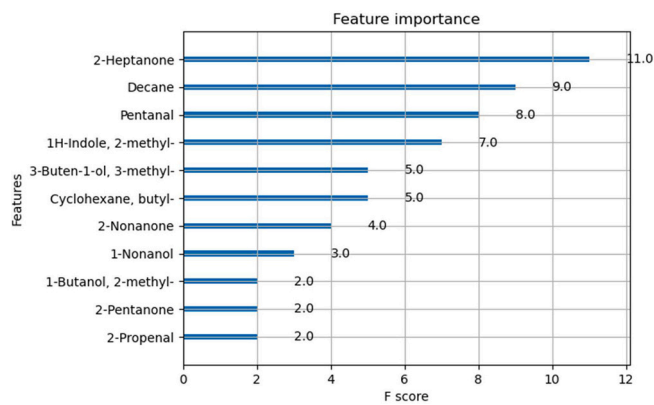


Fig. 6. Feature importance score of volatile compounds for TBARS.

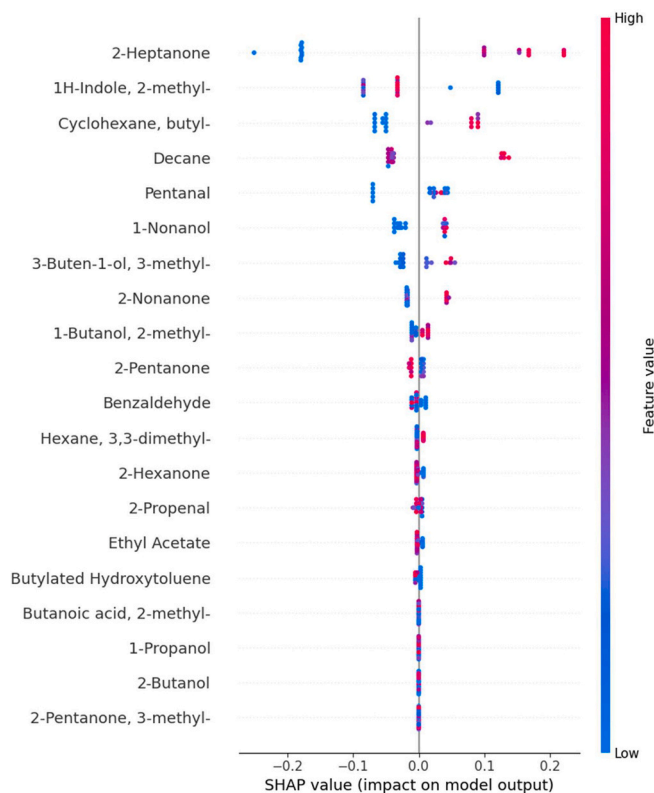


Fig. 7. Magnitude and direction of the effects of volatile compounds on the TBARS indicated by SHAP values. In each instance, the given explanation is represented by a single dot on each polyphenol column. The y-coordinate of the dot indicates the direction and magnitude of the effect of its volatile compounds' concentration on the SHAP value.

### 3.6.3. SHAP analysis of volatile compound contributions to TBARS prediction

To identify volatiles strongly influencing lipid oxidation (TBARS), SHAP interpretation was applied to 62 compounds. Global F-scores ranked 2-heptanone (11.0), decane (9.0), and pentanal (8.0) as top contributors (Fig. 6). These are well-established lipid oxidation products; for example, 2-heptanone is a methyl ketone linked to rancid/warmed-over flavour (Xi et al., 2023). Decane is an inert hydrocarbon formed during advanced peroxidation, as it has been identified in thermally processed meat products with a high odour threshold limiting its direct contribution to aroma (Yao et al., 2024). Conversely, pentanal is an early, potent straight-chain aldehyde with a strong grassy/fatty note, which has been confirmed as a key aroma compound with a high

odour activity value. Their dominance confirms that variation in volatiles reflects lipid autoxidation. MFA results corroborate this, showing control samples' separation along positive F1 driven by sharp increases in these markers, while ASP-treated samples remained stable due to near-complete suppression.

A gradient boosting model (Fig. 7) predicted TBARS with high accuracy ( $R^2 > 0.93$ ), and SHAP values identified 2-heptanone as the strongest positive contributor, followed by 2-methyl-1H-indole, butyl-cyclohexane, decane, and pentanal—all significantly higher in control and late BHT samples by Day 10 ( $p < 0.05$ ; Table S6). In contrast, volatiles such as 3-buten-1-ol, 3-methyl- and short-chain alcohols/ketones, along with residual BHT, showed strong negative SHAP values, lowering predicted rancidity. Interestingly, 3-buten-1-ol, 3-methyl was  $>3\times$  higher in ASP samples than in control or BHT by Day 10 ( $p < 0.001$ ), indicating ASP's profile was characterised by suppression of oxidation markers and presence of mild microbial metabolites with low rancidity.

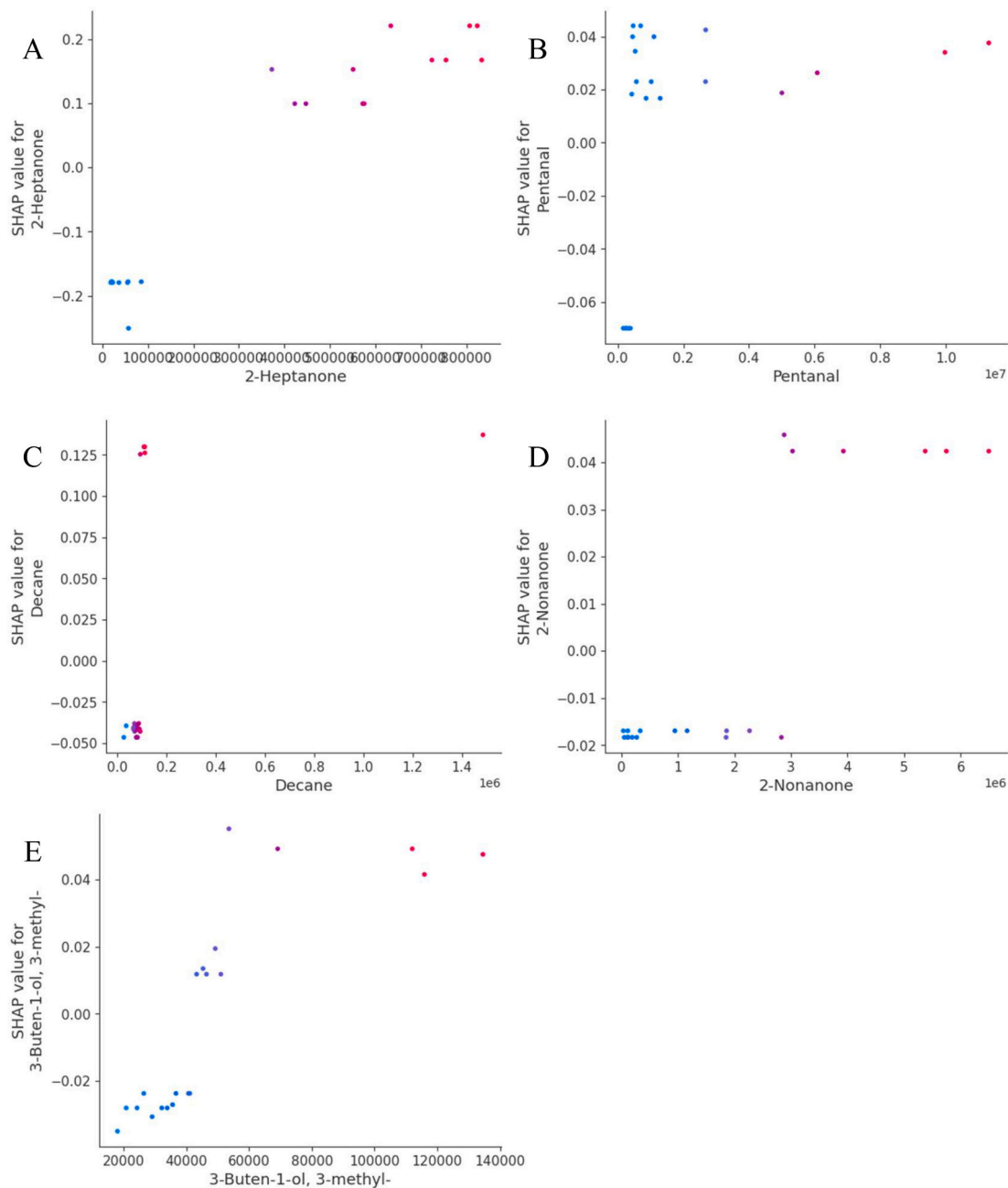
Fig. 8A shows concentration-dependent effects. 2-heptanone sharply increased predicted TBARS at high levels, consistent with SPME-GC-MS data (Table S6). Control samples exceeded this threshold by Day 10, BHT samples increased moderately, while ASP samples remained well below, suggesting ASP's broad-spectrum protection via mechanisms such as metal chelation or enzyme inhibition Falowo et al. (2014).

Fig. 8B illustrates pentanal's effect, a classic secondary product of linoleic acid oxidation (Brunton et al., 2000). At low concentrations, SHAP values were near zero, but increased steeply beyond a threshold, indicating strong prediction of lipid oxidation. ANOVA results (Table S6) confirm this: pentanal increased significantly in control and BHT samples from Day 0 to Day 10, while ASP-treated patties maintained low levels, remaining in the low-impact region and preventing rancidity. This confirms the ability of ASP to prevent the accumulation of this potent marker of rancidity.

Fig. 8C shows decane, a neutral hydrocarbon and reliable end-product of advanced lipid peroxidation (Garcia-Llatas et al., 2013; Ross et al., 2006). SHAP values were negligible at low concentrations but increased sharply above threshold. ASP-treated samples maintained low decane throughout storage, while control and BHT samples exceeded  $4 \times 10^6$  area units by Day 10 ( $4.410 \times 10^6$  and  $4.507 \times 10^6$ ), confirming ASP's efficacy against late-stage oxidation (treatment  $\times$  time interaction  $p < 0.001$ ; Table S6). This confirms that ASP effectively prevented the late-stage accumulation of this marker of severe lipid oxidation, whereas neither the untreated control nor the synthetic BHT treatment was able to do so by the end of refrigerated storage.

Fig. 8D shows 2-nonanone, another methyl ketone marker of advanced oxidation. Previous studies showed that methyl ketones such as 2-heptanone, 2-octanone, and 2-nonanone are reliable markers of advanced lipid oxidation in beef and pork, particularly under refrigerated storage (Domínguez et al., 2019). As with the other pro-oxidant volatiles, SHAP values remained near zero at lower concentrations and then increased markedly. ASP samples stayed below  $1.839 \times 10^6$  area units, while control and BHT reached  $3.027 \times 10^6$  and  $3.926 \times 10^6$  by Day 10, placing them in the high-impact zone ( $p < 0.05$ ; Table S6). These findings align with reports that BHT loses efficacy over time due to volatilisation or depletion (Dai et al., 2023), whereas polyphenol-rich extracts provide sustained protection via regeneration and multi-pathway scavenging (Jongberg et al., 2013; Maqsood et al., 2013).

Fig. 8E shows 3-methyl-3-buten-1-ol, a volatile alcohol with strongly negative SHAP values, indicating inhibition of lipid oxidation. ASP-treated samples achieved the highest concentrations ( $1.159 \times 10^6$  area units on Day 10;  $p < 0.001$ ) as seen in Table S6, while BHT and control remained below  $1.4 \times 10^5$ . Although previously noted as a spoilage marker in fish (Tan et al., 2024), similar alcohols have been linked to the protective action of phenolic-rich plant extracts, such as the marked inhibition of lipid oxidation demonstrated by caper and purslane extracts in ground beef (Bayındır et al., 2025), and the protective effects of rosemary phenolics (Jongberg et al., 2013) in meat systems. This study provides the first SHAP-based evidence that ASP promotes this



**Fig. 8.** SHAP-dependence plots displaying the top candidates for key volatile compounds influencing TBARS. The vertical axis is the effect of each instance on the predicted value. The horizontal axis is the SPME results of volatile compounds. Colour of the dots indicates the concentration of the strongest interacting volatile compounds.

compound, contributing mechanistically to superior oxidative stability compared with BHT and controls.

The findings demonstrate that fermented–roasted avocado seed powder not only functions as an effective natural antioxidant but also exemplifies a circular bioeconomy approach in food preservation. The valorisation of avocado seeds, an abundant agro-industrial byproduct, into a high-value functional ingredient aligns with United Nations Sustainable Development Goal 12 (Responsible Consumption and Production), particularly Target 12.3 on reducing food waste and Target 12.4 on promoting safer alternatives to hazardous synthetic additives. This strategy supports sustainable food systems by transforming waste into a clean-label solution for meat preservation.

While SHAP identifies features with high predictive importance rather than chemical mechanisms, our SHAP outputs (e.g., strong

contributions from  $\gamma$ -linolenic acid, pentanal, and selected lipid-derived volatiles) are consistent with the antioxidant behaviours expected from ASP's phenolic constituents enriched by *Lactiplantibacillus plantarum* fermentation and optimised roasting. Zhao and co-authors demonstrated that these processing steps significantly increase total phenolics and CUPRAC/FRAP-measured reducing capacity, with ASP containing catechin-type flavanols and phenolic acids (e.g., protocatechuic, ferulic) that are known to quench lipid-phase radicals and intercept peroxy chains (Zhao et al., 2022). Such compounds act predominantly via electron-transfer (ET)/hydrogen-atom transfer (HAT) pathways and, in some cases, metal chelation, thereby lowering the propagation rate of polyunsaturated fatty acid oxidation and suppressing formation of aldehydic markers captured in our volatile panel (Apak et al., 2007; Prior et al., 2005). Accordingly, the SHAP-highlighted predictors can be

predicted as directional evidence that ASP's phenolic matrix enhanced by fermentation/roasting contributes to oxidative stabilization through established radical-scavenging and chelation routes, even though SHAP itself does not resolve reaction stoichiometry or specific binding events. To advance from predictive importance to mechanistic attribution, future work can couple SHAP with targeted phenolic quantitation (LC-MS), lipid-phase radical kinetics (e.g., ORAC lipid-peroxidation assays), and metal-chelating activity tests to map feature importance onto compound-level reactions within the beef patty matrix (Apak et al., 2007; Prior et al., 2005; Zhao et al., 2022).

#### 4. Conclusion

This study demonstrates that fermented–roasted avocado seed powder (ASP) is an effective natural antioxidant for delaying lipid oxidation in refrigerated beef patties, offering a sustainable, clean-label alternative to synthetic antioxidants like BHT. Measurement of reducing capacity by CUPRAC confirmed ASP's superior reducing antioxidant potential, which correlated with a 21% reduction in TBARS compared to the control after 10 days. Although ASP's final TBARS value was slightly higher than BHT, its long-term protective effect was comparable, highlighting its potential as a robust natural preservative. ASP also improved physicochemical quality by maintaining redness ( $a^*$ ) and conserving key unsaturated fatty acids. Furthermore, microbiological analysis revealed that ASP significantly suppressed microbial growth throughout storage. This antimicrobial effect, coupled with its established antioxidant capacity, demonstrates that ASP extends the shelf life of beef patties through a dual mechanism of enhancing both oxidative and microbial stability. Multivariate and SHAP analyses showed ASP better preserved oleic acid and other PUFAs than BHT or control, suggesting its mechanism of action may involve not only reductive capacity but also stabilization of the lipid matrix.

Volatile analysis revealed distinct antioxidant mechanisms: BHT provided rapid initial suppression, while ASP delivered sustained protection, likely due to polyphenols and Maillard reaction products formed during fermentation and roasting. SHAP interpretation reinforced these findings, identifying volatiles and fatty acids as key predictors of oxidative stability. In summary, ASP is a safe, effective natural antioxidant that inhibits lipid oxidation, maintains colour, and preserves nutritionally important fatty acids, while enabling food waste valorisation.

However, this study has several limitations. First, this study did not include sensory evaluation to directly assess the flavour, aroma, and texture of the ASP-treated patties. While the reduction in lipid oxidation-derived volatiles (e.g., aldehydes) suggests potential flavour stability benefits, direct human perception is essential to confirm these positive effects and to rule out any potential negative sensory impacts introduced by the ASP itself. Therefore, consumer or trained panel sensory testing is a critical priority for future research. Second, the findings are limited to ground beef stored under refrigeration for 10 days and may not be directly generalisable to other meat types, processing conditions, or extended storage periods. Third, while the observed pH decline and TVC reduction indicate antimicrobial potential, the specific activity of ASP against spoilage or pathogenic bacteria warrants further investigation. Future work should optimise processing conditions, test ASP in other meat systems, and conduct sensory evaluations to confirm consumer acceptance, particularly regarding its unique volatile profile.

#### CRedit authorship contribution statement

**Zhiyu Zhao:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elphege Toulon:** Writing – review & editing, Writing – original draft, Investigation. **Uche Ejorh:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation. **Tony Chen:** Writing – review & editing, Writing – original draft, Validation,

Resources, Methodology, Formal analysis. **Kevin Kantono:** Writing – review & editing, Writing – original draft, Validation, Supervision, Formal analysis, Data curation. **Rothman Kam:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation. **Nazimah Hamid:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

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#### Declaration of competing interest

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

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#### Appendix A. Supplementary Data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2026.118583>.

#### Data availability

Data will be made available on request.

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