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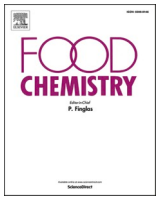

Origin and mitigation of catechins-induced discoloration during pea protein isolation while inhibiting off-flavor formation

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ABSTRACT

This study elucidates the mechanisms of catechin-induced discoloration during pea protein isolation via alkaline solubilization–isoelectric precipitation, where catechins are applied to inhibit off-flavor formation. Catechins were also combined with various mitigation strategies, including reduced protein solubilization pH, sodium bisulfite (SB), gallic acid (GA), Duralox®, and their effects were evaluated using untargeted metabolomics, lipid-oxidation markers (HODE, MDA) and volatile compounds. Untargeted metabolomics identified extractable catechin-derived oxidized dimers (m/z 570–610) as compounds strongly associated with brown color development. Lowering solubilization pH reduced pigment formation but diminished catechins retention and increased free-radical signals. Catechins–SB combinations most effectively suppressed discoloration, lipid oxidation and formation of volatile off-flavors which was consistent with improved sensory attributes. GA offered additional protection but caused concentration-dependent darkening, whereas Duralox® showed limited efficacy. Overall, targeted antioxidant systems, particularly catechins–SB combinations, offer an effective strategy to control oxidation and improve sensory quality in pea protein.

1. Introduction

Pea protein isolates (PPI) are widely used in plant-based foods due to their favorable nutritional profile, functional properties, and sustainable production. However, the conventional pea protein extraction process, typically involving alkaline solubilization followed by isoelectric precipitation, can induce lipid oxidation, particularly in the presence of unsaturated fatty acids (USFAs) and residual lipids (Gao et al., 2020; Sharifi et al., 2026). This oxidative degradation leads to the formation of volatile organic compounds (VOC), including aldehydes, e.g., hexanal, nonanal; alcohols, e.g., 1-pentanol, and ketones, which contribute to undesirable "beany" and "green" off-flavors that limit consumer acceptance and application in mildly flavored food matrices (Gultekin Subasi et al., 2024). Lipid oxidation may occur enzymatically via lipoxygenase (LOX), which converts USFAs into oxylipins that further degrade into volatiles, or through non-enzymatic pathways such as autoxidation and photooxidation via free radical reactions in the presence of oxygen or light (Trindler et al., 2022). Because both pathways contribute to off-flavor formation, complete inhibition remains a major challenge.

In our previous study, four antioxidant systems: coffee extract, caffeic acid, catechins, and Duralox®, were screened to inhibit lipid oxidation and off-flavor formation during pea protein isolation. Catechins were particularly effective in suppressing lipid oxidation markers such as HODE and hexanal. This was mostly because catechins showed dual enzymatic and non-enzymatic inhibition, markedly reducing LOX activity and radical formation (Villacis-Chiriboga et al., 2026). Although the addition of catechins during pea protein extraction substantially improved oxidative stability and reduced green and beany flavor perception, it also introduced a pronounced color darkening in the recovered proteins which can limit its application and needs further understanding and optimization.

Color development in phenolic-treated protein systems has been widely documented and mostly related to oxidative reactions. Catechins

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specifically undergo oxidation under alkaline conditions, leading to the formation of various chromophoric compounds (Germann et al., 2019b), especially quinones. For instance, Germann et al. (2019a) demonstrated that catechin and epicatechin are converted into catechinic acid and other chromophores during cocoa powder alkalization, contributing to the red coloration observed in alkalized cocoa powders. Given the complexity of these interacting mechanisms, comprehensive metabolomic analyses are essential to elucidate the specific pathways and compounds responsible for color formation in phenolic–protein systems. Therefore, tracking the formation of quinones by untargeted metabolomic analysis is crucial for understanding their biosynthetic origins, reaction dynamics, and roles in complex matrices, as well as for identifying novel intermediates and reaction products that contribute to color development and oxidative processes.

The rate of catechins oxidation and the associated color change are strongly influenced by pH, with oxidation being more favorable under alkaline conditions. This process involves the conversion of the catechol moiety in the B-ring of catechins to *o*-quinones, which represents the initial step in the oxidation pathway (L. Y. Chen et al., 2018). This suggests that elevated pH used during protein isolation could accelerate oxidative pathways and promote color darkening. Therefore, adjustment of the solubilization pH may represent a practical strategy to mitigate discoloration, although this approach remains largely unexplored in pea protein systems.

As an alternative mitigation strategy, the use of multi-component antioxidant systems has gained attention to balance oxidative inhibition and color stability. Combining antioxidants with distinct redox properties can provide synergistic protection by regulating the oxidative–reductive balance within the system. In this regard, several studies have investigated complementary antioxidants to mitigate catechin browning. For instance, Tan et al. (2025) demonstrated that gallic acid (GA), a naturally occurring compound in tea, can inhibit browning by maintaining the pH below 4. Similarly, Kuijpers et al. (2012) reported that sodium bisulfite (SB) reacts with *o*-quinones to form colorless addition products. In addition to generating soluble oxidation products, catechin-derived *o*-quinones may react with nucleophilic amino acid residues present in pea proteins through non-covalent or covalent reactions, potentially contributing to pigment retention within the protein matrix and influencing the effectiveness of antioxidant interventions. Therefore, we hypothesized that conditions that suppress quinone formation or promote quinone scavenging, such as reduced solubilization pH or the addition of nucleophilic antioxidants, would decrease the accumulation of oxidation-derived chromophores while maintaining the ability of catechins to inhibit lipid oxidation and off-flavor formation during pea protein isolation.

Although SB is an effective quinone scavenger, its practical application in food systems may be constrained by regulatory requirements, allergen labeling obligations, and consumer acceptance considerations. Accordingly, SB was included in the present study primarily as a mechanistic reference compound to evaluate the extent to which quinone trapping can mitigate catechin-induced discoloration during pea protein isolation. Previous studies have largely focused on the chemical prevention of quinone formation and browning reactions, whereas the effects of these mitigation strategies on lipid oxidation, volatile formation, and sensory properties remain poorly understood. Furthermore, to our knowledge, no study has systematically evaluated these relationships in pea protein systems, leaving an important knowledge gap regarding the interactions among antioxidants, phenolic compounds, proteins, and their collective influence on color stability and product quality.

Taken together, these considerations highlight the need for a comprehensive investigation integrating both chemical interventions and process optimizations to better control lipid oxidation while avoiding color formation during pea protein extraction. Such an integrated approach could improve understanding of the relationships between enzymatic oxidation, radical-mediated oxidation, catechin autoxidation, and their combined effects on color and sensory quality. With this information in mind, the objectives of this study were:

(i) to identify the key compounds generated during pea protein extraction in the presence of catechins that may contribute to color formation.
(ii) to evaluate the effectiveness of combining catechins with different antioxidants, including SB, GA, Duralox® oxidation management blend (a mixture of rosemary extract, ascorbic acid, tocopherols and citric acid) (Wu et al., 2021), and adjusting solubilization pH in mitigating catechins-induced discoloration during pea protein extraction.
(iii) to assess the impact of these modifications on lipid oxidation, the formation of volatile beany flavor compounds in the pea proteins and their sensory properties.

The findings of this study aim to support the development of antioxidant-assisted extraction strategies that enhance both oxidative stability and sensory quality in plant-based protein ingredients.

2. Materials and methods

2.1. Pea seeds and their preprocessing

Yellow pea (*Pisum sativum* L. var. Ingrid) was harvested in 2024, and was dried to a final moisture of 14% by air blowing at 30 °C. Upon arrival at the laboratory, the seeds were manually cleaned to remove any impurities and then stored in airtight containers at −80 °C. Dehulling was carried out using a Satake TM05C(2)-1 abrasive mill (Satake, Japan), followed by air separation to remove remaining hull fragments. The resulting dehulled peas were milled into fine flour with a particle size of 500 μm using a Retsch ZM 200 mill (Retsch, Haan, Germany) operating at 12000 rpm. The flour was then sealed and stored at −80 °C to preserve its quality.

2.2. Protein extraction

2.2.1. Classic wet fractionation

Pea protein was extracted using the alkaline solubilization and isoelectric precipitation method following the protocol described by Sajib et al. (2023). Pea flour was suspended in water at a ratio of 1:10 ($w.v^{-1}$). After one hour of incubation at room temperature (RT) the pH of the system was adjusted to 8.5 using 2 M NaOH and stirred for an additional hour. The solubilized protein was then separated from the starch fraction by centrifugation (4000 $\times g$ for 20 min at 4 °C). Protein was precipitated by lowering the pH to 4.5 with 2 M HCl and recovered by centrifugation (4000 $\times g$ for 20 min at 4 °C). The resulting protein was neutralized to pH 7.0, freeze-dried and stored at −80 °C until further analysis.

2.2.2. Wet fractionation in the presence of catechins and at different solubilization pH values

To understand the effect of protein solubilization pH on discoloration in the presence of catechins, pea flour was suspended in water at a ratio of 1:10 ($w.v^{-1}$) containing green tea catechins (Polyphenon 60, Sigma Aldrich, USA) at 1000 ppm (homogenate weight). After one hour of incubation at RT, the pH of the system was adjusted to 8.5 or 7.0 using 2 M NaOH and stirred for an additional hour. The pH values of 8.5 and 7.0 were selected to represent a conventional alkaline extraction condition previously optimized for pea protein recovery and a reduced-pH condition expected to limit catechin oxidation during extraction. The rest of the solubilization and precipitation steps were followed as described in section 2.2.1.

2.2.3. Wet fractionation in the presence of catechins in combination with other antioxidants

The protein was extracted in the presence of catechins in combination with other antioxidants. In all cases, the antioxidants were dissolved in water before mixing with the flour to avoid any type of oxidation. Prior to flour addition to 10 ratios of water ($w.v^{-1}$), different treatments were prepared by adding catechins at 1000 ppm (homogenate weight) combined with 0.1 and 1.0% sodium bisulfite (SB) (MW = 104.06 g. mol^{-1}; Sigma Aldrich, Germany), 0.1 and 1.0% gallic acid (GA) (MW = 170.12 g·mol^{-1}; Sigma Aldrich, Germany) or 75% Duralox® oxidation management (Kalsec, USA). After one hour of incubation at RT, the pH of the system was adjusted to 7.0 using 2 M NaOH and stirred for an additional hour. The rest of the solubilization and precipitation steps were followed as described in section 2.2.1.

A detailed description of the experimental setup is provided in Fig. 1.

2.3. CIELAB colorimetry determination

The color attributes of the protein samples were analyzed using a CR-400 colorimeter (Konica Minolta Sensing, Japan). The protein was placed in a small petri dish for measurement, with five independent readings recorded for each sample ($n = 5$). Color measurements were obtained within the CIELAB system within the dimensionless coordinates L* (between 0 and 100), a* and b*(between −128 and + 127), applying the following instrument settings: D65 illuminant, 2° standard observer, and d/0° measurement geometry. The device was operated in Specular Component Included (SCI) mode in accordance with the manufacturer's guidelines. Prior to analysis, the colorimeter was calibrated using the supplied white reference tile. To ensure uniformity of background reflection, all samples were positioned on a white plastic plate (30 × 30 × 1.3 cm) during measurements.

Color differences between the protein samples and the commercial

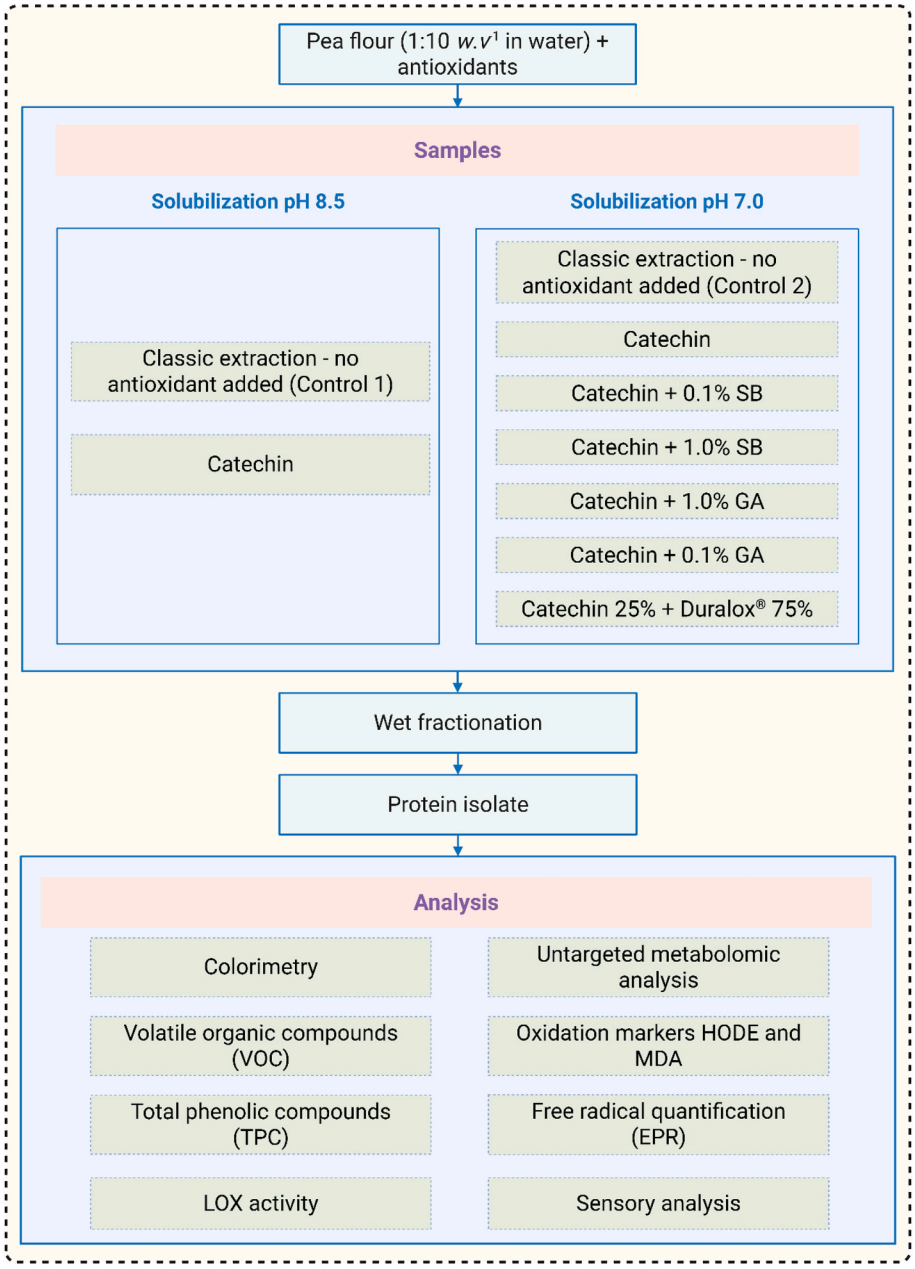

Fig. 1. Schematic overview of the experimental setup used in this study. SB: sodium bisulfite; GA: gallic acid; VOC: volatile organic compounds; TPC: total phenolic compounds; LOX: Lipoxygenase; HODE: hydroxyoctadecadienoic acid; MDA: malonaldehyde; EPR: electron paramagnetic resonance spectroscopy.

benchmark were quantified using the CIELAB color difference metric ΔE*ab (CIE76), calculated as the Euclidean distance between mean L*, a*, and b* values [Eq. 1], which was used as a straightforward metric for comparing relative color differences among samples.

$$\Delta E^*_{ab} = \sqrt{\left(L^*_{reference} - L^*_{sample}\right)^2 + \left(a^*_{reference} - a^*_{sample}\right)^2 + \left(b^*_{reference} - b^*_{sample}\right)^2} \quad (1)$$

2.4. Metabolomic analysis of pH-induced chromogenic compounds

The potential color-responsible compounds in pea protein were determined using untargeted metabolomic analysis. Pea protein was diluted in MeOH 80% (1:10 $w.v^{-1}$), vortexed for 5 s, sonicated for 20 min, centrifuged at 6000 x*g* for 10 min at 4 °C and filtered through a 0.45 μm syringe filter. The recovered supernatant, containing low-molecular-weight metabolites potentially associated with discoloration, was stored at −80 °C until analysis (Raita et al., 2025).

The analysis was performed using a Waters Xevo MRT coupled to a Waters Acquity Premier UPLC FTN. Metabolites were separated on a Waters HSS T3 column (1.8 μm, 2.1 × 100 mm) using a water–acetonitrile gradient containing 0.04% formic acid in both mobile phases. The gradient started at 5% B (acetonitrile with 0.04% formic acid), increased to 100% B over 6 min, and was held at 100% B for 4.5 min, followed by re-equilibration at initial conditions for 1.5 min. The flow rate was 0.4 mL.min^{-1}, the column temperature was maintained at 45 °C, and the injection volume was 1.0 μL.

Mass spectrometric data were acquired in negative electrospray ionization mode using data independent MSe acquisition over a mass range of 50–1200 *m/z* at a scan rate of 10 Hz. The high-energy function was operated with a collision energy ramp of 20–50 eV. The capillary voltage was set to 1.5 kV and the cone voltage to 30 V. The source temperature was 120 °C, and the desolvation temperature was 550 °C. Desolvation and cone gas flows were set to 800 and 50 L.h^{-1}, respectively. A lock mass correction was applied using leucine enkephalin (*m/z* 554.26202) infused in negative ion mode and sampled automatically at 1 min intervals.

Data was processed in MS-DIAL (v5.5.251021). Centroided raw data files (mzML format, triplicates) were imported into the software, and SWATH acquisition mode was selected. The collision type was set to CID/HCD. MS1 and MS2 mass tolerances were set to 0.01 Da and 0.25 Da, respectively. Data were acquired over a mass range of 50–1200 Da, and multithreading was enabled using 8 threads. Peak detection was performed with a minimum peak height of 4000 (amplitude) and a mass slice width of 0.1 Da. Peak smoothing was conducted using a linear weighted moving average with a smoothing level of 3 scans and a minimum peak width of 5 scans. For MS/MS deconvolution, an abundance cutoff of 1000 was applied. Feature alignment across samples was performed with a retention time tolerance of 0.1 min and an MS1 tolerance of 0.01 Da.

Under alkaline conditions, catechins undergo rapid oxidation at its catechol B-ring, forming a semiquinone intermediate that subsequently converts into an *o*-quinone, which can then participate in hydration, intramolecular cyclization, and oxidative coupling reactions that yield dimers and higher oligomers (L. Y. Chen et al., 2018; Danilewicz, 2013). Guided by these known transformation pathways, the untargeted metabolomic analysis was performed in negative ionization mode because catechins and their oxidation products typically exhibit superior ionization efficiency and detection sensitivity under these conditions as a result of the facile deprotonation of phenolic hydroxyl groups (De Paepe et al., 2013).

The resulting peak list and alignment matrix were exported for downstream statistical analysis, conducted in the R based online tool MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/), applying partial least squares–discriminant analysis (PLS-DA) to identify metabolites with high variable importance in projection (VIP) scores as key contributors to decoloration, using VIP scores calculated from Component 1 of the PLS-DA model, as this component captured the largest variation associated with the reddish coloration.

2.5. Measurement of volatile organic compounds

Selected volatile compounds associated with beany off-flavors, as reported by Gultekin Subasi et al. (2024), were analyzed using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC–MS). For each analysis, 0.5 g of each freeze-dried pea protein isolate was dispersed in 5 mL of Milli-Q water in 20 mL amber glass vials. Volatiles were extracted from the headspace using a 75 μm CAR/PDMS fiber at 60 °C for 40 min under continuous stirring (500 rpm). After extraction, the fiber was thermally desorbed and introduced into the GC–MS system in splitless mode for 5 min. Chromatographic separation and detection were carried out using a Shimadzu TQ8030 GC–MS equipped with a ZB-1701 column (30 m × 0.32 mm, 1 μm; Phenomenex, Torrance, CA). Helium was used as the carrier gas at a flow rate of 1.5 mL·min^{-1}. The inlet temperature was maintained at 300 °C, while the oven temperature was programmed to ramp from 35 °C to 260 °C. The MS transfer line and ion source were set at 265 °C and 200 °C, respectively, with mass scanning in the range of 30–500 amu. Ethylpyridine was used as the internal standard because it is absent from pea protein matrices, provides a stable and reproducible chromatographic response under the HS-SPME-GC–MS conditions employed, and does not interfere with the detection of the target volatile compounds. Quantification was based on relative peak areas normalized to the internal standard and compared against external calibration standards (isovaleraldehyde, valeraldehyde, 3-methyl-1-butanol, 1-pentanol, hexanal, 1-hexanol, 2-pentylfuran, 1-octen-3-ol, benzaldehyde, 2-methoxy-3-isopropyl pyrazine, and nonanal). Results were expressed as micrograms of each volatile compound per gram of dry pea protein (μg·g^{-1} DW).

2.6. Quantification of hydroxyoctadecadienoic acids (HODE) and malonaldehyde (MDA) using LC-MS

Hydroxyoctadecadienoic acid (HODE) levels were assessed using a derivatization-based LC-MS method previously validated for pea protein matrices (Villacis-Chiriboga et al., 2026). In brief, protein samples were diluted in water at a ratio of 1:5 ($w.v^{-1}$) and homogenized by vortexing for 10 s. A 50 μL aliquot of each sample was transferred into 1.5 mL microcentrifuge tubes and reacted with equal volumes of 3-nitrophenylhydrazine (3-NPH; 2 mg·mL^{-1} in MeOH), and EDC-6 (6 mg·mL^{-1} in MeOH containing 6% pyridine). The tubes were sealed and incubated in the dark at RT for 1 h under agitation (2000 rpm) using a multi-tube vortexer (DVX-2500, VWR, USA).

Post-reaction, samples were centrifuged at 15,000 ×*g* for 10 min. To isolate hydrophobic oxidation products, 200 μL of the supernatant was extracted three times with 500 μL of dichloromethane. Each extraction involved vortexing for 30 s followed by centrifugation at 16000 ×*g* for 2 min. The combined organic layers were stored at −80 °C until LC-MS analysis. Quantification was performed using a standard curve of 13-HODE (0.005–1 μM), processed identically to the samples. Chromatographic separation was achieved on a Kinetex C18 column using a binary mobile phase: 20 mM acetic acid in water (A) and methanol (B). The gradient program spanned 40 min, beginning at 70:30 (A:B), ramping to 95% A over 8 min, held for 10 min, then increased to 98% A by 25 min, held briefly, and returned to initial conditions. Detection was carried out using a QTRAP 6500+ mass spectrometer (Sciex, Singapore) operating in both positive and negative electrospray ionization modes. Results were expressed in μg HODE per gram of dry protein (μg·g^{-1} DW).

Malondialdehyde (MDA) was quantified following the protocol described by Tullberg et al. (2016), with minor modifications. Samples were diluted 1:5 in Milli-Q water and briefly vortexed. One milliliter of the homogenate was combined with 20 μL of BHT (0.1 g·mL^{-1} in

MeOH), 40 μL of EDTA (0.02 M), and 500 μL of 0.25 M HCl. After mixing and standing for 5 min at RT, samples were centrifuged at 16000 ×*g* for 2 min. A 400 μL aliquot of the supernatant was then reacted with 25 μL of DNPH (2 mg·mL^{-1} in MeOH), vortexed, and incubated for 1 h at RT.

Aldehydes were extracted using 500 μL of dichloromethane, followed by centrifugation (16,000 ×*g*, 2 min). This step was repeated three times, and the organic layers were pooled. Samples were dried under vacuum (SpeedVac) and reconstituted in 250 μL of methanol. MDA concentrations were determined using a calibration curve (0.3–60 μM), and peak areas were compared against those of the standards.

2.7. Total phenolic composition

Total phenolic content (TPC) in pea protein was assessed using a standardized method adapted from Slinkard and Singleton (1977). Each sample was suspended in a methanol–water solution (70:30, *v.v*$^{-1}$) containing 1% trifluoroacetic acid (1:10 *w.v*$^{-1}$ ratio). The suspension was sonicated for 5 min, vortexed, and centrifuged at 5000 ×*g* for 5 min at 4 °C. Subsequently, 500 μL of the resulting extract was mixed with 2.5 mL of Folin–Ciocalteu reagent (FC) (10%, *v.v*$^{-1}$) and allowed to react for 2 min. Then, 2 mL of sodium carbonate solution (75 g.L^{-1}) was added, and the mixture was incubated at 50 °C for 15 min. Absorbance was measured at 760 nm. Quantification was performed using a gallic acid (GA) calibration curve ranging between 10 and 100 μg.L^{-1} prepared under identical conditions. TPC values were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry pea protein (mg GAE.g^{-1} DW).

2.8. Free radical quantification

Free radicals were extracted and detected following a method previously optimized for pea (Villacis-Chiriboga et al., 2026). Pea protein was suspended in ethyl acetate:methanol (3:2 *v.v*$^{-1}$) at a 1:10 (*w.v*$^{-1}$) ratio and 100 μL of 200 mM N-tert-butyl-α-phenylnitrone (PBN) dissolved in ethanol was added. After vortexing for 10 s, the sample was incubated at 50 °C for 30 min in a water bath. Then, the samples were centrifuged (4000 ×*g*, 10 min, 4 °C), the supernatant was evaporated and then resuspended in 150 μL of the same solvent mixture prior to analysis. Microcapillaries (6.5 cm in length) were filled with 50 μL of the sample, and both ends were sealed with paraffin wax. A calibration curve of DPPH dissolved in EtOH (10 μM - 1 mM), each concentration with 100 μL of 200 mM PBN was used to determine spin concentration, with Avogadro's number (6.02214076 × 10^{23} mol^{-1}) used in the calculation. Electron paramagnetic resonance (EPR) measurements were performed in a Spinscan X-band EPR spectrometer (LINEV Systems, Germany) at 9.40 GHz, 10 mW power, 10 dB attenuation, 200 μT modulation amplitude, and 109 kHz modulation frequency, at RT. Data were processed with e-Spinoza (v1.1.02) and Origin 8.5, using double integration of spectra to determine spin concentration. Results were expressed as the number of spins per gram of isolated protein DW.

2.9. Assessment of lipoxygenase (LOX) activity

In this work, LOX activity measurements were intended to compare the influence of the different antioxidant systems on endogenous pea LOX activity. The assay was not designed to determine residual LOX activity in the final protein isolates. LOX activity was determined using a modified version of the protocol described by Gultekin Subasi et al. (2024). In brief, 0.1 g of freeze-dried sample was extracted with 10 mL of phosphate buffer (10 mM, pH 6.5) under continuous stirring for 5 h at RT. The resulting enzyme-rich supernatant was collected by centrifugation at 9100 ×*g* for 10 min.

The substrate emulsion was prepared by combining linoleic acid (140 μL) and Tween 20 (140 μL) into 8 mL of phosphate buffer, followed by clarification with 1.1 mL of 0.5 M NaOH. The final volume was adjusted to 50 mL with phosphate buffer. This stock solution was diluted 1:40 (*v.v*$^{-1}$) with 0.1 M sodium borate buffer (pH 9.0) prior to use. For the assay, 50 μL of enzyme extract was mixed with 1.25 mL of the diluted substrate, and the change in absorbance at 234 nm was monitored over 10 min using a UV spectrophotometer.

LOX activity was expressed in units per gram of pea flour (U·g^{-1}·min^{-1}), where one unit (U) corresponds to the rate of increase in absorbance per minute. The activity was calculated using the following equation:

$$\text{LOX activity}\left(\frac{\text{unit}}{\text{g}}\text{pea flour.min}\right)=\frac{\Delta \text{OD}_{234\text{nm}}\times \text{V}_2}{\Delta \text{t}\times \text{V}_1}\times\frac{\text{V}_0}{\text{m}} \quad (2)$$

where ΔOD_{234nm} is the change in absorbance at 234 nm; Δt is the reaction time (min); V_0 is the total volume of enzyme extract (mL); V_1 is the volume of extract used in the assay (mL); V_2 is the total volume of the reaction mixture (mL); and *m* is the mass of pea flour (g).

2.10. Sensory evaluation

To preserve the sensory integrity of the samples and minimize flavor alterations due to oxidation, all food-grade protein dispersions were freshly prepared immediately prior to evaluation. Samples were dissolved in water at a concentration of 5% (*w.v*$^{-1}$) and assessed using Quantitative Descriptive Analysis (QDA), following the methodology outlined by Lawless & Heymann (2010) and international standards (ISO 6658:2017, ISO 8586:2023, ISO 8589:2007, ISO 13299:2016). Sensory assessments were conducted by the use of a trained analytical panel at Kristianstad University.

The panel consisted of nine trained assessors on the first day of evaluation and eight on the second day due to illness. Panelist selection and training adhered to ISO standards 8586:2023 and 6658:2017. During training, panelists collaboratively identified and defined relevant sensory attributes such as flavor, aroma, and color, based on a diverse set of protein samples exhibiting pronounced sensory differences (see Table 1). Panelists were then trained to use a continuous intensity scale ranging from 0 to 100 mm, with anchor terms “little” and “much” positioned at 10 mm and 90 mm, respectively, to ensure consistent scoring across individuals.

Evaluations were carried out in a sensory laboratory compliant with ISO 8589:2010, using EyeQuestion software (version 4.11.68, Logic8, The Netherlands) for data collection. The QDA was performed over two consecutive sessions, each lasting two hours. All samples were served and evaluated at ambient temperature. Every sample was evaluated in triplicate by each panelist. To reduce bias, samples were coded with

Table 1
Sensory attributes identified and targeted in the study and their definitions.

Category	Attribute	Description
Odor (O)	Pea sprout/Sugar pea	Green/grassy notes
	Yellow pea	Soaked yellow peas, beaniness
	Soil	Wet/damp, earthy
	Sulfur	Flatulence
Appearance (A)	Yellow	Color intensity (low to high)
	Brown	Color intensity (low to high)
	Turbidity	Opaqueness / opacity
Texture (Tx)	Smoothness	Soft texture in the mouth (before spitting)
	Roughness	Rough texture in the mouth (after spitting), astringency
Taste (T)	Sourness	Basic taste
	Bitterness	Basic taste
	Sweetness	Basic taste
	Umami	Basic taste
	Saltiness	Basic taste
Flavor (F)	Nutty	Unripe hazelnut
	Pea sprout/Sugar pea	Green/grassy notes
	Citrus	Lime zest

randomized three-digit numbers and presented monadically in a unique sequence for each assessor. Palate cleansing was maintained between samples using water and neutral-flavored wafers.

Ethical approval for the study was granted by the Ethics Board at Kristianstad University (Ref. U2024–2.3.4-725).

2.11. Statistical analysis

All samples and analyses were conducted in triplicate. Volatile profiles were compared and their relationships with sample type and antioxidant concentration explored through principal component analysis (PCA) and heatmap visualization using OriginPro 2023 (v10.0.0.154, 64-bit, OriginLab, MS, USA). Statistical evaluation was performed in the same software, applying ANOVA with a 95% confidence level. Descriptive sensory data were examined via two-way ANOVA, treating products and panelists as fixed factors, and significant differences were further identified using Tukey's post hoc test (95% confidence level).

3. Results and discussion

3.1. Effect of solubilization pH and antioxidants on mitigating discoloration

The CIELAB coordinates of the pea proteins isolated at different solubilization pH values and in the presence of catechins and in combination with other antioxidants are displayed in Table 2, while Fig. 2 depicts the surface color of the isolated proteins. To contextualize the color characteristics of the experimental pea protein isolates, a commercially available pea protein isolate was included as a reference. This sample exhibited the highest lightness (L* = 78.53), indicating a bright appearance, along with a moderate green-red component (a* = 4.66) and a relatively low yellow hue (b* = 26.67). Together, these coordinates correspond to a pale beige to light yellow coloration, providing a benchmark against which the visual quality of the experimental treatments can be compared.

The control samples, extracted without any antioxidant at pH 8.5 and 7.0, showed a marked color distinction compared to the commercial sample. The lightness value (L*) decreased markedly with a pH decrease from 8.5 to 7.0. The sample extracted at pH 7.0 presented an L* of 66.50, indicating a relatively darker and more opaque appearance compared with one isolated at pH 8.5. At pH 7.0, the extract exhibited a small but positive red–green coordinate (a* = 5.86), indicating a slight shift toward red tones. Under alkaline extraction (pH 8.5), the a* value decreased to 1.61, reflecting a reduced redness and a more neutral chromaticity. This reduction may be linked to pH-induced changes in chromophoric compounds or reduced contribution from oxidized pigments (Lukitasari et al., 2024). The b* coordinate showed the largest numerical difference between the two control samples. The protein extracted at pH 7.0 displayed a strongly positive b* value (49.79), indicating an intense yellow coloration, whereas at pH 8.5, the b* value decreased to 35.78, corresponding to a noticeably less yellow appearance. Decreases in b* under alkaline conditions could be attributed to a reduced stability of pigments like chlorophyll and carotenoids. These pigments tend to be more stable in acidic conditions, while alkaline conditions can lead to degradation and a color shift (Alves et al., 2025).

Besides the discoloration caused directly by pH changes, the addition of catechins to the extraction system exerted a significant effect on the color of the solubilized pea protein. However, as shown in Table 1 and Fig. 2, reducing the solubilization pH from 8.5 to 7.0 markedly limited the development of brownish color. Thus, the protein solubilized at pH 7.0 was significantly lighter (L* = 53.46) than the one isolated at pH 8.5 (L* = 39.33). The protein extracted at pH 8.5 was much more brownish than that extracted at pH 7.0 (17.44 vs 4.19, respectively). Both samples were yellowish, but the one isolated at pH 8.5 was more intensely yellow (35.99) than the sample extracted at pH 7.0 (28.58). This pronounced color shift under alkaline extraction conditions can be attributed to the oxidative transformation of catechins, specifically the oxidative cleavage of the heterocyclic ring in flavan-3-ols, which leads to the formation of novel intermediates and structural modifications that could be responsible for the development of darker pigments (L. Y. Chen et al., 2018). This result aligns with previous studies, such as that of Wang et al. (2022), who demonstrated that the color of (−)-epigallocatechin-3-gallate in a model beverage system becomes increasingly red as pH rises from 2.2 to 8.5. These findings reinforce the role of pH in modulating catechins chemistry and its visual impact on protein extracts. The reduction in discoloration observed at pH 7.0 compared with pH 8.5 demonstrated that lowering the solubilization pH was an effective mitigation strategy against catechin-induced color formation. Therefore, pH 7.0 was selected for the subsequent evaluation of SB, GA, and Duralox® to determine whether complementary antioxidant systems could provide additional color stabilization beyond that achieved through pH reduction alone.

The addition of SB during protein extraction at pH 7.0 further inhibited the catechins-induced discoloration and resulted in a light-yellow appearance, as indicated by high L* and b* values (L* = 63.41, b* = 44.94) and a modest red component (a* = 4.75) when 0.1% SB was present. Increasing the SB concentration to 1.0% led to a slightly darker (L* = 58.51), marginally redder (a* = 5.18), and comparably yellow (b* = 43.75) protein extract. The overall lightness and strong yellow hue observed in both cases suggest that SB effectively mitigated the red coloration typically associated with catechins oxidation. This effect can be explained by the chemical reactivity of SB with quinones through a Michael addition mechanism. This produces stable bisulfite–quinone adducts that block further polymerization into colored products (Sreeramoju & Digenis, 2018). In addition, SB can reduce quinones back to their original catechol form, effectively preventing the polymerization of quinones by converting them back to less reactive catechols (Danilewicz, 2015). SB also reacts with hydrogen peroxide generated during catechol oxidation, further limiting oxidative damage. Interestingly, SB accelerates catechol oxidation by reducing Fe^{3+} to Fe^{2+}, which enhances the catalytic cycle. However, the rapid conversion of quinones ensures they are immediately captured or reduced by SB, preventing their accumulation and subsequent browning (Danilewicz, 2013). Together, these mechanisms explain why the samples remained light and strongly yellow rather than developing the darker red-brown hues typical of catechins oxidation at neutral pH. Thus, the observed color

Table 2
Colorimetric data of pea protein samples isolated at various solubilization pH's and in the presence of catechins in combination with various antioxidants: CIELAB coordinates (L*, a*, b*).

Treatments	$L^*(D_{65})$	$a^*(D_{65})$	$b^*(D_{65})$	ΔE^*_{ab}
Commercial pea protein	78.53 ± 1.71^{a}	4.66 ± 0.18^{d}	26.67 ± 0.78^{g}	–
Control (pH 8.5)	73.74 ± 3.35^{b}	1.61 ± 0.32^{f}	35.78 ± 1.69	10.73
Control (pH 7.0)	66.50 ± 3.17^{c}	5.86 ± 0.29^{b}	49.79 ± 2.65	26.09
Catechins (pH 8.5)	39.33 ± 0.58^{g}	17.44 ± 0.25^{a}	35.99 ± 0.44^{d}	42.27
Catechins (pH 7.0)	53.46 ± 1.24^{f}	4.19 ± 0.11^{e}	28.58 ± 0.65^{f}	25.15
Catechins +0.1% SB (pH 7.0)	63.41 ± 0.38^{d}	4.75 ± 0.09^{d}	44.94 ± 0.47^{b}	23.72
Catechins +1% SB (pH 7.0)	58.51 ± 0.65^{e}	5.18 ± 0.15^{c}	43.75 ± 0.44^{b}	26.32
Catechins +0.1% GA (pH 7.0)	54.77 ± 1.63^{f}	5.21 ± 0.15^{c}	37.06 ± 0.80^{d}	25.94
Catechins +1% GA (pH 7.0)	37.19 ± 1.05^{g}	1.66 ± 0.10^{f}	12.20 ± 0.35^{h}	43.90
Catechins + Duralox® (pH 7.0)	62.40 ± 1.34^{d}	6.09 ± 0.25^{b}	51.06 ± 1.50^{a}	29.28

Data are expressed as mean ± SD ($n = 5$). SB = sodium bisulfite; GA = gallic acid. ΔE^*_{ab} = (CIELAB color difference metric CIE 76) was calculated using Eq. 1. Different letters in the same column indicate statistical significance in the CIELAB coordinates ($p < 0,05$; Fischer test).



Fig. 2. Surface color of the pea proteins isolated at different solubilization pHs and in the presence of catechins in combination with various antioxidants. SB: sodium bisulfite, GA: gallic acid.

stabilization upon SB addition supports the hypothesis that quinone formation is responsible for the red color change and demonstrates that SB can be used to control color development during pea protein extraction with catechins.

The addition of gallic acid (GA) at 0.1% during pea protein isolation in the presence of catechins resulted in a relatively high lightness (L* = 54.77), along with pronounced red (a* = 5.21) and yellow (b* = 37.06) components. In contrast, increasing the GA concentration to 1.0% led to a significant reduction in all color parameters (L* = 37.19, a* = 1.66, b* = 12.2), indicating a markedly darker and less chromatic appearance. These findings suggest that GA addition is effective in modulating color only up to a certain concentration. Beyond this threshold, further increases in GA promote excessive darkening, likely due to enhanced oxidation and complexation reactions. At lower concentrations, GA may stabilize catechins molecules by limiting oxidative degradation, thereby preserving a lighter and more vibrant color. This stabilization effect aligns with previous reports showing that GA can improve the color stability of other phenolic compounds (Lin et al., 2023). However, at higher concentrations, GA appears to accelerate the formation of darker pigments through oxidative pathways (Yuann et al., 2021). Additionally, interactions between GA and catechins may lead to the formation of complex structures with increased light absorption, contributing to the observed darkening (Di Mattia et al., 2009). Considering this counterproductive effect, the following experiments focused exclusively on a GA concentration of 0.1%.

The colorimetric analysis of the pea proteins isolated in the presence of catechins and Duralox® solution demonstrates a lightness (L*) value of 62.40, indicating a moderately high brightness, with a slight greenish hue (a* = 6.09) and a pronounced yellow component (b* = 51.06), resulting in a vivid yellow-green appearance. Duralox® is an antioxidant blend that protects against oxidative deterioration, and its composition typically includes rosemary extract, ascorbic acid, tocopherols and citric acid (Wu et al., 2021). The synergistic combination of these ingredients enhances the oxidative stability of sensitive compounds like catechins, effectively inhibiting their degradation and minimizing browning reactions. This stabilization is reflected in the preserved color parameters observed in the catechins + Duralox® system, supporting the visual quality of the pea protein. Overall, the data suggests that Duralox®, through its multi-component natural antioxidant system, is also partially effective in maintaining the desirable color and stability of catechins-containing formulations under neutral pH conditions.

Color differences between the experimental protein samples and the commercial pea protein were quantified using the CIELAB ΔE*ab metric. All treatments exhibited ΔE*ab values well above the commonly accepted perceptibility threshold (ΔE*ab ≈ 2–3), indicating clearly visible color differences relative to the commercial reference. Among the control samples, adjustment of the extraction pH alone resulted in substantial color changes, with ΔE*ab values of 26.09 and 10.73 at pH 7.0 and 8.5, respectively. This indicates that alkaline conditions strongly affect product color, most likely due to enhanced pigment solubilization and non-enzymatic browning reactions.

The incorporation of catechins led to the largest color deviations, with ΔE*ab values exceeding 25 in all cases and reaching values above 40 for samples produced at pH 7.0 and for those containing 1.0% GA. These large ΔE*ab values are consistent with the pronounced decreases in L* and increases in b*, reflecting significant darkening and yellow-brown coloration. This behavior can be attributed to the intrinsic color of phenolic compounds and to their susceptibility to oxidation and polymerization under alkalinization.

The addition of antioxidant agents such as SB, GA, or Duralox® reduced neither the magnitude of the color differences nor restored color properties comparable to the commercial benchmark. Instead, ΔE*ab values remained high (≈ 24–29), indicating that, under the conditions tested, these additives were insufficient to mitigate overall color changes induced by catechin addition and processing pH.

Overall, the results demonstrate that both the reduction of solubilization pH to 7.0 and the strategic addition of SB or GA (only at low concentration) besides catechins can mitigate discoloration induced during pea protein extraction by catechins, but none of the investigated treatments achieved color characteristics close to those of the commercial product. However, color measurement alone does not fully capture the extent of oxidative degradation. Therefore, it is essential to analyze quinones in these samples, as studies indicate that these molecules are direct oxidation products of phenolic compounds and serve as key indicators of oxidative stress and pigment transformation.

3.2. Untargeted metabolomic characterization of color compounds generated in pea protein due to catechins oxidation

Considering the positive effect of a reduction in solubilization pH

and SB addition on color stabilization of pea protein (Fig. 2), proteins extracted with two SB concentrations (0.1% and 1.0%) were compared with catechins-only controls using untargeted metabolomics to identify the compounds responsible for color formation during the extraction.

To capture the diversity of oxidation products, the analysis focused on four targeted *m/z* regions: 280–295 for metabolites assigned to monomeric catechins and quinone intermediates, 570–610 for features consistent with oxidized catechin dimers, 850–890 for features consistent with oxidized trimers, and > 1140 for metabolites assigned to higher oligomers and thearubigin-like polymers. These classes of compounds have been reported as contributors to red-to-brown pigment formation during alkaline flavan-3-ol oxidation (Deshaies et al., 2022; Prasain et al., 2009; Tan et al., 2020).

The heatmap (Fig. 3A) shows the hierarchical clustering of metabolites (rows) across four extraction conditions. Both metabolites and samples cluster according to similarity in metabolic profiles. A clear separation among the four protein extraction conditions is visible, with protein isolated in the presence of catechins at pH 8.5 forming a distinct cluster characterized by widespread high-intensity (red) signals. These signals may correspond to metabolites arising from oxidized catechins dimers and oligomers that have previously been associated with red-to-brown pigment formation. In contrast, samples extracted using a combination of catechins and SB (both 1.0% and 0.1%) exhibited predominantly lower-intensity (blue) values across the same metabolite clusters, indicating a strong suppression of oxidation-derived metabolites. The protein extracted in the presence of catechins at pH 7.0 clusters between the SB-treated samples and the untreated alkaline control, reflecting partial mitigation of catechin oxidation through pH reduction alone. Overall, the heatmap demonstrates that protein isolation at pH 8.5 + catechins produces the highest levels of oxidation-derived catechin metabolites, whereas SB is the most effective treatment, resulting in the strongest global reduction in these metabolites. Lowering pH also mitigates oxidation-derived catechins products, but less effectively than SB. These patterns indicate that catechin oxidation is strongly pH-dependent and that SB effectively suppresses the metabolite signatures associated with red discoloration and lipid oxidation, thereby explaining the visible surface color displayed in Fig. 2.

The PLS-DA analysis revealed that the top VIP features in negative mode were significantly enriched in the reddish pea protein extracts (pH 8.5 + catechins) and markedly reduced in samples extracted at

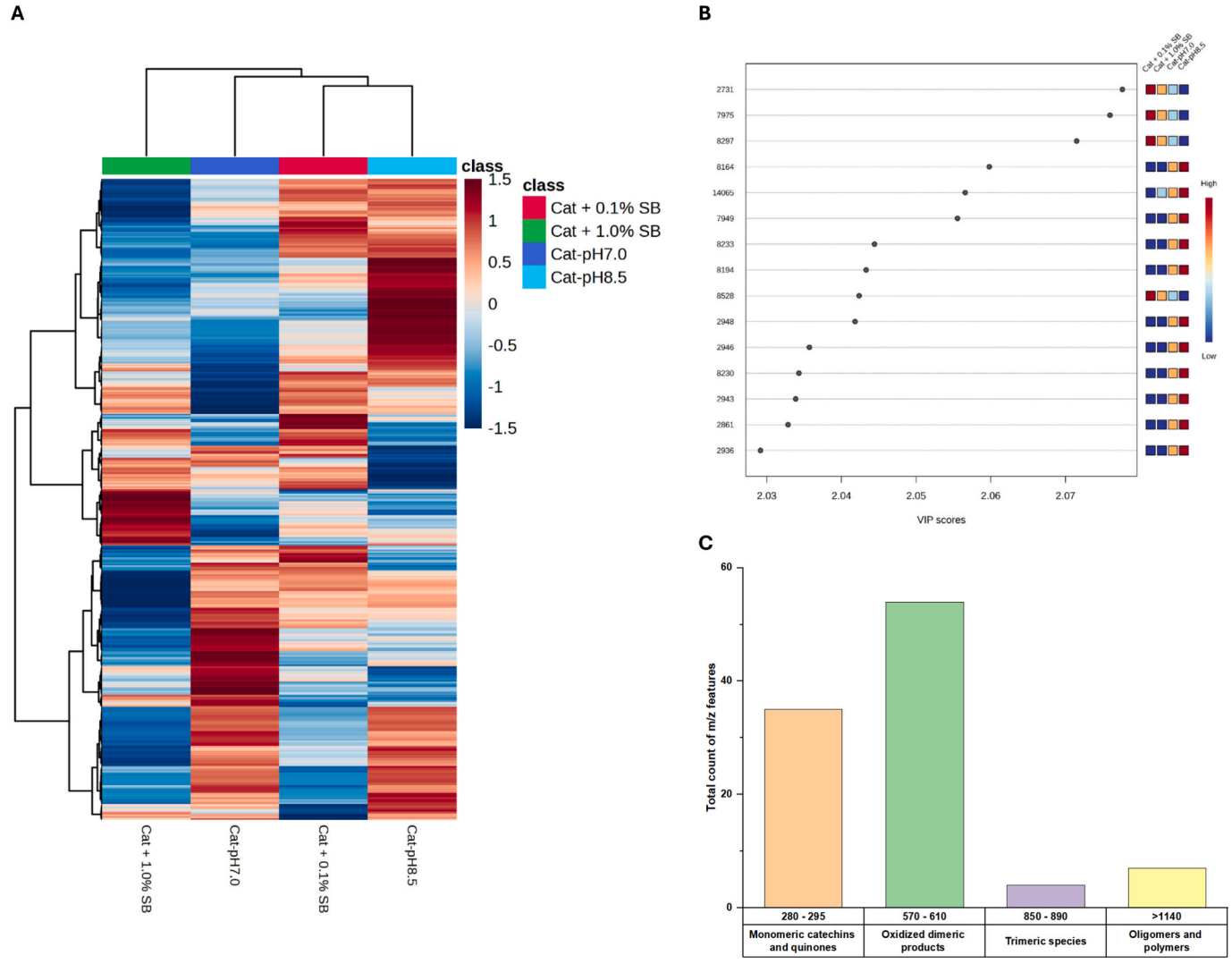

Fig. 3. Heatmap of quantified and normalized metabolites (rows) across pea protein extracted in the presence of catechins at pH 8.5, pH 7.0 and in combination with sodium bisulfite (SB) at pH 7.0. Color encodes abundance, with red = high and blue = low. Row and column dendrograms show hierarchical clustering based on similarity among metabolites and samples (a); PLS-DA Variable Importance in Projection (VIP) plot (Component 1) and corresponding heatmap of the top 25 features in negative ionization mode. Higher intensities (red) are observed in samples containing catechins at pH 8.5, whereas mitigation strategies result in substantially lower intensities (blue) (b); Number of features detected in each *m/z* range associated with catechin oxidation products (c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pH 7.0 or in the presence of SB (Fig. 3B). The clear predominance of dimeric oxidation products suggests that extractable catechin-derived oxidation products, particularly metabolites assigned to dehydrodicatechins and related quinone-coupling derivatives in the *m/z* 570–610 region, were closely associated with the red coloration observed in protein extracted in the presence of catechins. These compounds showed markedly elevated intensities in protein isolated in the presence of catechins at pH 8.5 and high VIP scores (>1.40). Conversely, their abundance decreased substantially when lower extraction pH (7.0) or SB was applied. This suppression pattern indicates that these mitigation strategies reduced alkaline-induced catechin auto-oxidation and subsequent dimer formation, thereby limiting the accumulation of red pigment precursors.

Among the first 100 metabolites with high VIP scores, 54 were assigned to oxidized dimers, followed by 35 monomeric catechins/quinones, four trimers and seven higher oligomers (Fig. 3C and the key metabolites are summarized in Supplementary material SS.1). Most were detected as deprotonated ions $[M–H]^-$, consistent with the acidic character of phenolic oxidation products (De Paepe et al., 2013). The oxidative behavior of catechins typically involves quinone-mediated coupling reactions, where catechol and pyrogallol groups generate dimers and higher oligomers through oxidative coupling (L. Y. Chen et al., 2018; Danilewicz, 2013). These reactions lead to dehydrocatechin-type A and B dimers, which exhibit characteristic visible-range absorption associated with reddish pigmentation (Osman et al., 2007). Similar mechanisms occur during black tea fermentation, where enzymatic catechins oxidation produces theaflavins and thearubigins, the red-orange pigments of black tea (Long et al., 2026).

Overall, the results suggest that extractable catechin-derived oxidation products, particularly metabolites assigned to dehydrodicatechins and related quinone-coupling derivatives, are important contributors to the red coloration observed during alkaline pea protein extraction. Their accumulation at pH 8.5 and suppression following pH reduction or SB addition further support the interpretation that controlling catechin oxidation is an effective strategy for limiting red pigment formation.

3.3. *Effect of modifications on formation of volatile organic compounds (VOC)*

Flavor is the primary sensory attribute of pea protein governing consumer acceptance and is driven by both volatile and non-volatile compounds. The volatile fraction, which is largely responsible for off-flavor perception, originates predominantly from lipid oxidation, with a secondary contribution from protein oxidation. Consequently, although pH adjustment and assistant antioxidant treatments have been applied to improve color and prevent discoloration induced by catechins, it is essential to ensure that these interventions do not compromise their effectiveness in mitigating lipid oxidation and the associated formation of off-flavors.

Table 3 shows that solubilization pH strongly influenced the VOC profile of the protein isolates. In particular, isovaleraldehyde, valeraldehyde, and 3-methyl-1-butanol were 3.2-, 3.3-, and 6.3-fold higher, respectively, in proteins solubilized at pH 8.5 compared to pH 7.0. The other compounds were more abundant at pH 7.0. This indicates that solubilization pH differentially modulates chemical pathways responsible for the generation of specific VOC. At pH 8.5, the more alkaline conditions promote protein unfolding and exposure of hydrophobic residues (Villacís-Chiriboga et al., 2023), facilitating interactions with lipid and carbohydrate-derived precursors. In parallel, higher pH accelerates amino acid degradation, including Strecker reactions, which yield branched-chain aldehydes and alcohols such as isovaleraldehyde and 3-methyl-1-butanol (Murat et al., 2013). Thus, the shift in VOC composition reflects a combined effect of protein conformational changes and enhanced precursor reactivity under alkaline conditions. On the other hand, pentanol, hexanal, hexanol, benzaldehyde, 1-octen-3-ol, pentylfuran, and nonanal were more abundant in protein isolated

Table 3
Quantitative comparison of volatile compounds (expressed in $\mu g.g^{-1}$ DW) in pea proteins isolated at different solubilization pH without and in the presence of different antioxidants determined by HS-SPME-GC–MS.

	Isovaleraldehyde	Valeraldehyde	3-methyl, 1-butanol	1-pentanol	Hexanal	1-hexanol	2-pentylfuran	1-octen-3-ol	Benzaldehyde	2-isopropyl-3methoxy-pyrazine	Nonanal
Control (pH 8.5)	0.26 ± 0.01^{bc}	0.26 ± 0.03^{ab}	54.63 ± 4.99^{a}	1.85 ± 0.04^{cd}	2.01 ± 0.13^{cd}	1.95 ± 0.09^{d}	0.20 ± 0.01^{ab}	1.96 ± 0.01^{ab}	0.12 ± 0.01^{b}	0.02 ± 0.00^{b}	0.10 ± 0.01^{ab}
Control (pH 7.0)	0.08 ± 0.03^{c}	0.08 ± 0.01^{b}	8.66 ± 4.87^{c}	7.33 ± 0.49^{a}	8.01 ± 0.48^{a}	2.11 ± 0.04^{cd}	0.58 ± 0.02^{a}	2.58 ± 0.01^{ab}	0.44 ± 0.01^{ab}	0.01 ± 0.02^{b}	0.34 ± 0.01^{a}
Catechins (pH 8.5)	0.01 ± 0.00^{c}	0.01 ± 0.01^{b}	7.26 ± 1.39^{c}	3.02 ± 0.31^{bc}	1.96 ± 0.07^{cd}	2.93 ± 0.03^{a}	0.06 ± 0.01^{b}	0.71 ± 0.14^{b}	0.13 ± 0.01^{b}	0.01 ± 0.00^{b}	0.02 ± 0.01^{b}
Catechins (pH 7.0)	0.14 ± 0.03^{c}	0.23 ± 0.03^{ab}	22.72 ± 14.77^{bc}	3.54 ± 0.17^{bc}	4.03 ± 0.04^{bc}	2.13 ± 0.01^{cd}	0.24 ± 0.01^{ab}	2.12 ± 0.16^{ab}	0.17 ± 0.02^{b}	0.02 ± 0.02^{ab}	0.17 ± 0.01^{ab}
Catechins + GA 0.1% (pH 7.0)	0.12 ± 0.01^{c}	0.03 ± 0.01^{b}	18.01 ± 2.07^{c}	2.69 ± 0.04^{bc}	2.79 ± 0.03^{bc}	2.14 ± 0.04^{c}	0.23 ± 0.00^{ab}	1.32 ± 0.04^{b}	0.16 ± 0.01^{b}	0.02 ± 0.00^{ab}	0.14 ± 0.01^{ab}
Catechins + SB 0.1% (pH 7.0)	0.12 ± 0.05^{ab}	0.17 ± 0.01^{a}	53.09 ± 0.34^{a}	0.33 ± 0.04^{cd}	0.55 ± 0.01^{bc}	2.23 ± 0.18^{b}	0.11 ± 0.01^{a}	1.18 ± 0.22^{a}	0.66 ± 0.04^{a}	0.02 ± 0.05^{a}	0.06 ± 0.01^{a}
Catechins + SB 1% (pH 7.0)	0.00 ± 0.00^{c}	0.11 ± 0.02^{b}	42.77 ± 11.43^{ab}	0.55 ± 0.26^{d}	0.11 ± 0.01^{d}	1.63 ± 0.01^{c}	0.06 ± 0.00^{b}	0.61 ± 0.02^{b}	0.08 ± 0.03^{b}	0.02 ± 0.01^{ab}	0.01 ± 0.00^{b}
Catechins 25% + Duralox® 75% (pH 7.0)	0.59 ± 0.05^{a}	0.04 ± 0.01^{b}	16.17 ± 0.87^{c}	1.81 ± 0.16^{cd}	1.85 ± 0.01^{cd}	2.07 ± 0.13^{cd}	0.19 ± 0.01^{ab}	1.37 ± 0.05^{b}	0.09 ± 0.04^{b}	0.01 ± 0.00^{b}	0.11 ± 0.01^{ab}

Data are expressed as mean ± SD. Values with different letters within a column indicate significant differences ($p < 0.05$) ($n = 2$). GA = gallic acid; SB = sodium bisulfite. Volatile compounds were identified by comparison of retention times and mass spectra with authentic analytical standards analyzed under identical HS-SPME-GC–MS conditions.

using solubilization at pH 7.0. This pattern is consistent with higher LOX activity in pea under near-neutral conditions (Liburdi et al., 2021). LOX catalyzes the oxidation of unsaturated fatty acids, generating oxylipins that break down into volatile off-flavor compounds, including aldehydes (hexanal, nonanal), alcohols (hexanol, pentanol), and furan derivatives (pentylfuran) (Gultekin Subasi et al., 2024).

As a general trend, the addition of catechins to the extraction system significantly decreased the VOC content in the samples, compared to the controls. Catechins mitigate VOC formation by inhibiting LOX, scavenging free radicals, and trapping reactive intermediates (dicarbonyls) (Villacis-Chiriboga et al., 2026) that would otherwise generate aldehydes, ketones, and other compounds responsible for beany, grassy, or rancid notes (Gultekin Subasi et al., 2024; Ishii et al., 2010; Soendjaja & Girard, 2024). When catechins were included in the extraction system, most VOC concentrations decreased compared to the controls without antioxidants, except for 2-isopropyl-3-methoxy-pyrazine, which increased by ~1.3-fold at pH 7.0 but decreased ~12-fold at pH 8.5 compared with their counterparts without catechins. At neutral pH, pyrazines can form dimers and polymers, which may affect their concentration and interactions with other compounds (Peral & Gallego, 2003). However, such processes would be expected to reduce volatility and free monomer availability. Therefore, the observed increase in 2-isopropyl-3-methoxy-pyrazine at pH 7.0 could be related to changes in matrix interactions or partitioning rather than aggregation phenomena. Overall, the amount of the studied VOC was between 1.2 and 22.1 times higher in the pea proteins extracted in the presence of catechins at pH 7.0 than in the proteins extracted at pH 8.5.

In line with these findings, the addition of SB at concentrations of 0.1% and 1.0% significantly ($p < 0.05$) reduced VOC levels compared with the protein extracted only in the presence of catechins, with the greatest reductions observed at 1.0%. At this concentration, compound levels were lowered by factors ranging from 0.6-fold for 1-pentanol to as much as 19-fold for nonanal. SB's ability to significantly reduce the detected VOC levels could be attributed to its chemical reactivity with carbonyl-containing compounds, forming stable, non-volatile bisulfite adducts. This reaction is well-documented in various contexts, including the conversion of aldehyde bisulfite adducts to nitriles (J. Zhu et al., 2012). This reaction effectively sequesters the volatiles, preventing their release into the headspace and thus reducing detectable off-flavors. The general reaction for aldehydes is schemed in Eq. 3:

$$R-CHO+HSO_3^- \rightleftharpoons R-CH(OH)SO_3^- \quad (3)$$

Considering that SB was included in a system where catechins were the main antioxidant, the reduction of VOC could be due to a synergistic effect. As shown previously, catechins mitigate the activity of LOX and scavenge free radicals (Villacis-Chiriboga et al., 2026), thereby preventing the degradation of polyunsaturated fatty acids (PUFAs) into volatile molecules. Subsequently, SB acts downstream, trapping already-formed volatiles through adduct formation (Sallay et al., 2009). This dual action allows catechins to slow the rate of VOC production, while SB more efficiently handles the reduced load of breakthrough volatiles. Although both catechins and SB have been extensively studied for their antioxidant properties, to our knowledge, no previous work has specifically evaluated their synergistic effects on volatile compound formation, emphasizing the importance of this research.

Interestingly, the addition of SB led to a significant increase in hexanol, benzaldehyde, and 2-isopropyl-3-methoxy-pyrazine, particularly at 0.1% compared to the control samples. For hexanol and benzaldehyde, this increase likely reflects insufficient SB concentration to achieve complete adduct formation. At 0.1%, SB may partially quench oxidative intermediates, yet the amount of VOC generated still exceeds its trapping capacity. In contrast, 2-isopropyl-3-methoxy-pyrazine is not a carbonyl compound (Peral & Gallego, 2003), and, therefore, is unaffected by SB's adduct-forming mechanism. These findings suggest that SB's effects on non-carbonyl VOC are compound-specific and may not always act synergistically with catechins across all volatile species. When GA was added at 0.1%, a significant ($p < 0.05$) reduction in VOC levels was observed compared to the controls. These results indicate a synergistic effect between catechins and GA in limiting the formation of specific VOC. The combination of these compounds appears to enhance overall antioxidant protection by stabilizing fatty acids and preventing oxidative damage, thereby reducing the generation of undesirable volatiles in pulse protein systems.

Overall, the modifications applied to mitigate catechins-induced discoloration in pea protein, specifically reducing solubilization pH to 7.0 and the incorporation of SB or GA, generally preserved or enhanced catechins' effectiveness in suppressing volatile off-flavor compounds in pea protein isolates. Alkaline solubilization at pH 7.0 substantially reduced total VOC levels while SB addition synergistically lowered most carbonyl-containing VOC through adduct formation, yielding pronounced reductions alongside catechins' upstream inhibition of oxidation.

3.4. Effect of antioxidant mixing on the formation of HODE and MDA

To assess lipid oxidation during pea protein extraction, HODE and MDA were quantified as markers of primary and secondary lipid oxidation, respectively. HODE reflects early lipid peroxidation and LOX activity, whereas MDA is formed during the degradation of polyunsaturated fatty acids at later staged of oxidation (Gläser et al., 2021; Z. Wang et al., 2019).

Protein extraction pH had a marked influence on HODE concentration (Fig. 4A). At pH 7.0, the HODE content reached 2.53 $\mu g.g^{-1}$ DW, which was 1.36 times higher than the value measured at pH 8.5 (1.87 $\mu g.g^{-1}$ DW). This trend closely paralleled the distribution of most volatile compounds. The observed differences can be partly explained by the pH dependence of LOX activity. LOX enzymes, which catalyze HODE formation, often exhibit higher activity under near-neutral conditions than under slightly alkaline conditions (Liburdi et al., 2021), resulting in greater oxidation of linoleic acid and HODE production.

The addition of catechins significantly reduced ($p < 0.05$) the HODE content of the pea protein isolates, with both treatments exhibiting an average value of 0.18 $\mu g{\cdot}g^{-1}$ DW, as compared to the controls. The slightly higher HODE content in the sample extracted in the presence of catechins at pH 7.0 could be attributed to the same mechanism observed in control samples, where a near-neutral environment favors LOX catalytic activity. The reduction in HODE levels compared to controls can be mechanistically explained by the antioxidant action of catechins, which trap reactive carbonyl intermediates, scavenge radicals, and chelate pro-oxidant metals, thereby interrupting key steps in lipid peroxidation and limiting HODE formation (Soendjaja & Girard, 2024).

The addition of SB and GA significantly influenced the HODE content of the protein extracts ($p < 0.05$), resulting in a ~ 5-fold reduction relative to the control samples. However, the HODE levels in protein extracted with SB and GA (0.26 $\mu g.g^{-1}$ DW) were similar to those observed when catechins were used alone, indicating no further improvement beyond the effect of catechins. Nevertheless, although the mixture of catechins, SB, and GA did not reduce HODE beyond that achieved by catechins alone, it also did not worsen lipid oxidation. This observation indicates that factors beyond HODE formation may contribute to the differences observed in volatile compound levels. The elevated HODE levels in the SB 1.0% treatment may indicate a partial shift in oxidation toward this more stable product (Sharifi et al., 2026), which could contribute to a reduced formation of certain VOC. At 0.1% SB + catechins, HODE was quantified at 0.76 $\mu g{\cdot}g^{-1}$ DW, which was 2.1 times higher than at 1.0% SB + catechins (0.36 $\mu g{\cdot}g^{-1}$ DW), while the addition of GA markedly reduced the HODE content to 0.16 $\mu g{\cdot}g^{-1}$ DW. Although the direct effect of SB on HODE levels in the presence of catechins has not been reported, SB is known to influence the stability and degradation of antioxidants and other bioactive compounds, potentially altering oxidative conditions. The present results suggest a

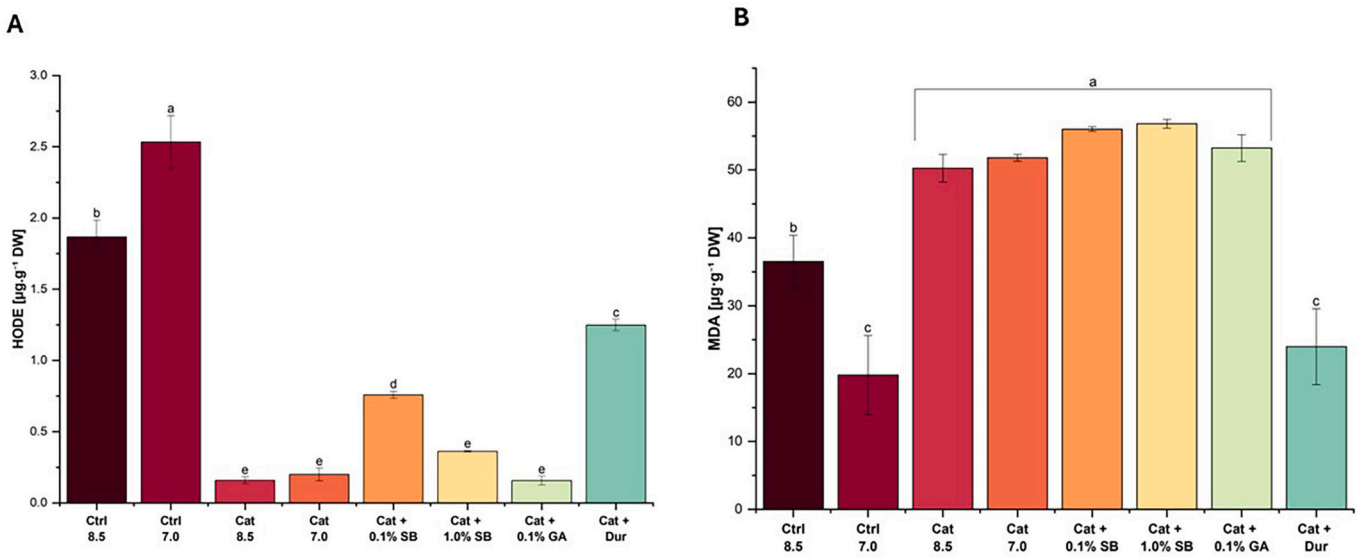

Fig. 4. Lipid oxidation products HODE (A) and MDA (B) in pea proteins isolated at different solubilization pH and in the presence of catechins and its combination with different complementary antioxidants. SB: sodium bisulfite; GA: gallic acid. Different small letters represent statistically significant differences across the samples ($p < 0.05$)

concentration-dependent effect of SB. Thus, at 0.1%, its protective capacity may be insufficient to fully suppress oxylipins production, and interactions with catechins may alter antioxidant efficiency, resulting in higher HODE levels. While at 1.0%, the stronger reducing capacity of SB more effectively limits HODE formation. The reduction in HODE upon GA addition indicates that GA brings the oxidation level down to that achieved by catechins alone, without providing additional enhancement. Previous studies have shown that combining antioxidants could enhance the overall antioxidant capacity through electron donation and radical stabilization (Mittal et al., 2023). Moreover, GA and catechins can effectively inhibit lipid oxidation by stabilizing colloidal properties and preventing both primary and secondary oxidation processes, thereby limiting HODE formation (Di Mattia et al., 2009). The parallel trends observed in HODE content and volatile composition suggest that both parameters are modulated by oxidative processes and the stabilizing effects of SB and GA.

The catechins-Duralox® system was the least effective antioxidant treatment among those evaluated, as the HODE content (1.25 µg·g^{-1} DW) was higher than the rest of the samples, but still significantly lower than the controls. In this sample, the inclusion of two antioxidants can play a parallel role. Catechins, on one hand, have demonstrated significant antioxidant activity and LOX mitigation, while Duralox® has been evaluated as less efficient, and has also demonstrated pro-oxidant activities (Villacis-Chiriboga et al., 2026). Hence, an imbalanced interaction between antioxidant and pro-oxidant effects may explain the reduced overall efficiency of the combined system.

An apparent inverse relationship was observed between HODE and MDA concentrations. While HODE is commonly considered a marker of primary lipid oxidation and MDA a marker of secondary lipid oxidation, the relationship between these compounds is complex and not necessarily linear. Their formation originates from different stages of lipid oxidation and may be influenced by distinct reaction pathways and reaction kinetics (Li Pomi et al., 2025).

The extraction pH had a significant impact on MDA levels: higher extraction pH was associated with higher MDA content (36.5 µg.g^{-1} DW at pH 8.5 vs. 19.8 µg.g^{-1} DW at pH 7.0). This observation is consistent with the increased oxidative stress associated with alkaline conditions, which can promote lipid peroxidation and the formation of secondary oxidation products such as MDA (Ma et al., 2019). Interestingly, samples containing catechins, SB or GA resulted in relatively consistent MDA levels across different extraction pH, ranging from 50.25 to 56.8 µg.g^{-1} DW. Catechins and GA are phenolic antioxidants known for their capacity to neutralize free radicals and ROS during extraction, thereby mitigating lipid oxidation in pea protein extracts. However, these antioxidants did not decrease the formation of MDA. This indicates that the different oxidation markers did not respond uniformly to the antioxidant treatments. The results demonstrate that MDA, HODE, and volatile compounds reflect different aspects of the oxidation process and may therefore respond differently to antioxidant interventions. Furthermore, the analytical method employed quantified only free MDA and did not assess potential interactions between MDA and the protein matrix. Therefore, matrix-dependent binding, release, or other transformations of MDA cannot be excluded and may have contributed to the observed concentrations. Consequently, evaluation of lipid oxidation in pea protein systems benefits from the combined assessment of multiple markers rather than reliance on a single compound.

Conversely, the combination of Duralox® with catechins resulted in a notably lower MDA concentration of 23.9 µg·g^{-1} DW. This reduction may be linked to the presence of ascorbic acid in Duralox®, which can act as a pro-oxidant in the presence of transition metals by reducing Fe^{3+} to Fe^{2+}, thereby catalyzing Fenton-type reactions and accelerating oxidative processes (Reis et al., 2006). However, the mechanisms underlying the lower MDA concentrations observed in this treatment cannot be conclusively established from the present results and therefore require further investigation.

Overall, the combined HODE, MDA, and VOC data indicate that catechins were the primary contributors to oxidative stabilization during pea protein isolation, whereas SB and GA largely preserved this protection without providing substantial additional suppression of lipid oxidation markers.

3.5. Total phenolic compounds

Fig. 5A presents the total phenolic compound (TPC) content in the protein samples subjected to various treatments, expressed as mg gallic acid equivalents (GAE) per g dry weight (DW). In the control samples at both pH 7.0 and 8.5, the TPC was minimal, approximately 0.52 mg GAE·g^{-1} DW, indicating that baseline extraction yields low levels of phenolics. Since no antioxidants were added in these samples, these values reflect the native phenolic composition of pea protein. These results are consistent with previously reported ranges for native pea phenolic content, which vary between 0.37 and 1.03 mg GAE·g^{-1} DW, as

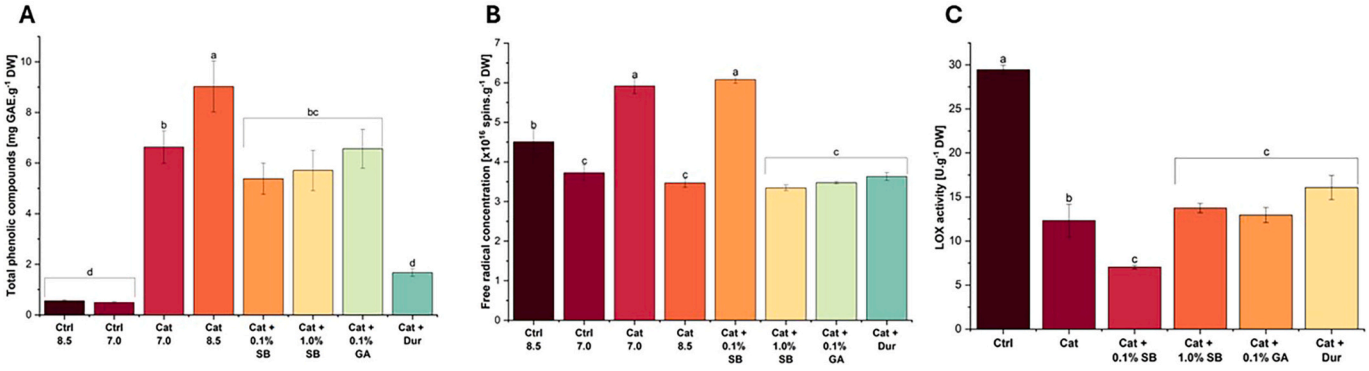

Fig. 5. Total phenolic content (TPC) (A) and free radical content (B) in pea protein isolate and LOX activity of their homogenates (C) from various extraction pH and in the presence of catechins and/or its combination with complementary antioxidants. Different small letters represent statistically significant differences across the samples (Fisher test; $p < 0.05$). GAE = gallic acid equivalent; SB = sodium bisulfite; GA = Gallic acid.

documented by Mohamed et al. (2022).

Catechin addition markedly increased TPC, with values of 9.02 and 6.63 mg GAE·g^{-1} DW for samples extracted at pH 8.5 and 7.0, respectively. Since the same catechin concentration was used in both treatments, the higher TPC at pH 8.5 indicates that extraction conditions influenced the abundance and/or reactivity of compounds detected by the FC assay, likely through increased protein unfolding and exposure of interaction sites (Dai et al., 2022). The higher FC values observed at pH 8.5 coincided with reduced VOC formation and lower oxidation marker levels.

The mixture of SB and catechins exhibited a TPC averaging 5.54 mg GAE·g^{-1} DW. Although SB is recognized as an antioxidant and the FC method is based on redox chemistry, the increase in SB concentration from 0.1% to 1.0% did not result in a significant rise in TPC. This outcome reflects the chemical specificity of the FC assay and the nature of SB itself. The FC method is designed to quantify phenolic compounds by reacting with phenolic hydroxyl groups, capable of reducing the FC reagent, producing a measurable color change (Slinkard & Singleton, 1977). SB, however, lacks phenolic hydroxyl groups. As a sulfur-based reducing agent SB scavenges reactive oxygen species and prevents oxidation, but its contribution to the FC response differs from that of phenolic hydroxyl groups (Sudha et al., 2022). Moreover, when compared to samples containing only catechins, the addition of SB resulted in a lower TPC. This reduction may be attributed to chemical interactions between SB and catechins, which can alter catechins structure or reduce their availability for detection by the FC assay. One such interaction is sulfonation, where a sulfonic acid group is added to the catechins molecule, typically at the C-4 position, forming 4β-sulfonated derivatives (Bonaldo et al., 2020). These modifications can alter the reactivity of catechin-derived compounds toward the FC reagent, thereby contributing to the lower measured TPC values. However, this apparent reduction in TPC does not reflect a loss in antioxidant efficacy. Despite the lower TPC values measured by the FC assay, the combination of catechins and SB still significantly reduced VOC and HODE formation, indicating that its ability to improve oxidative stability was maintained. Therefore, the lower FC response observed in the SB-containing samples should not be interpreted as evidence of reduced antioxidant performance.

The inclusion of GA at 0.1% modestly increased the TPC to 6.65 mg GAE·g^{-1} DW compared with the sample treated with catechins alone but not significantly ($p < 0.05$). During protein isolation, catechins may have undergone structural transformations, forming more complex molecules such as dimeric theaflavins or high-molecular-weight polymers (See section 3.2) through covalent cross-linking with GA. These reactions reduce the concentration of free monomeric catechins available for detection by the FC assay (Zuwei et al., 2025). Moreover, the interaction between GA and catechins is often antagonistic, potentially diminishing the overall antioxidant capacity and leading to a lower apparent phenolic content (Vicol & Duca, 2023). This antagonism may result from competitive binding or structural masking of reactive hydroxyl groups, which are essential for FC reactivity. This apparent decrease did not correlate with the formation of VOC or oxidation markers. In fact, these markers were significantly reduced in the GA–catechins-treated sample, suggesting that the antioxidant potential of the complexed molecules remained effective. This implies that while the FC assay may underestimate phenolic content due to structural modifications, the functional antioxidant activity, particularly in limiting lipid oxidation and off-flavor formation, was preserved. Conversely, the TPC detected in the sample extracted with Duralox® and catechins was the lowest among all antioxidant-enriched treatments, with a value of 1.67 mg GAE·g^{-1} DW, which is due to the lower catechins content in the system.

3.6. Free radical quantification via electron paramagnetic resonance

Free radicals play a pivotal role in the development of lipid oxidation-associated off-flavors in pea protein isolates (Villacis-Chiriboga et al., 2026). These reactive species initiate and propagate oxidative degradation of PUFAs, leading to the formation of VOC (Gultekin Subasi et al., 2024). Given their central role in oxidative deterioration, quantifying free radical content provides critical insight into the oxidative status of protein-and-lipid-rich systems. This is particularly important when evaluating the efficacy of antioxidant interventions during protein extraction, which enables the assessment of oxidative suppression at the molecular level, offering a mechanistic understanding of flavor stabilization strategies.

Fig. 5B depicts the EPR-detected free radical concentration in pea protein extracted under different conditions. A free radical content of 3.73×10^{16} spins·g^{-1} DW was observed in the protein isolated at pH 7.0, which was lower than the protein isolated at pH 8.5 (4.51×10^{16} spins·g^{-1} DW). This suggests that the more alkaline extraction condition promoted oxidative reactions during protein isolation. At higher pH, endogenous lipids are more prone to oxidation, and oxylipins may decompose more readily into radical species, contributing to the higher EPR signal. In addition, alkaline conditions can increase the reactivity of protein side chains, including thiols, thereby facilitating protein oxidation (Kurz et al., 2020; Turell et al., 2020). These observations are consistent with the oxidation markers, as the higher MDA content detected in proteins extracted at pH 8.5 indicates more extensive secondary lipid oxidation under alkaline conditions.

Protein extracted in the presence of catechins at pH 8.5 exhibited a lower free radical concentration than that extracted at pH 7.0 (3.47×10^{16} spins·g^{-1} DW vs. 5.92×10^{16} spins·g^{-1} DW, respectively). Notably, this suggests a potential role of catechins in modulating free radical levels in pea protein. As shown in Fig. 5A, the phenolic content of pea protein extracted in the presence of catechins at pH 8.5 was

significantly higher compared with the one recovered at pH 7.0. Studies have demonstrated that extractability and, therefore, the antioxidant activity of phenolic compounds increase with pH (L. Y. Chen et al., 2018). This is attributed to the enhanced electron-donating ability of phenols upon deprotonation in alkaline environments, which stabilizes them and improves radical scavenging activity. Moreover, in alkaline conditions, phenolic compounds can undergo polymerization reactions, forming new oxidizable hydroxyl moieties in their polymeric products, resulting in higher radical scavenging activity (Altunkaya et al., 2016). This reversal suggests that catechins mitigate free radical formation or persistence during pea protein isolation, but their effectiveness is affected by the protein isolation pH.

The relationship between SB concentration and free radical content during protein extraction at pH 7.0 reflects the dual role of SB as both a reducing agent and antioxidant. At 0.1% SB, the low concentration partially disrupts protein aggregates, exposing reactive groups (e.g., lysine or thiol residues) that facilitate redox cycling and oxygen interactions (Kurz et al., 2020), leading to a higher free radical content of 6.07×10^{16} spins·g^{-1} DW. In contrast, higher SB concentrations enhance protein stabilization and radical scavenging (Du et al., 2024), lowering free radical abundance to 3.34×10^{16} spins·g^{-1} DW. The TPC remained constant (~5.5 mg GAEq·g^{-1} DW), and lipid oxidation markers (HODE and MDA) showed little variation, suggesting that the effect of SB is driven mainly by changes in protein structure and redox environment. Supporting this, SB is known to dissociate high-molecular-weight protein aggregates (e.g., soy β-conglycinin), alter secondary structures, and promote lysine ionization (Zhang & Sun, 2010). Higher SB concentrations also favor covalent complexation with phenolic compounds, enhancing radical scavenging (Du et al., 2024), while preventing oxidation and covalent binding of phenolics to proteins under neutral conditions (Pickardt et al., 2011).

Addition of both GA at 0.1% and catechins + Duralox® during pea protein extraction at pH 7.0 maintained free radical abundance at relatively low levels of 3.47×10^{16} and 3.63×10^{16} spins·g^{-1} DW, respectively, and very close to the control samples recovered at pH 7.0 without catechins. This reduction aligns with the higher TPC content (6.57 mg GAEq·g^{-1} DW), and the low HODE levels observed in the presence of GA, indicating effective mitigation of oxidative processes by the catechins + GA mixture. Both catechins and GA are potent polyphenols with complementary antioxidant mechanisms: GA scavenges a broad range of reactive oxygen, nitrogen, and sulfur species through hydrogen donation (Mittal et al., 2023), while catechins provide additional radical-quenching capacity. Their combination enhances antioxidant performance through synergistic interactions, including the formation of stable protein–polyphenol complexes, which improve and radical scavenging (Liu et al., 2025). This synergy is particularly relevant during pea protein extraction, where minimizing oxidative damage is essential. Regarding the effect of Duralox®, the TPC in this protein was among the lowest in the proteins (1.63 mg GAEq·g^{-1} DW), which is consistent with its HODE, which was the highest among the samples. This divergence arises because Duralox® is less efficient at preventing lipid peroxidation of polyunsaturated fatty acids in pea protein extracts, as has been previously shown (Villacis-Chiriboga et al., 2026). More specifically, ascorbic acid in Duralox® can reduce Fe^{3+} to Fe^{2+}, enhancing metal-driven redox cycling (Reis et al., 2006). The synergy with catechins mitigates free radical abundance but is less effective against lipid-specific oxidation, explaining the high HODE content in Duralox®-treated samples.

3.7. Effect of antioxidant mixtures on LOX activity

Although LOX is ubiquitous in peas, its stability is strongly influenced by pH. Considering this, in this part of the study, solubilized pea flour was examined under different antioxidant systems while maintaining constant pH conditions in the homogenate without any pH adjustment. No further protein extraction steps were performed, as pH-shifting would reduce LOX activity and potentially mask the effects of the applied antioxidants. The results in Fig. 5C show that LOX activity was markedly affected by the presence of antioxidants and other molecules in the system.

The control sample, which lacked antioxidants, exhibited the highest LOX activity (29.5 LOX units·g^{-1} DW). This highest value represents the baseline for LOX activity, and thus it corresponds with the greatest volatile formation and the highest oxidation marker levels in the control group, regardless of solubilization pH. However, this activity decreased 2.3-fold after enriching the extraction medium with antioxidants.

Catechins inclusion in the system decreased LOX activity to 12.3 LOX units·g^{-1} DW. This reduction likely results from catechins acting as mixed non-competitive LOX inhibitors, binding at sites distinct from the active center and altering the enzyme's conformation, thereby decreasing its catalytic efficiency (Q. Cao et al., 2020). In addition, catechins chelate the non-heme iron in the catalytic site, interrupting the propagation of lipid peroxidation products (Mahesha et al., 2007). This inhibitory effect aligns with the observed decrease in HODE and volatile compounds when catechins were present in the protein solubilization medium. At the same time, an inverse correlation with TPC in the extract further highlights the influence of catechins concentration (measured as TPC) on LOX activity. Overall, the marked reduction in LOX activity following catechins addition stresses the central role of enzymatic oxidation inhibition in catechins efficacy. Since HODE is mainly formed through LOX-catalyzed reactions, its substantial reduction provides additional evidence of this inhibition.

Notably, the lowest LOX activities were observed in samples containing both catechins and SB at 0.1% and 1.0%, showing values of 7.04 and 11.04 LOX units·g^{-1} DW, respectively. Interestingly, the TPC in these samples was not among the highest, indicating that the addition of SB enhanced the inhibitory effect beyond that of catechins alone. Consistent with the previous results, the pronounced reduction in LOX activity was accompanied by the lowest levels of HODE detected and a marked decrease in VOC. Furthermore, its slightly acidic nature can disrupt the enzyme's conformational structure or active site functionality, thereby impairing LOX activity, as LOX typically exhibits optimal activity near neutral pH (Esan et al., 2023). Overall, these results suggest that in samples containing both catechins and SB, LOX inhibition was not solely driven by phenolic interactions, but also by the direct antioxidant and reducing properties of SB, which played a fundamental role in minimizing enzymatic oxidation.

A similar effect on LOX was observed after the addition of catechins and GA, resulting in 12.9 LOX units.g^{-1} DW. The inhibition mechanism of GA on LOX has been reported to be non-competitive, similar to that of catechins, as demonstrated by Lineweaver-Burk analyses (Al-Lehebe et al., 2022). This indicates that GA binds to a site distinct from the active site on the enzyme, modifying its conformation and reducing catalytic activity without directly competing with the substrate. However, when compared with other polyphenols, GA exhibits a weaker inhibitory effect on LOX activity than quercetin and catechins (Ye et al., 2023), placing it in a moderate position relative to catechins alone.

The LOX activity measured after the addition of catechins and Duralox® was relatively modest, ranking among the higher values within the antioxidant-treated samples (16.08 LOX units·g^{-1} DW). This elevated activity, second only to the control sample, corresponded with the oxidation markers HODE and MDA, which also followed the same trend. In this formulation, Duralox® accounted for the largest proportion of the antioxidant fraction compared to catechins (75% vs. 25%, respectively), likely overshadowing the contribution of catechins. Duralox® comprises rosemary extract, ascorbic acid, tocopherols, and citric acid (Wu et al., 2021), all recognized for their strong antioxidant capacities. However, despite this composition, the simultaneous increase in LOX activity and oxidation products suggests that non-enzymatic pathways, possibly driven by redox-active components such as ascorbic acid, played a compensatory or even dominant role in promoting downstream oxidation. Similarly, previous findings have shown

that Duralox® did not produce significant LOX inhibition in pea protein systems, as compared to catechins (Villacis-Chiriboga et al., 2026).

Overall, catechins alone effectively mitigated LOX activity, with no evident improvements when combined with other antioxidants. However, the inclusion of SB at 0.1% reduced LOX activity to the lowest level observed among the samples, demonstrating that enriching the extraction system with this compound not only limits pea protein discoloration but also suppresses LOX catalytic activity, the main mechanism underlying the improved oxidative stability.

3.8. Multivariate statistics

The PCA performed on volatile markers, enzymatic and radical oxidation indices, total phenolics, and color attributes yielded six principal components, of which the first three had eigenvalues of $\lambda_1 = 6.21$, $\lambda_2 = 5.36$, and $\lambda_3 = 3.82$, respectively. All exceeded the Kaiser criterion ($\lambda > 1$), confirming that they captured meaningful structure within the dataset. The first three principal components explained 85.51% of the total variance (PC1: 34.52%, PC2: 29.78% and PC3: 21.21%), indicating that these dimensions capture the dominant sources of variability in the dataset. The 3D plot in Fig. 6 reveals distinct oxidative pathways modulated by the treatments.

LOX and HODE load strongly and colinearly on the positive side of PC2, forming a coherent axis associated with lipoxygenase-mediated primary oxidation, together with 1-octen-3-ol. In contrast, the cluster comprising hexanal, nonanal, 2-pentylfuran, 1-pentanol, and isovaleraldehyde loads on the negative side of PC2, indicating a shared variance characteristic of secondary autoxidation, and the obtuse angles relative to LOX/HODE demonstrate a marked inverse association between enzymatic lipid oxidation and autoxidation-derived aldehydes.

Along PC1, TPC and the chromatic attributes a* and b* project negatively and remain largely orthogonal to PC2, confirming that variation in phenolic content and color development arises primarily from treatment effects. L* loads modestly on PC2, reflecting small but measurable lightness differences across treatments. The third component is driven predominantly by EPR and MDA, indicating a radical-driven oxidative dimension that is largely independent of both enzymatic oxidation (PC2) and aldehyde formation (PC2 negative). The partial alignment of EPR with 3-methyl-1-butanol and valeraldehyde suggests the presence of a distinct radical-linked chemistry, potentially related to Strecker-type aldehyde formation or radical-mediated alcohol generation, that does not align with the main lipid-oxidation axes.

The score plot further illustrates how treatments modulate oxidation pathways. The control sample, extracted at pH 8.5 projects strongly in the direction of the LOX/HODE vectors, indicating a high lipoxygenase activity and accumulation of primary oxidation products. When catechins were included at a solubilization pH 7.0, a shift away from this enzymatic-oxidation axis and from the aldehyde/furan cluster was seen, indicating suppression of both LOX activity and secondary volatile formation. Catechins + 1.0% SB significantly deviates from color coordinates a* and b*, reflecting an increased chromatic intensity, while simultaneously remaining distant from LOX/HODE, corroborating the positive effect of SB inclusion to the system in terms of color preservation and off-flavor formation. Catechins + 0.1% SB shows a partial shift toward positive PC3, consistent with a moderate increase in radical-related variance (Fig. 3B), as suggested by its proximity to the EPR/MDA axis. Catechins + 0.1% GA loads on negative PC1 alongside a* and b*, demonstrating a pronounced color effect, and simultaneously remains far from both aldehydes and LOX/HODE, suggesting effective suppression of both enzymatic oxidation and secondary aldehyde accumulation, while producing pea protein discoloration during extraction. Catechins + Duralox® occupies the negative PC2 region near

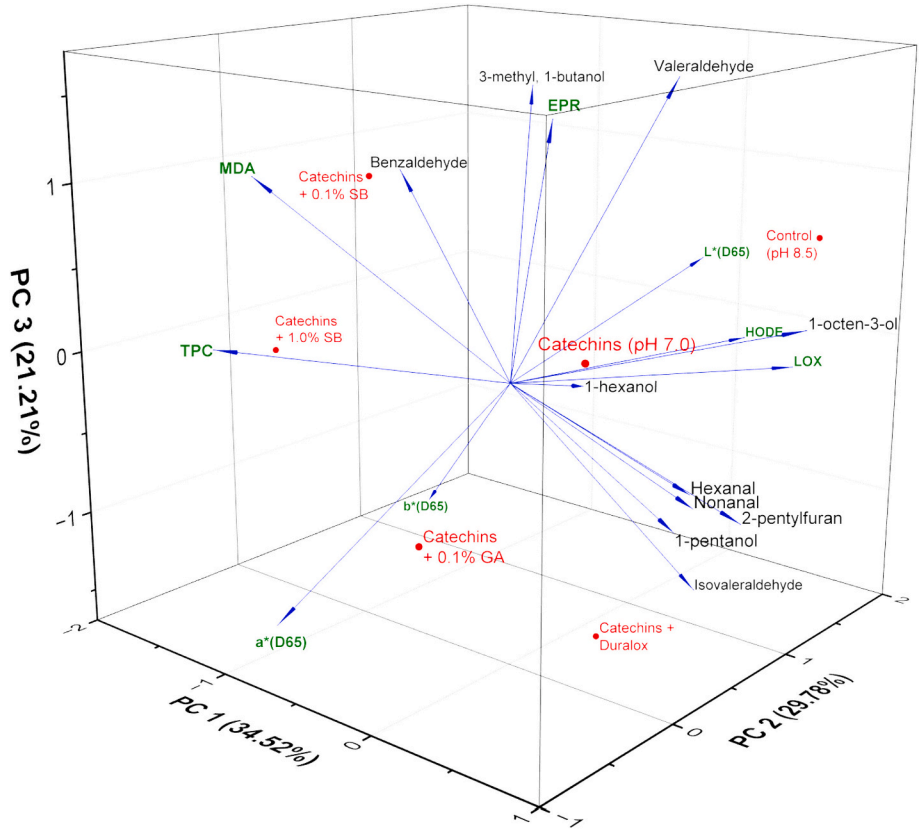

Fig. 6. PCA biplot of volatile compounds of pea proteins extracted at different solubilization pH values and with various antioxidant systems. EPR = free radical content via electron paramagnetic resonance; GA = Gallic acid; HODE = hydroxyoctadecadienoic acid; LOX = lipoxygenase; MDA = malonaldehyde; SB = sodium bisulfite; TPC = Total phenolic compounds.

some autoxidation-related volatiles but maintains relatively low PC3 values, indicating limited radical accumulation despite a modest presence of autoxidation products.

Overall, the PCA demonstrates that catechins-based interventions, modulated by pH or co-additives like SB or GA, consistently redirect samples away from discoloration but also the LOX/HODE axis and reduce off-flavor formation, while certain combinations (notably catechins at pH 7.0 and catechins + GA) also achieve substantial reductions in free-radical load (low EPR, low MDA).

3.9. Sensory analysis

Sensory analysis conducted by a trained analytical panel was employed as the primary validation tool to evaluate the extent to which the implemented treatments mitigated discoloration in the pea protein isolates while influencing texture, taste, and overall flavor perception as shown in Fig. 7.

Panelists mainly described the samples in terms of yellowness, browning and turbidity (Fig. 7A). Control proteins exhibited the highest yellowness values (scoring ~72), regardless of extraction pH. The protein extracted in the presence of catechins and Duralox®, showed the second highest yellowness, scoring 40.8. This relatively high value, despite catechins addition, was expected because of its low concentration, which is insufficient to cause noticeable discoloration. In agreement with the instrumental surface color measurements (Section 3.1), the addition of catechins during the extraction process, at both solubilization pH, provided a protein with the lowest yellowness (score = 21.0). As expected, the yellow color was relatively preserved after enrichment of the protein solubilization system with catechins and GA and SB. Thus, all the remaining proteins were evaluated with an average yellowness score of 29. An opposite trend was observed for the brown color. The control protein samples solubilized at pH 8.5 and 7.0 showed the lowest browning score (17.1). In contrast, the addition of catechins increased browning, with scores ranging from 41.3 to 76.3. Within this range, the samples containing 1.0% SB and the mixture of catechins + Duralox® exhibited the lowest browning score (44.3 and 51.5, respectively), whereas the addition of GA led to the highest browning score, reaching 76.3. Overall, these results corroborate those delivered in Table 1 and Fig. 2, showing that catechins addition to the system decreased the yellowness and increased brownness in the pea proteins, but this change was substantially mitigated when catechins were applied together with SB. However, the protein isolate with the addition of SB with catechins still did not completely replicate the color of the control. Finally, turbidity was assessed with a relatively

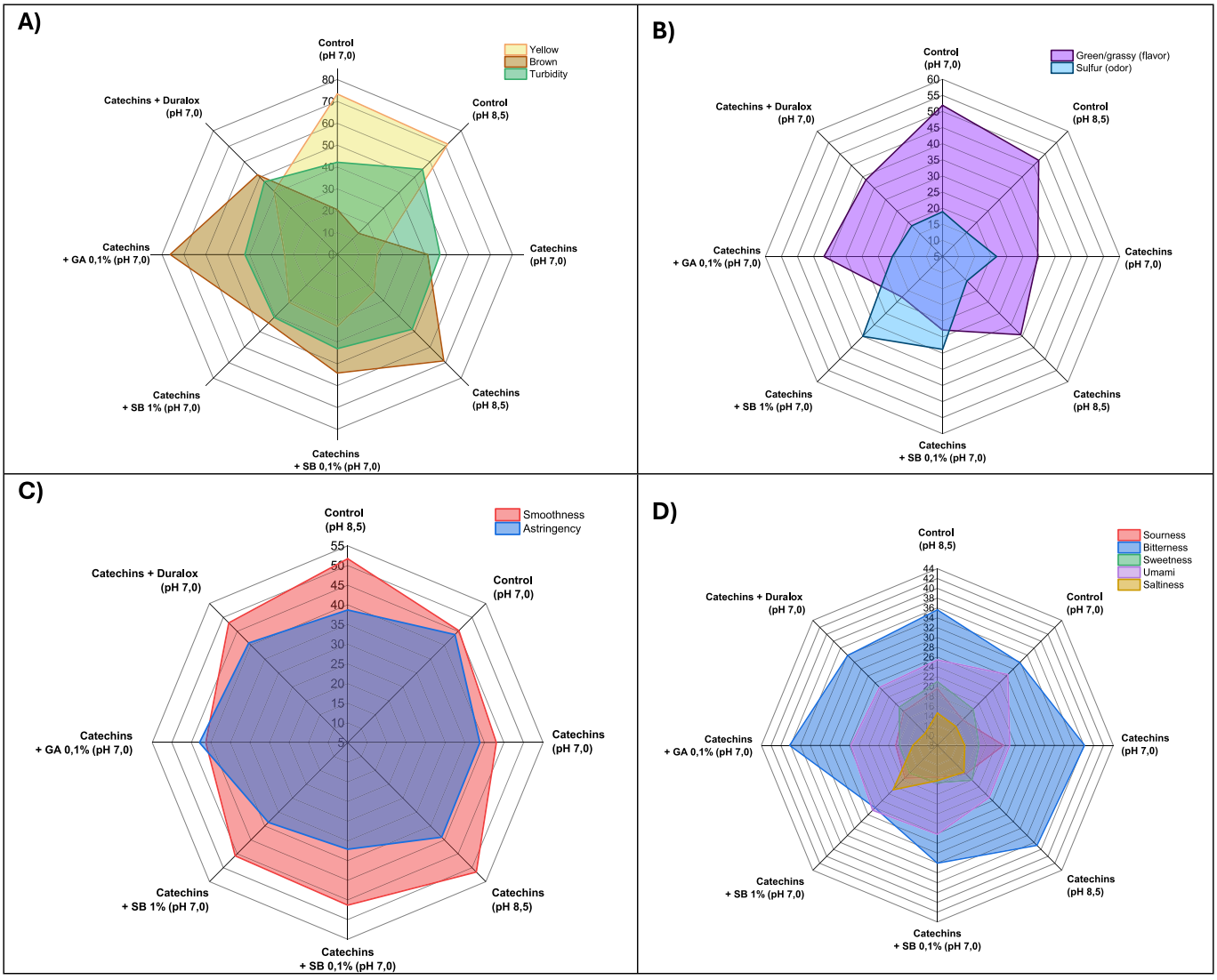

Fig. 7. Radar chart for the sensory attributes including color (A), flavor/odor (B), texture (C) and taste (D) of the pea proteins isolated at various solubilization pH and in the presence of different antioxidants. SB = sodium bisulfite, GA = Gallic acid.

homogeneous score, averaging 45.7. There was not a big effect from the addition of antioxidant on the turbidity of the proteins compared with the control.

Flavor and odor profiling revealed green or grassy flavor as the dominant notes (Fig. 7B), consistent with the presence of odor-active lipid oxidation products typically associated with pea protein (Sharifi et al., 2026). Control proteins solubilized at pH 8.5 and 7.0 exhibited high green/grassy scores (47.2 and 51.9, respectively), which aligns with elevated levels of aldehydes and furans (see Table 3) known to impart green and beany notes, including hexanal, nonanal, isovaleraldehye, valeraldehyde, and 2-pentylfuran. These compounds have extremely low odor threshold (ranging from $\mu g.L^{-1}$ to $ng.L^{-1}$) and are recognized as key contributors to beany perception in legumes and pea protein isolates, often exhibiting high odor activity values despite moderate absolute concentrations (Sharifi et al., 2026; van Gemert, 2011). Regarding odor, sulfur scores ranged between 15.6 and 40 and increased with SB addition, reaching the highest values in samples containing 0.1% and 1.0% SB (33.8 and 40, respectively). The remaining treatments exhibited an average sulfur score of 18.5 ± 2.3. These results indicate that although SB effectively suppressed green/grassy notes associated with lipid oxidation, this benefit was partially offset by the development of sulfur-related sensory attributes. Therefore, the sensory effect of SB should be considered as a trade-off between improved oxidative stability and increased sulfur perception. This effect was particularly pronounced at the higher SB concentration, highlighting the need to balance color stabilization and off-flavor suppression with the potential introduction of sulfurous notes when evaluating the practical applicability of sulfite-based strategies for pea protein processing.

The addition of catechins alone, or combined with Duralox® or GA, significantly reduced green/grassy notes (average score 28.1), coinciding with a marked decreased in LOX-derived aldehydes and furans in those samples (see Table 3). Catechins suppress lipid radical propagation and inhibit LOX activity, thereby limiting the formation of oxylipins and their subsequent cleavage into odor-active volatiles (Sharifi et al., 2026). Notable, the inclusion of SB further reduced greenness/grassiness, particularly at 1.0% (scoring 22.8), which correlated with a pronounced decrease in isovaleraldehyde, valeraldehyde, hexanal, 2-pentylfuran, 1-pentanol and nonanal relative to the control pea protein (pH 8.5). This verifies that the reductions observed in the quantity of VOC in the presence of this antioxidant mixture, due to inhibition of lipid oxidation, resulted in significantly noticeable improvement in the flavor profile of the pea proteins as well.

Plant-based proteins, particularly pea protein, often exhibit poor solubility and limited techno-functional properties, while frequently imparting undesirable mouthfeel characteristics such as pronounced astringency (Sarkar, 2024). This astringency arises largely from interactions between inherent phenolic compounds in the plant matrix and salivary proteins, leading to complex formation, precipitation of salivary proteins, disruption of the lubricating salivary film, and increased oral friction. These mechanisms result in reduced lubrication, dryness, and grittiness (Rossetti et al., 2009). In the present study, pea protein astringency scores ranged from 32.1 to 44.2 (Fig. 7C), confirming elevated levels, while the lowest score was observed in protein extracted with 0.1% SB. A recent study (Kew et al., 2026) highlighted high astringency in pea protein systems, with scores exceeding 25 on an unstructured 0–100 scale. Conversely, smoothness scores ranged from 41.3 to 53.5, with the lowest score in 0.1% GA, reflecting unpaired mouthfeel uniformity.

These differences stem from distinct physicochemical interactions modulated by the added antioxidants. Notably, the addition of catechins carries a risk of intensifying astringency and mouthfeel issues, especially considering the increased TPC, which promotes stronger protein-phenolic binding, thereby enhancing salivary protein precipitation, aggregation, and oral friction, exacerbating dryness, puckering, and reduced lubrication, key hurdles in pea protein applications (Sarkar, 2024). Sodium bisulfite modifies these aspects by cleaving disulfide bonds, increasing free sulfhydryl groups and surface hydrophobicity. This prevents covalent cross-linking and oxidative aggregation, improving protein solubility, dispersion, and reducing viscosity and elastic modulus. Ultimately, these changes enhance stability, smoothness, and overall mouthfeel while lowering perceived astringency (X. Zhu et al., 2016). In contrast, GA negatively impacts these properties. As a phenolic compound, it can form insoluble protein complexes, increase particle size, and disrupt textural uniformity, potentially amplifying astringency through enhanced protein-phenolic interactions and aggregation. GA has been reported to induce macroscopic aggregation in myofibrillar proteins (Y. Cao et al., 2016) and weaken gel networks in protein–starch systems (Han et al., 2021), which aligns with the observed reduced smoothness and contributes to worsened mouthfeel challenges in pea protein systems.

Fig. 7D shows the radar plots for taste attributes: sourness, bitterness, sweetness, umami, and saltiness. Bitterness was the dominant attribute, with an average intensity score of 34.7. The control sample showed an average bitterness score of 33.7, representing the natural baseline for this attribute in pea protein. As expected, bitterness increased after the inclusion of catechins and GA in the extraction system. With catechins, the bitterness score averaged ~37.5, showing only minor differences between proteins solubilized at pH 8.5 and 7.0. Similarly, GA increased bitterness up to 38.2. Pea protein is naturally bitter, due to the inherent presence of saponins, lipids, monoglycerides, and specific peptides generated during extraction (Agovi et al., 2022). Phenolic compounds, which are plant secondary metabolites commonly involved in defense mechanisms, can further modulate taste perception. Several studies summarized by Gonzalo-Diago et al. (2014) indicate that bitterness is strongly associated with small phenolic molecules. In the specific case of catechins, bitterness is linked to their activation of specific bitter taste receptors, such as hTAS2R39 (Narukawa et al., 2011). Therefore, although incorporating phenolic compounds during pea protein extraction offers advantages in terms of off-flavor mitigation, their contribution to increased bitterness may further reinforce this naturally undesirable sensory characteristic of pea protein and could negatively affect consumer acceptance. The lowest bitterness (score = 26.0) observed in protein extracted with 1.0% SB likely reflects extraction-driven transformations. SB, as a reducing agent, suppresses oxidative reactions and prevents the formation of bitter compounds such as oxidized lipids and peptides (Sudha et al., 2022). This aligns with previous studies showing that bisulfite prevents oxidative damage and protein carbonylation, both central to bitterness (MacAllister et al., 2013).

The addition of catechins to the extraction system did not significantly affect the umami and sweetness taste of the pea protein samples. Overall, umami scores ranged from 22.4 to 28.4, with the highest in the control protein sample extracted at pH 7.0.

The control samples displayed an average saltiness score of 14.0, which can be attributed to residual sodium hydroxide used during extraction and pH neutralization. Interestingly, the lowest score was observed in the protein extracted with the mixture Duralox® and catechins (11.5). As previously discussed, catechins represented the lowest fraction in this antioxidant system, while Duralox® may have contributed to reduced saltiness perception. This effect is likely due to the flavor-modulating properties of Duralox® components. Citric acid and ascorbic acid are sour-tasting compounds, and sourness interacts with saltiness in complex ways, often reducing the perceived intensity of salt (C. Chen et al., 2023). Both sour and salty tastes are mediated by ion channels rather than G protein-coupled receptors. Sour taste is detected by type III taste cells through proton-selective channels, while salty taste involves both type II and type III cells expressing sodium-selective channels. Their shared ionic basis may contribute to overlapping or suppressive interactions (Wilson & Kinnamon, 2025). The presence of catechins and GA conferred a saltiness score of approximately 14.3, regardless of extraction pH. Compared to the control proteins, this difference is not significant, indicating that catechins do not inherently

contribute to saltiness nor do they mask this taste attribute. In contrast, SB at 1.0% increased the saltiness score to 20.8. SB, an inorganic salt, introduces sodium ions (Na^+) that persists in the isolate post-purification, likely contributing to higher ash content and directly stimulating epithelial sodium channels, key Na^+ sensors in taste cells (Nomura et al., 2020). SB may also introduce sulfurous compounds that influence flavor perception through sulfur-derived volatiles, enhancing overall flavor intensity and perceived saltiness (Luo et al., 2024).

The sourness of the pea protein was dependent on the isolation pH. In the control samples, solubilization at pH 8.5 resulted in a higher sourness score than solubilization at pH 7.0 (19.6 vs 15.3, respectively). Sourness perception is primarily driven by the concentration of hydrogen ions (H^+) and protonated organic acid species in the system. The pH level influences the degree of protonation of organic acids. Hence, at higher pH values such as 8.5, a higher proportion of protonated acid species is observed compared with pH 7.0. These protonated species contribute more strongly to sour taste, leading to the higher sourness reported at pH 8.5 (Da Conceicao Neta et al., 2007). The sourness scores of the antioxidant-treated samples (all extracted at pH 7.0), averaged 16.5, which is higher than the control extracted at pH 7.0. This suggests that catechins influenced sourness perception in these samples. Notably, when pea protein was extracted at pH 7.0 in the presence of catechins alone, the sourness score increased considerably to 21.5. Catechins are less stable and more prone to degradation at neutral pH, generating acidic degradation products that may intensify sourness perception (Matić et al., 2024).

Taken together, the combined effects of catechins with SB or GA reveal distinct and contrasting sensory outcomes. Catechins consistently reduced yellowness and suppressed beany off-flavor, but it also intensified browning and increased bitterness. SB offered the most favorable overall profile, markedly reduced beaniness, astringency, and bitterness while improving smoothness, though accompanied by a predictable rise in perceived saltiness from added sodium. In contrast, GA generally deteriorated sensory quality, promoting browning, astringency, bitterness, and reduced smoothness.

4. Conclusions

This study investigated the efficacy of combining catechins with complementary antioxidant systems: sodium bisulfite (SB), gallic acid (GA), Duralox®, at lower protein solubilization pH in mitigating both color changes and lipid oxidation-associated off-flavor formation during pea protein extraction via alkaline solubilization and isoelectric precipitation. The untargeted metabolomic analysis demonstrated that oxidized dimeric products arising from phenolic structures were the molecular responsible for the color change induced in the pea protein isolated in the presence of catechins. Lowering the solubilization pH from 8.5 to 7.0 in the presence of catechins significantly mitigated catechins-induced discoloration. However, this shift also diminished their effectiveness in suppressing beany volatile compounds associated with protein and lipid oxidation, likely due to reduced retention of TPC in the protein isolate and a concomitant increase in EPR-detected free radicals. Among the tested complementary antioxidants, catechins combined with SB demonstrated the least level of discoloration but the most consistent suppression of lipid oxidation markers (HODE and MDA), volatile compounds, and LOX activity, while also improving sensory attributes such as reduced beaniness and bitterness, compared with the control. SB acted synergistically with catechins, resulting in enhanced oxidative control and improved mouthfeel. Duralox®, despite its multi-component antioxidant formulation, showed limited efficacy in preventing free radical accumulation, hexanal formation and reduced LOX activity mitigation, which could be due to a low TPC. Sensory analysis confirmed that antioxidant treatments, particularly catechins–SB combinations, reduced undesirable flavor notes while maintaining acceptable taste and appearance. It is important to say that although SB proved highly effective in mitigating discoloration and suppressing oxidation, it was included in this study primarily as a model quinone-scavenging system to investigate the role of quinone trapping in controlling catechin-induced discoloration during pea protein isolation, rather than as a proposed food ingredient for commercial application. Its practical use may be limited by regulatory requirements, allergen labeling obligations, and consumer preferences for clean-label ingredients. Additionally, SB addition was associated with increased sulfur-related sensory notes. Therefore, future work should investigate naturally derived or label-friendly quinone-scavenging systems capable of providing similar functionality.

Overall, the findings underscore the potential of targeted antioxidant systems to enhance the oxidative and sensory quality of pea protein isolate with minimum side effect on its color. Future studies should focus on optimizing antioxidant dosage and on evaluating the techno-functional and structural properties of the resulting protein isolates to determine their suitability for food applications.

CRediT authorship contribution statement

José Villacís-Chiriboga: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Elahe Sharifi:** Writing – review & editing, Investigation, Formal analysis. **Madeleine Jönsson:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Karin Wendin:** Writing – review & editing, Validation, Resources, Project administration, Investigation, Conceptualization. **Rounak Chourasia:** Writing – original draft, Software, Investigation, Formal analysis. **Otto Savolainen:** Writing – original draft, Software, Investigation, Formal analysis, Conceptualization. **Mehdi Abdollahi:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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.foodchem.2026.151868.

Data availability

Data will be made available on request.

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