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Cap and stipe extracts from *Pleurotus ostreatus* mushrooms reveal differences in enhancing the quality and stability of beef burgers during frozen storage

Milagros Maribel Coaguila Gonza [a], Juan D. Rios-Mera [b], Alan Giovanini de Oliveira Sartori [a], Chimenes Leal de Araújo [a], Sarah Mafeis de Jesus [a], Nils Leander Huamán-Castilla [c], Erick Saldaña [d], Carmen Josefina Contreras Castillo [a,*]

[a] *Departamento de Ciência e Tecnologia de Alimentos (LCA), Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo (USP), Piracicaba, Brazil*

[b] *Grupo de Investigación en Reformulación de Alimentos (GIRA), Instituto de Investigación de Ciencia y Tecnología de Alimentos (ICTA), Universidad Nacional de Jaén, Carretera Jaén-San Ignacio, km 24-Sector Yanuyacu, Jaén 06800, Peru*

[c] *Laboratorio de Tecnologías Sustentables para la Extracción de Compuestos de Alto Valor, Instituto de Investigación para el Desarrollo del Perú (IINDEP), Universidad Nacional de Moquegua, Moquegua 18001, Peru*

[d] *Sensory Analysis and Consumer Study Group, Escuela Profesional de Ingeniería Agroindustrial, Universidad Nacional de Moquegua, Prolongación Calle Ancash s/n, Moquegua 18001, Peru*

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ABSTRACT

Previous studies have demonstrated the effectiveness of whole *Pleurotus ostreatus (P. ostreatus*) mushrooms and mushroom-derived ingredients as natural antioxidants in meat products. However, the extraction of antioxidants from distinct anatomical parts of *P. ostreatus*, specifically the stipe and cap, and their comparative evaluation in meat systems have not been previously reported. In this study, extracts from the cap and stipe of *P. ostreatus*, produced using the Pressurized Liquid Extraction (PLE) method, were incorporated into a beef burger formulation as substitutes for the synthetic antioxidant sodium erythorbate. The extracts presented differences in total phenolic content and antioxidant capacity. In frozen burgers stored for up to 90 d, the stipe extract improved the stability of a^*, b^*, and chroma, as well as lipid oxidative stability, while the cap extract enhanced the stabilization of L^* and myoglobin. Both extracts showed performance similar to or superior to that of sodium erythorbate during frozen storage. The extracts did not cause significant changes in the texture profile, cooking loss and diameter reduction, but they increased the free amino acid content in the burgers, which could explain the improvement in the dynamic sensory profile, due to the dominance of the sensory attributes "tasty" and "seasoned". The findings demonstrate the potential of *P. ostreatus* to replace sodium erythorbate in beef burgers; however, the distinct mechanisms of action of each extract and their effects on nutritional quality require further investigation.

1. Introduction

Beef burgers are among the most well-known and widely consumed meat products worldwide, primarily due to their outstanding flavor, juiciness, aroma, and texture, resulting from the incorporation of ingredients such as salt, animal fat, and functional additives. However, these products are prone to the formation of oxidation compounds, primarily derived from proteins and fats, which reduce oxidative stability and, consequently, shorten shelf life. In addition, oxidation compounds can have harmful effects on the human body (Wang, Wu, Tu, & Xu, 2023). To counteract their formation, the use of synthetic antioxidants is a common practice in the food industry (Crippa, Larsson, Discacciati, Wolk, & Orsini, 2018; Echegaray et al., 2018).

Although synthetic antioxidants are used in meat products at the maximum doses permitted by regulations, their regular consumption may be associated with adverse health effects, including gastric and intestinal allergies, hypersensitivity, asthma, and potential links to carcinogenic processes (Hasler, 2000; Pereira, Inácio, Pereira, & De

* Corresponding author.
E-mail address: ccastill@usp.br (C.J.C. Castillo).

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Angelis-Pereira, 2015; Rios-Mera, Arteaga, Ruiz, Saldaña, & Tello, 2024). Consequently, growing consumer concerns about health have driven the reformulation of meat products to reach clean label alternatives, that is, products made without synthetic additives (Selani, Herrero, & Ruiz-Capillas, 2022).

Over the years, academic efforts have focused on developing plant-based alternatives to synthetic antioxidants that have shown positive effects on the oxidative stability of meat products. Various plant parts, including leaves, stems, roots, fruits, bark, and seeds, have been evaluated (Aminzare et al., 2019). However, their incorporation may negatively affect sensory attributes due to the strong flavors often associated with plant-derived antioxidants (Aminzare et al., 2019; Grasso, Estévez, Lorenzo, Pateiro, & Ponnampalam, 2024).

Fortunately, certain natural sources provide antioxidant activity while also enhancing flavor. Among these, edible mushrooms are noteworthy. Commercially important species such as *P. ostreatus* (oyster mushroom) are promising functional ingredients owing to their rich content of bioactive compounds, including polysaccharides, polyphenols, flavonoids, amino acids, vitamins, and fatty acids (Barbosa et al., 2020; Tong et al., 2009). Consequently, *P. ostreatus* exhibits antioxidant, anti-inflammatory, and hypolipidemic effects (Xia, Fan, Zhu, & Tong, 2011), as well as antitumor activity (Wisbeck et al., 2017). In addition, it stands out for its ability to enhance umami flavor, attributed to its content of free amino acids (such as glutamic acid and aspartic acid), 5′-nucleotides, and volatile compounds (Ren, Zheng, Zhao, Hu, & Yang, 2022; Sun et al., 2020). However, the production of *P. ostreatus* generates considerable waste, particularly from the stipe, which accounts for 25 %–33 % of the mushroom's fresh weight (Chou, Sheih, & Fang, 2013).

The use of *P. ostreatus* by-products as natural antioxidants aligns with the most sustainable and necessary approach in the formulation of meat products (Grasso et al., 2024). However, it has been shown that mushroom extracts, including *P. ostreatus,* exhibit different antioxidant potentials depending on the mushroom part, such as the cap and the stipe (Radzki, Tutaj, Skrzypczak, Michalak-Majewska, & Gustaw, 2024). Although the whole *P. ostreatus* mushrooms may contain higher overall levels of bioactive compounds, industrial processing routinely separates caps and stipes, generating distinct by-product streams with different chemical compositions (Ayuso, Quizhpe, Peñalver, García-Pérez, & Nieto, 2025). Because stipes are often removed due to their greater toughness and lower palatability, they are less exploited commercial processing workflows (Li et al., 2018). Evaluating each part individually is therefore necessary to understand their specific antioxidant contributions and to determine whether such downgraded fractions can serve as targeted natural antioxidant sources in meat formulations. Moreover, replacing synthetic antioxidants with extracts obtained from different edible mushroom parts remains underexplored in meat products. Addressing this gap, the present study demonstrates that selecting the mushroom part for antioxidant extraction is critical to improving the quality attributes of beef burgers. This finding is consistent with Estévez (2021), who emphasized that a key requirement for the application of natural antioxidants in meat products is the careful selection of an appropriate antioxidant source.

Among the emerging green extraction technologies, pressurized liquid extraction (PLE) stands out as an environmentally friendly method for recovering bioactive compounds. Traditionally, these compounds have been extracted using hazardous organic solvents (Gilbert-López et al., 2015). In contrast, PLE utilizes food-grade solvents, such as water, ethanol, or CO_2, at elevated temperatures and pressures, which enhance mass transfer, improve extraction yields, and reduce solvent consumption and waste generation (Herrero, del Pilar Sánchez-Camargo, Cifuentes, & Ibáñez, 2015; Mazzutti, Rodrigues, Mezzomo, Venturi, & Ferreira, 2018). These advantages make PLE an attractive option for obtaining natural antioxidants from agro-industrial by-products.

Therefore, this study aimed to evaluate the antioxidant potential and flavor-enhancing capacity of extracts obtained via PLE from the cap and stipe of *P. ostreatus* for use in the formulation of beef burgers. Product stability during frozen storage was assessed to determine the viability of *P. ostreatus* as a natural antioxidant alternative. Specifically, instrumental color, myoglobin redox state, lipid and protein oxidation, pH, texture profile analysis. The extracts did not cause significant changes in the texture profile, cooking loss, diameter reduction, and the free amino acid profile were determined, and a Temporal Dominance of Sensations (TDS) sensory analysis was conducted.

2. Materials and methods

2.1. Raw materials and ingredients

Beef (*Quadriceps femoris*: 74.61 % moisture, 21.97 % protein, 2.3 % fat, and 1.14 % ash), pork back fat (13.46 % moisture, 3.38 % protein, 82.98 % fat, and 0.22 % ash), and household table salt (commercial food-grade salt, predominantly composed of NaCl). The extracts used in this study were obtained from *P. ostreatus* by-products, specifically the stipes and cap portions, The extracts used in this study were obtained from *P. ostreatus* by-products, specifically the stipes and cap portions. Prior to extraction, the samples were disinfected by immersion in an aqueous sodium hypochlorite solution (100 ppm free chlorine) for 10 min, rinsed with running water, and subsequently freeze-dried. Extraction was carried out using the PLE method with an ASE 150 system (Dionex, Thermo Fisher, San José, CA, USA). A stainless-steel extraction cell (100 mL) was prepared by placing a cellulose filter at the bottom, followed by 10 g of quartz sand as a base layer. Subsequently, a premixed blend of 70 g of quartz sand and 2 g of the sample was added. Water was used as the extraction solvent, and the process was conducted at 150 °C. The extraction parameters, based on the method reported by Huaman-Castilla et al. (2019), included a pressure of 10 MPa, a single extraction cycle, a flush volume of 150 %, a nitrogen purge of 250 s, and a static extraction time of 5 min. Initially, three independent extractions were performed for each mushroom fraction (stipes and caps). Subsequently, the contents of selected polyphenols, total polyphenols, and antioxidant activity (assessed by DPPH and ORAC assays) were evaluated for each extract. Additionally, further extractions were performed to obtain the required volume for the burger formulations. After extraction, the obtained extracts were lyophilized and vacuum-sealed for further use. For the analysis of free amino acids, HPLC-grade reagents were used, while all other reagents were of analytical grade.

2.2. Phenolic content and antioxidant capacity of mushroom extracts

Selected polyphenols (gallic acid, catechin, caffeic acid, resveratrol, and vanillin) were quantified following Huaman-Castilla et al. (2019) with minor modifications. A Vanquish Flex UHPLC system (Thermo Scientific, Germany) was used, coupled to an Orbitrap mass spectrometer equipped with a HESI-II source (Thermo Scientific, USA). Freeze-dried extracts were reconstituted in ultrapure water, filtered (0.22 μm), and 1 μL was injected onto a Poroshell EC-C18 column (150 × 2.1 mm; 1.9 μm) maintained at 30 °C. *mobile* phases were A (water +0.1 % formic acid) and B (acetonitrile +0.1 % formic acid). The gradient program was as follows: 0–15 min, 5 % B; 15–33 min, 40 % B; 33–53 min, 5 % B for re-equilibration; flow rate, 0.20 mL/min. Quantification was performed by external calibration with solutions of authentic standards analyzed under identical chromatographic conditions. Results were expressed as μg of polyphenol per gram of dry sample.

The total phenolic content was determined using the Folin–Ciocalteu colorimetric method, as previously described by Magalhães, Santos, Segundo, Reis, and Lima (2010). A calibration curve was prepared using gallic acid as the standard, with concentrations ranging from 5 to 50 μg/mL. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample.

To evaluate the antioxidant capacity of the extracts, two assays were

performed. The Oxygen Radical Absorbance Capacity (ORAC) assay was conducted according to Huamán-Castilla et al. (2021), using Trolox as the standard to construct a calibration curve (8–40 μM), and results were expressed as μmol Trolox equivalents (TE) per gram of freeze-dried extract. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay was performed follow to Abderrahim, Arribas, Gonzalez, and Condezo-Hoyos (2013), and antioxidant activity was expressed as IC_{50}, defined as the concentration (mg of extract per mL of reaction medium) required to inhibit 50 % of the radical signal. All assays were conducted in triplicate

2.3. Beef burger manufacture

Four different formulations of beef burgers were developed to evaluate the effects of synthetic and natural antioxidants during frozen storage (Table 1). The experimental treatments included: control (negative control without any antioxidant), sodium erythorbate (0.01 % sodium erythorbate), stipe (0.5 % *P. ostreatus* stipe extract), and cap (0.5 % *P. ostreatus* cap extract). Both mushroom extracts were incorporated as freeze-dried powders. A total of 240 burgers were prepared: five burgers per treatment x four treatments (control, sodium erythorbate, stipe, and cap) x four sampling points (0, 30, 60, 90 d) x three independent replicates performed on different days. The 0.5 % inclusion level for both mushroom extracts was selected based on preliminary antioxidant screening using ORAC and DPPH assays, benchmarked against sodium erythorbate. These assays indicated that extract inclusion levels of approximately 0.2–0.4 % would be required to approach the in vitro antioxidant capacity of 0.01 % sodium erythorbate, depending on the assay and the anatomical fraction. Based on this comparison, a common inclusion level of 0.5 % was selected to provide a conservative antioxidant dose and to allow a controlled comparison of the functional performance of the cap and stipe extracts under identical formulation conditions. Using the same extract concentration minimized formulation-related confounding effects associated with changes in the relative proportions of beef, fat, and other ingredients, which could alter the amount of oxidizable substrates available for antioxidant action. Standardizing the antioxidant mass across treatments therefore allowed differences in oxidative stability to be attributed to the intrinsic properties of the extracts rather than to formulation variability.

The base formulation for all treatments consisted of lean beef (70 %), pork back fat (20 %), water (7.5 %), and salt (2.0 %). No spices or flavoring agents were added to avoid potential interference with the antioxidant and flavor-enhancing properties provided by the mushroom extracts.

For burger preparation, lean beef and pork fat were ground separately. Salt was first added to the meat and mixed manually for 2 min to facilitate protein extraction. Subsequently, the remaining ingredients were incorporated according to the treatment and mixed by hand for an additional 3 min to ensure homogeneity. Burger portions (100 g) were manually formed in a mold (10 cm diameter × 1 cm thickness) and then vacuum-packaged. Samples were stored at −20 °C and analyzed on days 0, 30, 60, and 90 to evaluate changes in instrumental color, myoglobin redox state, lipid oxidation, protein oxidation, and pH. All other analyses were performed exclusively on day 0.

Table 1
Formulation of beef burger treatments.

Ingredient (%)	Treatments			
	Control	Sodium erythorbate	Stipe	Cap
Beef	70.70	70.69	70.20	70.20
Pork back fat	20.20	20.20	20.20	20.20
Water	7.58	7.58	7.58	7.58
Salt	1.52	1.52	1.52	1.52
Sodium erythorbate	0	0.01	0	0
Stipe extract	0	0	0.50	0
Cap extract	0	0	0	0.50

2.4. pH

The pH of the raw burger samples was measured using a pH meter (Hanna® HI 98163, Hanna® Instruments Inc., Woonsocket, USA, manufactured in Romania, RO), equipped with a glass penetration electrode. The meter features automatic temperature compensation (ATC) to ensure accurate measurements under varying temperature conditions. Before measurement, the pH meter was calibrated with buffer solutions at pH 4.0, 7.0, and 10.0. A 100 g raw burger sample was used for each measurement, and pH was determined directly with the penetration electrode, without dilution (Rios-Mera et al., 2019). Measurements were taken at three different points in the sample to ensure consistency and representativeness.

2.5. Instrumental color and myoglobin redox state

Instrumental color was assessed after the raw burger samples were allowed to bloom for 30 min at 4 °C, which promotes surface oxygenation. A portable spectrophotometer (MiniScan XE Plus, model 45/0-LV, Hunter Associates Laboratory, USA), featuring a 2.54 cm aperture and a 10° observation angle, was used in conjunction with the Easy Match QC system for measurements. The device was calibrated using black-and-white ceramic reference tiles provided by the manufacturer.

Color parameters were recorded in the CIELab space, including lightness (L^*), redness (a^*), and yellowness (b^*), by averaging readings from five random surface points on each sample, avoiding visible fat and connective tissue. Chroma (C^*) and hue angle (h^*) were calculated from the a^* and b^* values using the following equations, as recommended by AMSA, 2012 and Saldaña, Siche, Castro, Huamán, & Quevedo, 2014:

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (1)$$

$$h^* = \text{arctangent}\left(b^*/a^*\right) \quad (2)$$

To evaluate the oxidative state of myoglobin, the relative proportions of metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb) were estimated based on spectral reflectance data, following the equations proposed by King et al. (2023):

$$\%\text{MMb} = \left\{1.395 - \left[\frac{A_{572} - A_{700}}{A_{525} - A_{700}}\right]\right\} \times 100 \quad (3)$$

$$\%\text{DMb} = \left\{2.375 \times \left[1 - \frac{A_{473} - A_{700}}{A_{525} - A_{700}}\right]\right\} \times 100 \quad (4)$$

$$\%\text{OMb} = 100 - (\%\text{MMb} + \%\text{DMb}) \quad (5)$$

In these equations, A represents the absorbance, calculated as the log of 1/R, where R is the reflectance value at the respective wavelengths.

2.6. Lipid oxidation

Lipid oxidation in raw burger samples was evaluated using the thiobarbituric acid reactive substances (TBARS) method, following the procedure described by Sørensen and Storgaard Jørgensen (1996), with specific modifications adapted for this study. Approximately 5 g of burger was weighed and transferred into a conical tube, to which 15 mL of a trichloroacetic acid (TCA) solution containing propyl gallate and EDTA (7.5 % TCA, 0.1 % propyl gallate, 0.1 % EDTA) was added. The samples were homogenized using an Ultra-Turrax homogenizer (IKA, T25D, Germany) at 3500 rpm for 45 s, and the homogenate was then filtered through Whatman No. 1 filter paper (125 mm). An aliquot of 5 mL of the filtrate was mixed with 5 mL of 0.02 M thiobarbituric acid

(TBA) solution, and the mixture was heated at 100 °C for 40 min in a water bath. After rapid cooling in an ice bath, the absorbance was measured at 532 nm and 600 nm using a UV–Vis spectrophotometer (Mini 1240, Shimadzu, Japan). The malondialdehyde (MDA) content was quantified using a standard calibration curve prepared with 1,1,3,3-tetraethoxypropane (TEP). Results were expressed as milligrams of MDA per kilogram of burger sample (mg MDA/kg).

2.7. Protein oxidation

Protein oxidation in raw burger samples was evaluated by measuring carbonyl content, following the method described by Oliver, Ahn, Moerman, Goldstein, and Stadtman (1987), with specific modifications adapted for this study. Approximately 1 g of raw burger sample was homogenized in 10 mL of 0.6 M NaCl using an Ultra-Turrax homogenizer (IKA T25D, Germany) at 3000 rpm for 60 s. Two aliquots of 150 μL were transferred into separate Eppendorf tubes. Both aliquots were precipitated with 1 mL of 10 % trichloroacetic acid (TCA), vortexed, and centrifuged at 5000 rpm for 5 min at 4 °C (Eppendorf 5810R centrifuge). One pellet was resuspended in 1 mL of 2 N HCl (protein reference), while the other was treated with 1 mL of 0.2 % 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl for carbonyl detection. Samples were incubated at room temperature for 1 h, with manual shaking every 20 min. After incubation, 1 mL of 10 % TCA was added, and the mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. The resulting pellet was washed twice with 1 mL of ethanol:ethyl acetate solution (1:1, *v*/v), vortexed, and centrifuged at 10,000 rpm for 5 min. The remaining solvent was evaporated under a gentle stream of nitrogen (N_2). The dried pellet was resuspended in 1.5 mL of 6 M guanidine hydrochloride prepared in 20 mM sodium phosphate buffer (pH 6.5) and centrifuged at 2000 rpm for 2 min at 4 °C. Absorbance was measured at 280 nm (for protein content) and 370 nm (for carbonyl groups) using a UV–Vis spectrophotometer. Carbonyl concentration was calculated using an extinction coefficient of 22,000 $M^{-1}{\cdot}cm^{-1}$, and results were expressed as millimoles of carbonyl per milligram of protein (mM carbonyl/mg protein).

2.8. Texture profile analysis

Texture profile analysis (TPA) was performed using a TA-XT texture analyzer (Stable Micro Systems, Godalming, UK), based on the methodology described by Selani et al. (2016), with specific modifications adapted to the present study. Initially, the burgers were cooked on an electric hot plate at 150 °C until their internal temperature of the burgers reached 75 °C, ensuring even cooking on both sides. The samples were cooled to room temperature. Cylindrical samples of the cooked burgers, 2.5 cm in diameter and 1 cm in height, were prepared and compressed to 75 % of their original height at a speed of 20 cm/min using a 3.5 cm-diameter flat probe (Stable Micro Systems, Godalming, UK).

Four key texture parameters were evaluated during the test, following the method of Saldaña et al. (2015). Hardness (N) was defined as the maximum force recorded during the first compression cycle, representing the sample's resistance to deformation. Springiness was measured as the distance the sample recovered between the end of the first compression and the beginning of the second, reflecting its ability to return to its original shape. Cohesiveness was calculated as the ratio of the area under the second compression curve to that under the first, indicating the internal structural integrity of the sample. Finally, chewiness (N) was calculated as the product of hardness, cohesiveness, and springiness, representing the energy required to chew the sample until it is ready for swallowing.

2.9. Cooking loss and diameter reduction

Cooking losses and diameter reduction were evaluated on five burgers per batch, following the methodology described by Sánchez-Zapata et al. (2010), with modifications. Cooking losses (CL) were determined by calculating the difference in weight between raw and cooked samples, expressed as a percentage according to Eq. 6:

$$\mathrm{CL}\,(\%) = \frac{\text{Raw weight (g)} - \text{Cooked weight (g)}}{\text{Raw weight (g)}} \times 100 \tag{6}$$

Diameter reduction (DR) was measured before and after cooking and expressed as a percentage based on Eq. 7, as described by Rios-Mera et al. (2019):

$$\mathrm{DR}\,(\%) = \frac{\text{Raw diameter (cm)} - \text{Cooked diameter (cm)}}{\text{Raw diameter (cm)}} \times 100 \tag{7}$$

2.10. Free amino acid profile

For the extraction of free amino acids (FAA), the methodology described by Rabie, Peres, and Malcata (2014) was followed. Approximately 5 g of the burger sample was homogenized with 10 mL of 0.1 % 2-mercaptoethanol and 10 mL of 10 % (*w*/*v*) trichloroacetic acid. The homogenates were allowed to rest for over 3 h, then centrifuged at 6500 ×*g* for 20 min at 4 °C. The supernatant was recovered, filtered through filter paper, and the pH of the filtrate was adjusted to 7. The FAA determination was performed using the method described by Wu and Meininger (2008) and Hackbarth et al. (2018), with specific adjustments adapted to the conditions of the present study. Analyses were carried out using high-performance liquid chromatography (HPLC) coupled with a fluorescence detector (RF-20 A) (Shimadzu, Kyoto, Japan), employing a C18 reversed-phase column (Agilent LC-18, 250 mm × 4.6 mm, 5 μm) as the stationary phase.

The mobile phase consisted of two solvents: Phase A, composed of 100 mM sodium acetate (90.49 %), methanol (9 %), tetrahydrofuran (0.5 %), and hydrochloric acid 6 N (0.0048 %); and Phase B, composed of 100 % methanol. Gradient elution was applied as follows: 0–15 min, 14 % B; 15–20 min, 14–30 % B; 20–24 min, 30–35 % B; 24–26 min, 35–47 % B; 26–34 min, 47–50 % B; 34–38 min, 50–70 % B; 38–40 min, 70–100 % B; 40–50 min, 100 % B; 50–51 min, 14 % B; and 51–56 min, 14 % B. The flow rate was set at 1 mL/min, and the column temperature was maintained at 25 °C using a CTO-10 AC oven. All samples were filtered through 0.20 μm membranes before injection into the chromatographic system.

For amino acid pre-column derivatization, a solution containing *o*-phthaldialdehyde (OPA), methanol, borate buffer, and *β*-mercaptoethanol was used, and also filtered through a 0.20 μm membrane. Fluorescence detection was performed at 340 nm excitation and 455 nm emission. Amino acids were identified by comparing their retention times with those of authentic standards (Sigma-Aldrich, St. Louis, USA). Concentrations of each analyte were determined using calibration curves, expressed as milligrams of free amino acids per gram of dry sample (mg FAA/g DW).

2.11. Microbiological analysis

The microbiological analysis was conducted to ensure the food safety of the burgers intended for sensory evaluation. According to the methods described by the American Public Health Association (APHA, 2015), the following groups were analyzed: aerobic mesophilic bacteria, psychrotrophic bacteria, molds and yeasts, total coliforms, *Escherichia coli*, and *Salmonella* spp. Aerobic mesophilic bacteria were quantified

using the pour plate method, in which 1 mL of a serial dilution (10^{-1} to 10^{-6}) was inoculated onto PCA plates and incubated at 35 °C for 24 h. Psychrotrophic bacteria were enumerated using the spread plate technique, where 0.1 mL of the sample was plated on PCA and incubated at 7 °C for 7 d. Molds and yeasts were counted on acidified Potato Dextrose Agar (PDA) and incubated at 25 °C for 5 d. Total coliforms and *E. coli* were determined on chromogenic Brilliance™ *E. coli*/coliform agar incubated at 37 °C for 24 h, with colonies distinguished by color. Detection of *Salmonella* spp. involved pre-enrichment in buffered peptone water, selective enrichment, and plating on XLD and Hektoen agars, followed by biochemical confirmation.

2.12. Temporal Dominance of Sensations (TDS) and overall liking of cooked beef burgers

Eight sensory experts with prior experience in sensory analysis of meat products participated in a free listing task (de Araújo et al., 2025), tasting four formulations (control, sodium erythorbate, stipe, and cap) in random order. Each expert generated a list of temporal sensory attributes based on their perception of each formulation. A consensus session followed, during which synonyms were merged, and hedonic terms were removed to define operational descriptors. The final lexicon included terms like tender, juicy, tasty, fatty, salty, and seasoned. These descriptors were used in the TDS analysis to evaluate the formulations and ensure consistency in consumer sensory evaluation.

A total of 130 consumers, including students, researchers, and staff from the ESALQ/USP, voluntarily participated in the study. The sensory analysis was conducted using the TDS technique. Before the sensory analysis, consumers read a consent form to indicate their consent to participate. A brief familiarization session was then held, during which a general explanation of how the TDS method works was provided. The sensory analysis protocol was approved by the Ethics Committee of ESALQ/USP with number 6.995.367 was obtained for experimentation with human subjects, and informed consent was obtained from all participants prior to their participation. Participants initiated the test by clicking the “Start” button, and throughout the chewing process, they identified the sensory attributes they perceived as dominant at each moment, allowing them to reselect them as their perceptions changed. The test had no time limit, and participants could end it by clicking “Stop” when they no longer identified any dominant sensations. Water and crackers were offered as a palate cleanser between samples. After completing the TDS evaluation, consumers rated overall liking using a 9-point hedonic scale (Peryam & Pilgrim, 1957). Data collection was conducted entirely using the *Compusense Cloud* software on Android tablets.

2.13. Statistical analysis

For the physicochemical analysis conducted at time 0, a Completely Randomized Block Design (CRBD) was used. The sources of variation considered in this design were the treatments and the blocks, defined as batches of burger production processed on three different dates, with three replicates per date. The treatment was treated as a fixed effect to evaluate differences between experimental conditions, while the block was treated as a random effect to control for the inherent variability across different production dates (Biffin et al., 2020).

For the analyses carried out over the storage period, an RCBD with a factorial structure was employed, with treatment, storage time, and their interaction as fixed effects, and production batches (defined as blocks) as random effects. This design allowed evaluation of the independent effects of treatments and storage time on physicochemical properties, as well as their potential interaction. Instrumental data were analyzed using analysis of variance (ANOVA), and when significant differences ($P < 0.05$) among treatments were detected, Tukey's test was used for pairwise comparisons of means to identify which treatments differed significantly. Total phenolic content, antioxidant capacity (ORAC and DPPH), and the concentrations of selected individual polyphenols were analyzed using Student's *t*-test. All analyses were conducted in RStudio (version 4.2.0) at the 5 % significance level. In this analysis, the extract type (stipe or cap) was treated as a fixed factor, and differences between groups were evaluated based on the residual variability among replicates.

For the TDS data, dominance rates were calculated using standardized time and plotted as band diagrams for each attribute (Merlo et al., 2019). The standardized durations of the dominant attributes were subsequently analyzed using ANOVA, with samples (fixed) and consumers (random) as factors. When the effect of the sample factor was statistically significant, the Tukey test was applied for pairwise comparisons at a 5 % significance level. Acceptance data were analyzed similarly using ANOVA, with treatments as fixed effects and consumers as random effects. Since only one session was conducted for the sensory evaluation, the session factor was not included as a random term. The Tukey test was used for pairwise comparisons at a 5 % significance level.

3. Results and discussion

3.1. Phenolic content and antioxidant capacity of mushroom extracts

The content of selected phenolic compounds, total phenolic, and antioxidant capacity of *P. ostreatus* extracts are shown in Table 2. For selected phenolic compounds, no statistically significant differences were observed between cap and stipe; however, the cap extract presented higher numerical higher values for all quantified polyphenols. This trend affected total phenolic content, with the cap extract statistically superior to the stipe extract. The results are similar to those reported by Radzki et al. (2024), who observed that the content of phenolic compounds in *P. ostreatus* caps was almost three times higher than in the stipe; furthermore, *P. ostreatus* was superior compared to other species such as *A. bisporus*, *L. edodes*, *P. nameko*, and *P. eryngii*, which motivates its use for obtaining antioxidants. The presence of gallic acid and caffeic acid in *Pleurotus ostreatus* has been previously reported; furthermore, gallic acid and its lower-molecular-weight derivatives exhibit greater antioxidant activity, supporting their contribution to the observed activity in the analyzed extracts (Selli, Guclu, Sevindik, & Kelebek, 2021).

The cap extract exhibited higher total phenolic content and ORAC values, along with a lower DPPH IC_{50} (mg/mL), indicating greater antioxidant capacity than the stipe extract ($P < 0.05$). In the DPPH assay, antioxidant activity is reported as the IC_{50}, the concentration required to inhibit 50 % of the radical signal; lower values indicate greater antioxidant potency (Krümmel, Rodrigues, Vitali, & Ferreira, 2022). While the DPPH is a free radical that reacts with various

Table 2
Quantification of individual phenolics, total phenolic content and antioxidant capacity in mushroom extracts.

Variable	Cap	Stipe
Gallic acid (μg/g)	2.23 ± 0.14^{a}	1.70 ± 0.03^{a}
Caffeic acid (μg/g)	0.22 ± 0.08^{a}	0.07 ± 0.00^{a}
Catechin(μg/g)	0.23 ± 0.08^{a}	0.06 ± 0.08^{a}
Procyanidin B2 (μg/g)	0.08 ± 0.04^{a}	0.04 ± 0.03^{a}
Resveratrol (μg/g)	0.84 ± 0.35^{a}	0.34 ± 0.29^{a}
Vanillin (μg/g)	0.16 ± 0.00^{a}	0.26 ± 0.06^{a}
Hydroxytyrosol (μg/g)	0.04 ± 0.03^{a}	0.27 ± 0.08^{a}
Oleuropein (μg/g)	0.05 ± 0.00^{a}	0.03 ± 0.01^{a}
Total phenolic content (mg GAE/g)	14.50 ± 0.35^{a}	8.40 ± 0.13^{b}
ORAC (μMeq trolox/g)	336.33 ± 8.24^{a}	290.70 ± 1.70^{b}
DPPH IC50 (mg/mL)	1.09 ± 0.15^{b}	1.73 ± 0.13^{a}

All values are expressed on a freeze-dried extract basis. Individual phenolic compounds: μg/g of dry extract, total phenolic content: mg GAE/g of dry extract, ORAC: μMeq trolox/g of dry extract, and DPPH IC_{50} as mg of extract per mL of reaction medium. Different letters in the same row represent the effect ($P < 0.05$) of the type of mushroom extract.

antioxidants in a food sample via electron- and hydrogen-transfer mechanisms, the ORAC assay measures the ability of antioxidants to scavenge peroxyl radicals, which are the main free radicals produced during lipid oxidation in foods (Abeyrathne, Nam, & Ahn, 2021). Our results are consistent with those reported by Radzki et al. (2024) in their evaluation of antioxidant capacity using the FRAP and DPPH methods, in which these authors observed greater antioxidant capacity in the caps than in the stipes.

The differences in the antioxidant profile of *P. ostreatus* cap and stipe extracts suggest that their application in burgers will differ in terms of oxidative stability and other relevant parameters. The application and comparison of antioxidant extracts from different parts of edible mushrooms in meat products have not been explored. In the search for better-performing substitutes for synthetic antioxidants, the present study evaluated the substitution of sodium erythorbate, a widely used antioxidant in the meat products industry.

3.2. Instrumental color

Color is one of the primary sensory attributes influencing consumer acceptance of meat products, as it is closely associated with freshness and perceived quality (Mancini & Hunt, 2005). In the present study, the instrumental color parameters *L**, *a**, *b**, *C** (chroma), and hue were evaluated over 90 d of frozen storage to determine the impact of *P. ostreatus* extracts on the color stability of burgers. As shown in Table 3, storage time, treatment, and their interaction had significant effects on all color parameters ($P < 0.05$). The discussion focuses on the interaction between treatment and storage time. This interaction indicates that the effect of each antioxidant was not constant over storage time but varied across days, therefore color responses should be interpreted dynamically rather than based solely on main effects.

The interaction between storage time and treatment significantly affected the color stability of the burgers, with variations observed across time points and treatments. The trend in the color parameters *L**, *a**, *b**, and chroma was a decrease at 30 d, followed by stabilization, resulting in values statistically similar to those at time zero. However, the magnitude and recovery of these changes differed across treatments, evidencing the significant interaction. This pattern was more noticeable for the lightness of the burgers, especially in those containing cap extract, where significantly higher values were observed at 60 d compared with time zero, indicating a greater protective effect of the cap extract on product lightness. However, for *a**, *b**, and chroma, the stipe extract showed better performance, particularly up to 60 d of storage. Conversely, hue results were the opposite of those for the other color measures. For all treatments, hue increased at 30 d and then stabilized. The increase in hue for burgers with *P. ostreatus* extracts was significantly lower than that for the other treatments at 30 d, suggesting less color loss and better burger stability. Therefore, *P. ostreatus* extracts helped stabilize burger color, especially the stipe extract, which improved redness, yellowness, and color saturation, particularly during the sharp changes observed at 30 d of storage. Then, these parameters tended to increase. Additionally, hue values at 60 and 90 d stabilized and closely resembled those at day 0, demonstrating recovery and sustained color stability.

Based on the findings, two conclusions can be drawn. First, burger discoloration was not linear. We initially hypothesized a steady decrease in *a**, *b**, and chroma with a concomitant increase in hue; however, even in the control treatment, these parameters tended to stabilize after 30 d of storage. Georgantelis, Blekas, Katikou, Ambrosiadis, and Fletouris (2007) reported linear decreases in *a**, *b**, and chroma, with a concomitant increase in hue, in beef burgers stored at −20 °C for 180 d, while *L** varied according to the antioxidant applied (rosemary extract, chitosan, α-tocopherol). In contrast, our results are consistent with those of Deng, Han, Gao, Ye and Liu (2021), who observed nonlinear discoloration in beef stored at −18 °C under temperature fluctuations of ±0.5, 1.0, and 2.0 °C, with a sharp decrease in *a** during the first 10 d, followed by stabilization up to 90 d. In our study, the freezing chamber fluctuated by ±2 °C, which likely contributed to the non-linear color changes. Temperature variation should therefore be considered a critical factor in maintaining color stability in frozen meat products. The vacuum environment further modulates color stability by limiting oxygen availability, thereby favoring DMb formation and reducing O2Mb stability during frozen storage. This packaging condition amplifies the influence of antioxidant treatments and temperature fluctuations on the DMb–O2Mb–MMb balance, providing a mechanistic basis for the nonlinear discoloration patterns described above (Ledward, 1985). In addition to the non-linear discoloration behavior, the similarity in color values between the Control and the sodium erythorbate treatment can be attributed to erythorbate's reduced effectiveness at frozen temperatures (Sepe et al., 2005). Although erythorbate is a strong reducing agent

Table 3
Effect of antioxidant type on the instrumental color parameters in beef hamburgers during frozen storage.

Variable	Time (Day)	Treatment				*P*-value			
		Control	Sodium erythorbate	Stipe	Cap	SEM	Tim.	Tr.	Tim*Tr
*L**	0	51.8^{ab}	50.2^{abcd}	49.9^{abcd}	47.1^{bcde}	0.02	<0.0001	0.017	0.040
	30	45.4^{de}	50.3^{abc}	44.3^{e}	45.6^{cde}				
	60	51.3^{ab}	50.6^{ab}	49.9^{abcd}	52.5^{a}				
	90	50.6^{ab}	50.6^{ab}	50.3^{abcd}	49.2^{abcd}				
*a**	0	29.2^{a}	20.4^{e}	28.1^{ab}	27.2^{ab}	3.30	<0.0001	<0.0001	<0.0001
	30	7.1^{h}	8.9^{gh}	12.1^{f}	10.5^{fg}				
	60	23.5^{cd}	23.4^{cd}	25.1^{bc}	21.3^{de}				
	90	23.2^{cde}	22.6^{cde}	21.4^{de}	21.5^{de}				
*b**	0	22.5^{a}	18.2^{bc}	22.6^{a}	21.3^{a}	1.48	<0.0001	<0.0001	<0.0001
	30	13.4^{g}	13.5^{fg}	14.2^{defg}	13.9^{efg}				
	60	16.9^{bc}	16.5^{bcd}	18.4^{b}	16.7^{bc}				
	90	17.2^{bc}	16.8^{bc}	16.0^{bcde}	15.9^{cdef}				
Chroma	0	36.9^{a}	27.3^{d}	36.0^{a}	34.6^{ab}	3.36	<0.0001	<0.0001	<0.0001
	30	15.2^{e}	16.2^{e}	18.7^{e}	17.4^{e}				
	60	28.9^{cd}	28.6^{cd}	31.1^{bc}	27.0^{d}				
	90	28.9^{cd}	28.2^{cd}	26.8^{d}	26.8^{d}				
Hue	0	37.7^{de}	41.8^{d}	38.8^{de}	38.0^{de}	3.35	<0.0.001	0.005	<0.0.001
	30	61.9^{a}	57.1^{ab}	49.7^{c}	53.0^{bc}				
	60	35.8^{e}	35.7^{e}	36.3^{de}	38.1^{de}				
	90	36.6^{de}	36.6^{de}	36.9^{de}	36.5^{de}				

Different letters indicate significant differences (Tukey's test, $P < 0.05$) within each variable (*L**, *a**, *b**, Chroma, Hue). SEM: standard error of the mean. Tim: time effect; Tr: treatment effect; Tim*Tr: interaction between time and treatment.

in fresh or chilled meat, its capacity to maintain myoglobin in the ferrous state decreases when freezing promotes pigment denaturation, iron release, or reductant depletion. This is consistent with our myoglobin derivative results, where erythorbate neither limited the increase in MetMb nor maintained a stable OxyMb fraction. Similar findings in frozen beef patties also report diminished reductant activity, resulting in color characteristics comparable to controls (Sepe et al., 2005; Suman et al., 2005).

Second, the stipe extract performed better than the cap extract. The superior performance of the stipe extract, despite its lower in vitro antioxidant capacity, may reflect qualitative compositional differences and how specific molecules interact with pro-oxidant sites in meat. Previous research shows that the chemical structure of polyphenols influences their reactivity with proteins and pro-oxidants, including heme-containing myoglobin (Cheng, Lin, Tang, Yang, & Liu, 2022; Wu, Bak, Goran, & Tatiyaborworntham, 2024). Moreover, certain phenolic compounds can affect the redox state of myoglobin and thereby influence color stability (Palanisamy, Singh, Zhang, Kim, & Benjakul, 2025). Instrumental color reflects underlying chemical changes in the product, primarily myoglobin oxidation, which is a major driver in beef burgers (Estévez, 2021; Gonza, Cavalcante, Saldana, de Oliveira Sartori, & Castillo, 2023; Grasso et al., 2024). Other constituents, such as lipids, may also contribute to color modification. Faustman, Sun, Mancini, and Suman (2010) proposed that lipid and myoglobin oxidation are interrelated, with reactive species from one pathway promoting oxidation in the other. Wang, Tu, Zhou, Lu, and Xu (2021) described a broader framework for rabbit meat, in which microbial spoilage, myoglobin oxidation, lipid oxidation, and protein oxidation collectively influence color. Understanding these chemical changes is therefore essential for interpreting color quality.

To clarify why the stipe extract improved burger color stability more than the cap extract despite its lower phenolic content and antioxidant capacity, we note that a high concentration of phenolic compounds does not necessarily increase antioxidant activity; in some cases, it can exert pro-oxidant effects (Estévez, 2021), potentially compromising burger color. Nevertheless, this does not appear to be the case here, as both mushroom extracts reduced the magnitude of color changes during storage compared with sodium erythorbate and the control, even though the absolute a^* values were not higher. This result partially aligns with Packer et al. (2015), who reported that guava extract reduced lipid oxidation in cooked chicken meat despite having lower phenolic content and antioxidant capacity than beetroot extract. Although the color parameters (L^*, a^*, b^*) showed no significant differences between antioxidant treatments, these findings suggest that antioxidant efficacy is not solely determined by phenolic content or chemical assays of antioxidant capacity; rather, it depends on the antioxidant source and its mechanism of action. In line with this view, Estévez (2021) has proposed that identifying specific oxidized amino acids (such as carbonyls, kynurenines, and dityrosines) can provide complementary information on myoglobin chemistry, offering deeper insight into how antioxidants influence the color of meat products. Therefore, color differences associated with antioxidant type likely stem from chemical phenomena occurring within the meat matrix, which are addressed in the following sections.

3.3. Chemical forms of myoglobin

Color changes in meat are primarily due to the redox dynamics of myoglobin. This protein exists in its ferrous, non-oxygenated form, deoxymyoglobin (DMb), which is characterized by a purplish-red color. Upon exposure to oxygen, myoglobin is converted to the ferrous, oxygenated form, oxymyoglobin (OMb), which gives the meat a bright cherry-red appearance. The oxidation of the heme iron in myoglobin replaces oxygen with water, forming metmyoglobin (MMb), which imparts a brown color to the meat (Faustman & Suman, 2017; Mancini & Hunt, 2005). In this study, beef accounted for 70 % of the burger formulation; therefore, chemical changes in myoglobin may explain the observed variations in instrumental color. As shown in Table 4, the three redox forms of myoglobin evaluated (DMb, OMb, and MMb) exhibited significant differences, and a notable interaction between treatments and storage time was detected, indicating that the oxidation pathway varied depending on both time and antioxidant type.

DMb increased significantly in all treatments from 60 d of storage, although the increase was less evident in burgers containing *P. ostreatus* extracts. This increase suggests that oxygen availability in the burgers decreases over time. This was evident in the OMb results, which were inversely related to DMb; that is, significant decreases were observed across all treatments throughout frozen storage. However, the cap extract tended to stabilize OMb levels better than the other treatments, as no significant differences were observed between 0 and 90 d. At the same time, this treatment showed the lowest DMb index at 90 d, suggesting greater oxygen availability and better myoglobin stability. Abrupt decreases in OMb were also observed at 30 d, similar to those observed for the color parameters L^*, a^*, b^*, and chroma, described in the previous section. Concomitantly, there was a significant increase in MMb at 30 d, similar to that observed in the hue results. This increase suggests oxidation of the iron present in myoglobin; however, it was significantly lower in burgers containing *P. ostreatus* extracts after 30 d of storage. Likewise, MMb levels tended to stabilize across treatments; however, this stabilization was insufficient in burgers containing sodium erythorbate, where MMb levels remained significantly lower than at 0 d. In short, *P. ostreatus* extracts improved myoglobin stability by attenuating the sharp increase in MMb observed at 30 days, when oxidation was most pronounced. The extracts effectively limited oxidative changes

Table 4
Interaction effect of storage time and antioxidant treatment on myoglobin redox states in beef burgers during frozen storage.

Variable	Time (Day)	Treatment				*P*-value			
		Control	Sodium erythorbate	Stipe	Cap	SEM	Tim.	Tr.	Tim*Tr
%DMb	0	5.2^{fg}	6.7^{fg}	0.6^{g}	3.6^{g}	1.00	<0.0001	<0.0001	0.034
	30	9.4^{efg}	8.9^{efg}	6.5^{fg}	5.3^{fg}				
	60	31.1^{ab}	37.2^{a}	21.7^{cd}	17.0^{cde}				
	90	18.8^{cd}	24.6^{bc}	20.2^{cd}	12.9^{def}				
%OMb	0	69.9^{ab}	61.0^{abcd}	72.5^{a}	68.6^{abc}	5.76	<0.0001	<0.0001	<0.0001
	30	26.4^{h}	38.4^{gh}	54.1^{def}	51.7^{def}				
	60	46.6^{efg}	42.4^{fg}	56.8^{cde}	59.4^{bcd}				
	90	57.3^{cde}	51.3^{def}	54.7^{de}	62.9^{abcd}				
%MMb	0	24.9^{ef}	32.3^{d}	26.8^{def}	27.8^{de}	4.93	<0.0001	<0.0001	<0.0001
	30	64.2^{a}	52.8^{b}	39.4^{c}	43.1^{c}				
	60	22.2^{ef}	20.4^{f}	21.5^{ef}	23.6^{ef}				
	90	23.9^{ef}	24.1^{ef}	25.1^{ef}	24.2^{ef}				

Superscripts indicate significant differences (Tukey's test, $P < 0.05$) across all treatment ×time means within the same variable (%DMb, %OMb, %MMb). Differences are not interpreted by main effects due to significant Time*Treatment interaction.
SEM: standard error of mean; Tim.: time effect; Tr.: treatment effect; Tim*Tr: interaction effect;

during the critical mid-storage period, contributing to overall color stability.

The similar patterns of change between OMb and MMb and the color parameters suggest that changes in myoglobin redox state explain the burgers' color, in accordance with established concepts in meat science (Estévez, 2021; Grasso et al., 2024). Notably, unlike the color results, the contribution of the stipe extract was not higher than that of the cap extract; instead, the latter tended to stabilize myoglobin levels with respect to DMb and OMb, which could be associated with its higher total phenolic content and antioxidant capacity. In this sense, beyond the myoglobin redox state, other factors, such as lipid and protein oxidation, may also contribute to determining the final color of the burgers (Faustman et al., 2010; Gonza et al., 2023; Wang et al., 2021).

3.4. Lipid oxidation

In meat products, lipid oxidation is commonly monitored by measuring malondialdehyde (MDA), a product of fatty acid autoxidation that correlates with sensory analysis as an indicator of oxidative flavor (Pegg, Kerrihard, & Shahidi, 2024). In this study, MDA was quantified using the TBARS method (Fig. 1). Lipid oxidation measurements showed significant interactions ($P \leq 0.05$); therefore, the discussion focuses on the interaction between freezing time and treatment.

In the control treatment, TBARS values increased significantly ($P < 0.05$) up to 30 d, then stabilized until 90 d. In contrast, treatments with erythorbate, stipe, and cap showed no significant increase over the 90 d storage period and remained significantly lower ($P < 0.05$) than the control. These results indicate that natural *P. ostreatus* extracts effectively preserved antioxidant properties during storage. Both *P. ostreatus* extracts were comparably effective in delaying lipid oxidation in beef burgers. These findings are consistent although the whole *P. ostreatus* mushrooms may with previous studies showing that mushroom-based ingredients can act as effective natural antioxidants in meat products, protecting against lipid oxidation during storage (Saldaña & Rios-Mera, 2025). However, unlike those studies, in which whole mushrooms or non-fractionated flours were incorporated, our work specifically evaluates antioxidant extracts obtained separately from the stipe and cap of *P. ostreatus*, representing a novel anatomical comparison.

The interaction between the stipe extract and sodium erythorbate at 30 d suggests greater effectiveness than the cap extract, in agreement with the color results. This finding further supports the existence of differences in antioxidant mechanisms between mushroom parts (Radzki et al., 2024). Understanding the molecular mechanism of antioxidant action is essential for their application in meat products (Estévez, 2021); however, this was not the objective of the present study. Previous research on *P. ostreatus* has shown that its phenolic-rich extracts exert antioxidant effects primarily through radical scavenging, reducing power, and metal-chelating mechanisms (Effiong, Umeokwochi, Afolabi, & Chinedu, 2024). Rather, the aim was to comprehensively evaluate the protective effect of *P. ostreatus* extracts on the physicochemical and sensory quality of a meat product. In this regard, although MDA levels increased during storage, they remained below sensory rancidity thresholds reported for pork and beef products, typically ranging from 2.3 to 2.5 mg MDA/kg (Wood et al., 2008; Zhang et al., 2019). Even in the control treatment, values remained low, suggesting that other factors, such as prolonged freezing and vacuum packaging, may have contributed to slowing lipid oxidation in burgers, in agreement with the findings of Coombs, Holman, Friend, and Hopkins (2017) and Kanner (1994).

However, as reviewed by Jacobsen (2019), TBARS measurement alone does not provide a complete picture of oxidation products. A more comprehensive assessment, including protein oxidation (Estévez, 2021), can offer a more accurate explanation for the changes observed in the quality traits of beef burgers.

3.5. Protein oxidation and pH

Protein carbonylation is one of the main chemical changes associated with protein oxidation, affecting texture, color, aroma, flavor, water-holding capacity, and biological functionality in meat systems (Estévez, 2011). In the present study, both the antioxidant type and freezing storage, as well as their interaction, significantly affected carbonyl formation in beef burgers (Figs. 2–3). For all treatments, protein oxidation increased significantly with longer frozen storage; however, the control treatment exhibited the highest levels of oxidation. The *P. ostreatus* extracts showed an effect similar to that of sodium erythorbate, further supporting their potential as natural alternatives to this synthetic additive.

Another factor related to protein functionality is pH. Prior studies have shown that protein deterioration in burgers during frozen storage is linked to increased pH (Ashrafi, Ahari, Asadi, & Mohammadi Nafchi, 2024; Zahid et al., 2020). In line with this background, a significant increase in pH was observed for all treatments (Table 4), with the control showing higher values than the antioxidant treatments from 30 d onward. Notably, burgers containing *P. ostreatus* extracts exhibited lower pH at 90 d ($P < 0.05$) than the other treatments, suggesting that

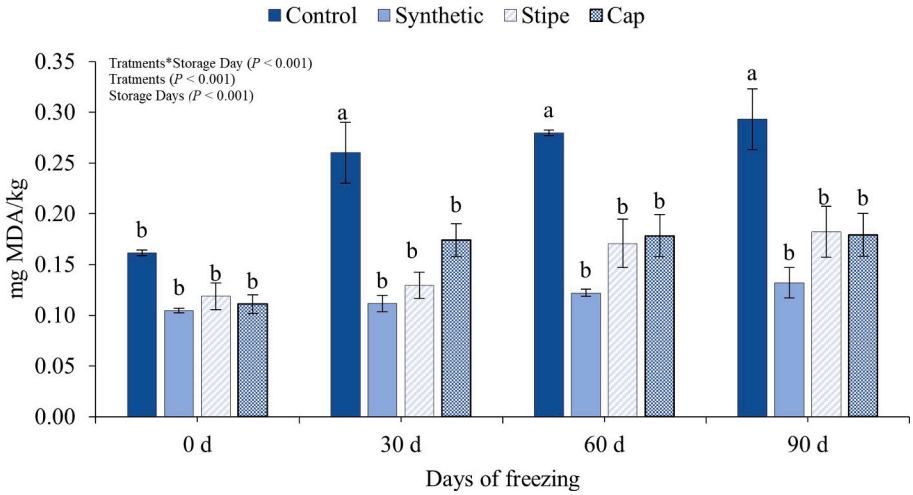


Fig. 1. Effect of antioxidant type on lipid oxidation (TBARS, mg MDA/kg) in beef burgers during frozen storage (0–90 days). Treatments: Control (no antioxidant), Sodium erythorbate (0.01 %), *P. ostreatus* stipe extract (0.5 %), and *P. ostreatus* cap extract (0.5 %). Error bars correspond to the standard error. Different letters [a-b] indicate a significant difference (Tukey's test).

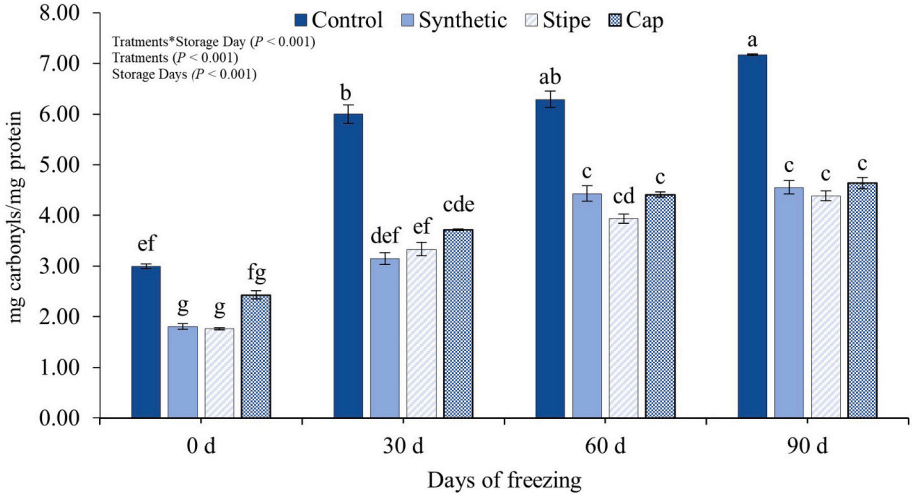


Fig. 2. Effect of antioxidant type on protein oxidation in beef hamburgers during frozen storage (0–90 days). Treatments: Control (no antioxidant), Sodium erythorbate (0.01 %), *P. ostreatus* stipe extract (0.5 %), and *P. ostreatus* cap extract (0.5 %). Error bars correspond to the standard error. Different letters [a-b] indicate a significant difference (Tukey's test).

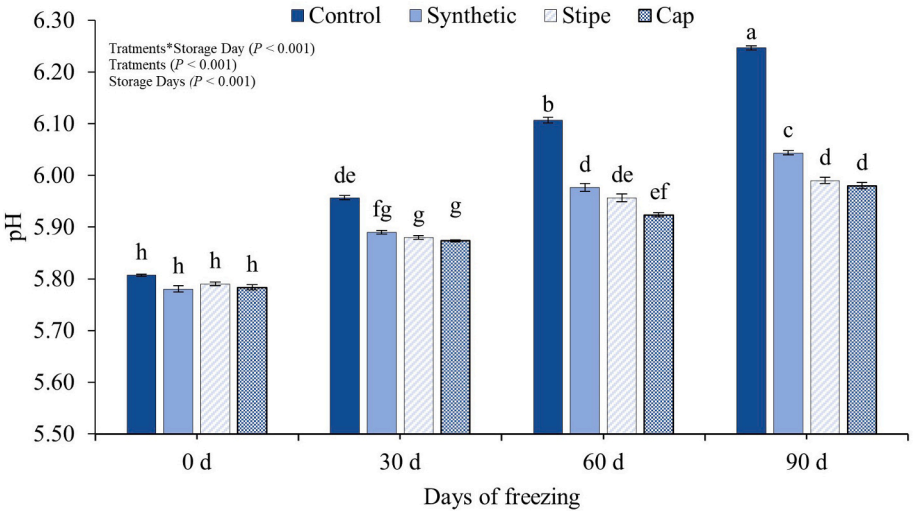


Fig. 3. Effect of antioxidant type on pH in beef hamburgers during frozen storage (0–90 days). Treatments: Control (no antioxidant), Sodium erythorbate (0.01 %), *P. ostreatus* stipe extract (0.5 %), and *P. ostreatus* cap extract (0.5 %). Error bars correspond to the standard error. Different letters [a-b] indicate a significant difference (Tukey's test).

these natural extracts protected against protein structural modifications during prolonged freezing, with potential positive implications for meat quality.

In terms of the sensory quality of raw meat products, color is a primary aspect. Thus, instrumental color and the changes possibly associated with this parameter reveal distinct mechanisms in the two types of *P. ostreatus* extracts during frozen storage. The stipe extract favored redness (a^*), yellowness (b^*), and chroma, at the same time, delayed lipid oxidation similar to sodium erythorbate, while the cap extract promoted better stabilization of lightness (L^*) and myoglobin, with a similar effect to the stipe extract on protein oxidative stability and pH. The differences between total phenolic content and antioxidant capacity, while informative, do not fully explain the extracts' overall antioxidant behavior. Therefore, a more detailed evaluation of the antioxidant constituents of the extracts is necessary, including factors related to their mechanism of action, such as the partitioning potential, location and mobility in the reaction media, polarity, molecular size, as well as the interactions of the antioxidants with the components of the meat product, through assessments of electron transfer, hydrogen transfer and metal chelation (Packer et al., 2015; Shahidi & Zhong, 2011). Since *P. ostreatus* extracts showed advantages over sodium erythorbate and therefore have the potential to replace it, elucidating the mechanism of action of each extract is necessary to explain the protective effect not only in terms of meat product quality but also to evaluate its nutritional effects (Estévez, 2021).

On the other hand, mushrooms, in addition to their antioxidant properties, have the potential to enhance flavor in meat formulations (Saldaña & Rios-Mera, 2025), which motivated the present study to explore *P. ostreatus* extracts in beef burgers. For this type of product, the dominance of sensory attributes related to flavor and juiciness, such as "tasty," "seasoned," "salty," and "juicy," as well as adequate texture, directly impacts consumer acceptance (Rios-Mera et al., 2020). In this regard, understanding the physicochemical changes that affect sensory characteristics is key to establishing appropriate processing parameters for burger reformulation.

Table 5
Effect of antioxidant type on cooking losses, diameter reduction, and instrumental texture properties in beef burgers.

Variable	Treatment					*P*-value
	Control	Synthetic	Stipe	Cap	SEM	
Cooking losses (%)	28.55	28.42	28.90	26.18	0.44	0.05
Diameter reduction (%)	1.39	0.90	1.02	0.90	0.08	0.06
Hardness (N)	99.38	105.12	94.08	109.24	2.34	0.06
Springiness (mm)	0.64	0.69	0.68	0.76	0.02	0.21
Cohesiveness	0.33	0.33	0.32	0.32	0.00	0.96
Chewiness (N)	20.56	24.03	19.92	26.62	1.11	0.08

The SEM (Standard Error of the Mean) is provided for each comparison.

3.6. Texture profile analysis, cooking loss and diameter reduction

The instrumental texture profile (hardness, springiness, cohesiveness, and chewiness) and cooking losses and diameter reduction are shown in Table 5. No differences ($P > 0.05$) were observed between treatments for the analyzed parameters, suggesting that *P. ostreatus* antioxidants helped maintain the burgers' physical structure and retain components such as water and fat during cooking. Similar results were reported by Torres-Martínez et al. (2022), who used *P. ostreatus* extract as a substitute for butylhydroxytoluene in pork patties and observed that the instrumental texture evaluated immediately after processing showed no statistical differences between treatments; however, the treatment containing the highest concentration of *P. ostreatus* (5 %) reduced cooking losses. Although no statistical differences were found among treatments, burgers containing the cap extract showed numerically lower cooking losses and higher hardness and chewiness values (Table 5). A similar pattern was observed by Sheng et al. (2024) in sous-vide-cooked chicken patties marinated with Lentinus edodes powder at concentrations ranging from 0 % to 14 %, with the highest concentrations (12 % and 14 %) resulting in the lowest cooking losses and the greatest hardness and chewiness. Thus, the effects on texture, cooking loss and diameter reduction will depend on the type of meat product, the type and concentration of the mushroom extract, and other factors. For this study, the impact of the observed trends on sensory quality will be considered.

3.7. Free amino acid profile

Free amino acids are major contributors to the overall taste profile of meat. They are associated with sour, sweet, bitter, salty, and umami tastes and, in addition to their sensory roles, provide essential nutrients for human health (You et al., 2024). Table 6 presents the free amino acid profile of the burgers. A total of 19 amino acids were identified, including 10 non-essential amino acids (ASP, GLU, ASN, SER, GLN, GLY, ARG, TYR, ALA, ORN) and 9 essential amino acids (HIS, THR, TRP, MET, VAL, PHE, ILE, LEU, and LYS) (Dewangan, Berdimurodov, & Verma, 2023). The most abundant amino acids, in descending order, were glutamine, alanine, lysine, and glutamic acid, which are reported to contribute to flat/salty/bitter, sweet, sweet/bitter, and umami/sour flavors, respectively (You et al., 2024). Significant differences ($P < 0.05$) were observed for most amino acids, except tyrosine, ornithine, and lysine. Where differences occurred, burgers containing *P. ostreatus* extracts showed higher concentrations of free amino acids than those containing sodium erythorbate or no antioxidant. Notably, the cap extract led to higher levels of aspartic acid and glutamic acid than the stipe extract ($P < 0.05$), both of which are key contributors to umami flavor (You et al., 2024).

Previous studies suggest that the cap has a richer amino acid or free amino acid content than the stipe in mushroom species such as *Lentinula edodes*, *Tricholoma matsutake*, *Agaricus bisporus*, *Pleurotus ostreatus*, and *Hypsizygus marmoreus* (Chen et al., 2015; Cho, Choi, & Kim, 2010; Jai-Sik, Young-Hoi, Myung-Kon, Young-Soo, & Hee-Suk, 1989; Park et al., 2017), which could be related to higher metabolic activity and protein biosynthesis in the cap compared to the stipe (Park et al., 2017). However, there is also evidence suggesting that the stipe may have a higher total content of free amino acids than the cap, as observed by Dospatliev et al. (2018) in *Morchella esculenta*. In addition to the part of the mushroom, these variations can be due to factors such as species, maturity stage, quality grade, storage time, and processing methods (Zhang, Venkitasamy, Pan, & Wang, 2013). The findings of this study suggest that the extracts from *P. ostreatus* have a balanced profile of essential and non-essential free amino acids, which could enhance the nutritional and sensory quality of the burgers.

Table 6
Effect of antioxidant type on free amino acids in beef burgers.

Amino acid (mg/100 g)	Control	Sodium Erythorbate	Stipe	Cap	SEM
Aspartic acid (ASP)	1.56^{c}	1.61^{c}	3.09^{b}	5.86^{a}	0.71
Glutamic acid (GLU)	13.04^{c}	14.25^{c}	20.21^{b}	25.72^{a}	2.07
Asparagine (ASN)	3.58^{ab}	3.34^{b}	4.24^{ab}	4.37^{a}	0.18
Serine (SER)	6.64^{b}	6.96^{b}	9.66^{a}	10.01^{a}	0.62
Glutamine (GLN)	93.83^{b}	95.34^{b}	102.09^{a}	101.14^{a}	1.46
Histidine (HIS)	6.31^{b}	6.44^{b}	8.78^{a}	8.02^{ab}	0.43
Glycine (GLY)	9.74^{b}	10.14^{ab}	11.03^{a}	10.45^{ab}	0.19
Threonine (THR)	5.21^{b}	5.49^{b}	7.75^{a}	7.84^{a}	0.50
Arginine (ARG)	9.4^{b}	9.73^{b}	12.31^{a}	12.69^{a}	0.60
Tyrosine (TYR)	2.24^{a}	2.29^{a}	3.12^{a}	3.35^{a}	0.20
Alanine (ALA)	70.78^{b}	72.94^{b}	83.55^{a}	83.18^{a}	2.37
Tryptophan (TRP)	1.08^{b}	1.12^{b}	1.51^{a}	1.55^{a}	0.09
Methionine (MET)	2.98^{b}	3.02^{b}	3.73^{a}	3.70^{a}	0.15
Valine (VAL)	5.51^{b}	5.77^{b}	8.35^{a}	8.28^{a}	0.55
Phenylalanine (PHE)	4.25^{b}	4.42^{b}	6.50^{a}	6.64^{a}	0.46
Isoleucine (ILE)	3.56^{b}	3.76^{b}	5.75^{a}	5.40^{a}	0.39
Leucine (LEU)	6.67^{b}	6.83^{b}	10.30^{a}	9.71^{a}	0.67
Ornithine (ORN)	10.81^{a}	11.27^{a}	11.27^{a}	9.83^{a}	0.24
Lysine (LYS)	17.97^{a}	16.32^{a}	17.27^{a}	17.39^{a}	0.24

Values are expressed as mg/ 100 g of raw burger (fresh weight basis). Different letters in the same row represent the effect ($P < 0.05$) of treatment (control, sodium erythorbate, stipe, cap). The SEM (Standard Error of the Mean) is provided for each comparison.

3.8. Sensory analysis

Consuming foods like meat involves mastication and salivation, releasing flavors evaluated by dynamic sensory methods like TDS, which collects data during a single evaluation. TDS has been used, as in the present study, to characterize chicken burgers containing pink pepper extract as a natural antioxidant (Menegali et al., 2020). In this work, the dominance of sensations of the evaluated attributes is shown in Fig. 4. But before analyzing the results, it is important to mention that the microbiological quality of the burgers ranged from 3.66 to 4.56 log CFU/g for aerobic mesophilic bacteria, 5.15 to 5.49 log CFU/g for psychrotrophic bacteria, <2 log CFU/g for molds and yeasts, <0.48 log MPN/g for total coliforms and *Escherichia coli*, and absence of *Salmonella* spp., being within the limits for human consumption (APHA, 2015) and guaranteeing its consumption for sensory evaluation.

As shown in Fig. 4, the treatments differed in the dominance of the sensory attributes tender, fatty, tasty, and seasoned. Among the most notable differences was the attribute 'tender', which was not perceived as dominant in burgers containing the cap extract. This observation aligns with the instrumental texture results, which showed numerically higher hardness and chewiness values for this formulation (Table 5). Another difference observed was that, in the control treatment, the fatty sensory attribute showed higher scores at the end of the evaluation, while burgers containing antioxidants exhibited higher perceived flavor intensity. These differences may be related to the greater lipid oxidation in the control burger (Fig. 1), which can lead to unfavorable sensory effects arising from the lipid fraction and its oxidation products. All antioxidant-treated burgers showed a greater dominance of the attribute

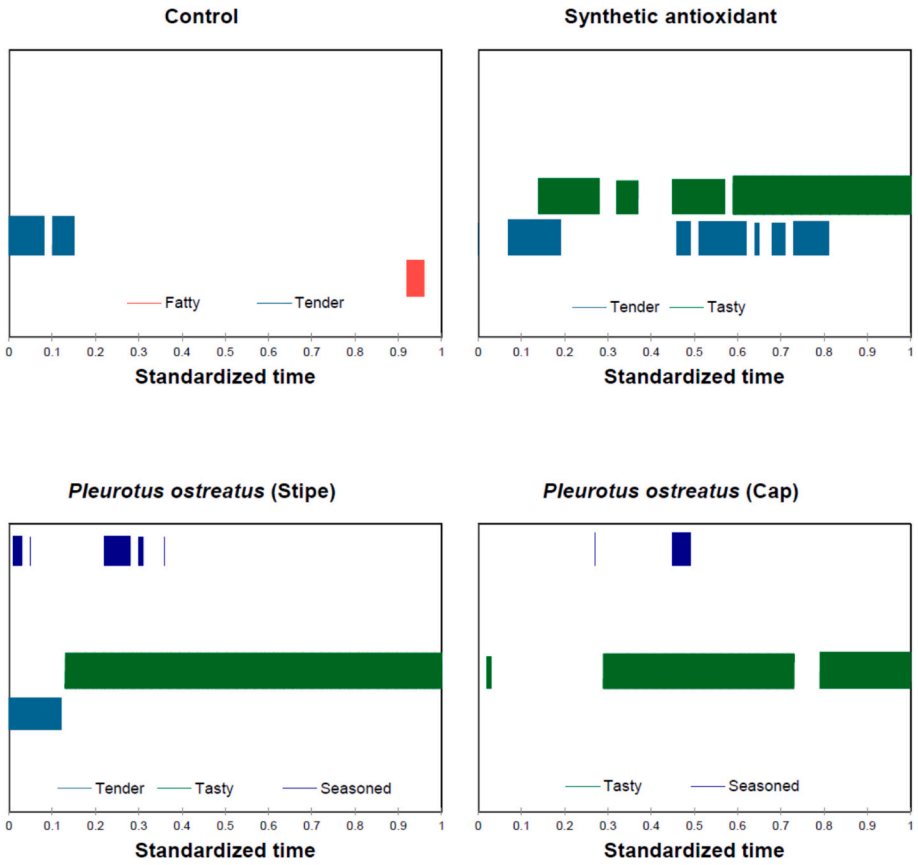


Fig. 4. Temporal Dominance of Sensations (TDS) profiles of beef burgers with different antioxidant sources, illustrating the sequence and dominance of sensory attributes (tender, fatty, tasty, and seasoned) during consumption. Treatments: Control (no antioxidant), sodium erythorbate (0.01 %), *P. ostreatus* stipe extract (0.5 %), and *P. ostreatus* cap extract (0.5 %).

"tasty" compared with the control. This dominance may be associated with the lower lipid and protein oxidation observed in these treatments, which likely reduced the formation of off-flavors and preserved desirable flavor compounds during mastication.

In contrast, burgers formulated with *P. ostreatus* extracts exhibited an additional and sustained dominance of the attribute "seasoned", which was not perceived in the control or sodium erythorbate treatments. This effect may be attributed to the higher abundance of free amino acids in the burgers containing *P. ostreatus* extracts (Table 6), as free amino acids are known contributors to seasoning-like and umami-related sensory perceptions. It is important to clarify that spices were intentionally omitted from all formulations to avoid confounding effects. This design allowed the sensory impact of *P. ostreatus* extracts to be evaluated in isolation, ensuring that the perceived "seasoned" or savory character is attributable to the mushroom extract rather than external seasoning ingredients. Future studies could extend this approach by directly comparing mushroom-treated burgers with conventionally spiced formulations to quantify relative sensory contributions.

Overall, the results strengthen the multifunctional role of edible mushrooms in meat systems (Saldaña & Rios-Mera, 2025), demonstrating that the antioxidant and flavor-enhancing effects of *P. ostreatus* can even surpass those of sodium erythorbate. Specifically, it is demonstrated that the antioxidant and sensory benefits of *P. ostreatus* in beef burgers even surpass those of the synthetic antioxidant sodium erythorbate. The stipe of mushrooms is considered waste, accounting for 25 % to 33 % of the fresh weight (Chou et al., 2013). The use of the stipe of *P. ostreatus* can be a clean-label, sustainable solution for beef burger formulations, in line with current Sustainable Development Goals and consumer demands.

3.9. Overall liking

Fig. 5 shows the consumer ratings of overall liking for the burgers, evaluated on a 9-point hedonic scale. The results indicate significant differences among treatments. Burgers with stipe extract of *P. ostreatus* received the highest scores (7.13), differing significantly from the control (6.47). Meanwhile, sodium erythorbate (6.82) and cap extract (6.98) showed intermediate values, not significantly different from either the stipe extract or the control. Overall, all treatments achieved

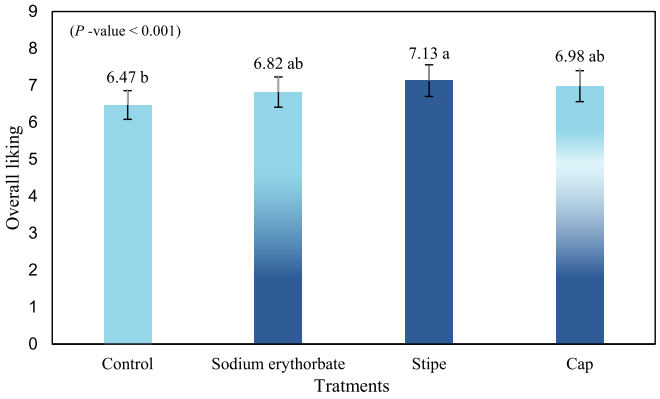


Fig. 5. Overall taste ratings of burgers with synthetic antioxidants and *Pleurotus ostreatus* extracts on a 9-point hedonic scale. Treatments: Control (no antioxidant), Sodium erythorbate (0.01 %), *P. ostreatus* stipe extract (0.5 %), and *P. ostreatus* cap extract (0.5 %). Error bars correspond to the standard error. Different letters $^{a\text{-}b}$ indicate a significant difference (Tukey's test).

mean scores close to 7 on the 9-point scale, suggesting moderate to high consumer acceptance.

These comparable ratings indicate that the inclusion of *P. ostreatus* extracts or the synthetic antioxidant (sodium erythorbate) did not adversely affect consumer perception. In particular, both stipe and cap extracts had no negative effect on overall liking, indicating that these natural antioxidants can improve product quality without compromising sensory acceptance. Overall, these findings underscore the potential of *P. ostreatus* extracts as natural antioxidants in beef burgers, maintaining consumer acceptance while enhancing product stability and sensory appeal.

4. Conclusion

Cap and stipe extracts of *P. ostreatus* improved the quality of beef burgers, although the extracts differed in the characteristics evaluated. The combined effect of the treatments and freezing for up to 90 d caused changes in instrumental color (L^*, a^*, b^*, chroma, hue), myoglobin redox state, lipid and protein oxidation, and pH, with a greater predominance of positive effects of the stipe extract on a^*, b^*, chroma, and lipid oxidation, while the cap extract improved the stabilization of lightness (L^*) and myoglobin. Both extracts showed effects similar to or superior to those of sodium erythorbate during freezing storage, suggesting the potential of *P. ostreatus* to maintain the oxidative stability of beef burgers. Burgers containing *P. ostreatus* extracts showed a modest increase in free amino acid abundance and were characterized by higher intensities of the "tasty" and "seasoned" attributes, which contributed to a slightly enhanced sensory profile compared with the control. Further research should investigate the mechanisms of action of each *P. ostreatus* extract and their contributions to the nutritional quality of meat products, to comprehensively assess their potential as natural substitutes for synthetic antioxidants.

Consent form

The sensory analysis protocol was approved by the Ethics Committee of ESALQ/USP with number 6.995.367 was obtained for experimentation with human subjects, and informed consent was obtained from all participants prior to their participation.

CRediT authorship contribution statement

Milagros Maribel Coaguila Gonza: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Juan D. Rios-Mera:** Writing – review & editing, Writing – original draft, Investigation. **Alan Giovanini de Oliveira Sartori:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Chimenes Leal de Araújo:** Methodology, Investigation, Formal analysis. **Sarah Mafeis de Jesus:** Investigation, Formal analysis. **Nils Leander Huamán-Castilla:** Validation, Methodology. **Erick Saldaña:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Carmen Josefina Contreras Castillo:** Writing – review & editing, Software, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

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Data availability

Data will be made available on request.

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