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OLIVE LEAF POWDER AS A NATURAL ANTIOXIDANT ADDITIVE IN BEEF BURGERS

PÓ DE FOLHA DE OLIVEIRA COMO ADITIVO ANTIOXIDANTE NATURAL EM HAMBÚRGUERES BOVINOS

POLVO DE HOJA DE OLIVO COMO ADITIVO ANTIOXIDANTE NATURAL EN HAMBURGUESAS DE CARNE BOVINA

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ABSTRACT
Beef burgers are widely consumed meat products but due to their composition, are highly susceptible to lipid oxidation, leading to quality deterioration and reduced shelf life. There is a growing trend toward replacing synthetic antioxidants with natural alternatives. Olive leaves (Olea europaea L.), a by-product of the olive oil industry, are rich in bioactive compounds with strong antioxidant activity. This study evaluated the potential of olive leaf powder (OLP) as a natural antioxidant additive in beef burgers. Four formulations were prepared: a control without OLP and three treatments containing 0.8%, 1.6%, and 3.2% OLP. Burgers were stored under refrigeration (4 ± 1 °C) for 7 days. Antioxidant activity (by ABTS and DPPH scavenging assay), lipid oxidation (TBARS), pH, color, texture, cooking loss, shrinkage, and diameter reduction were analyzed. Olive leaves exhibited high levels of phenolic compounds and strong antioxidant capacity. The improvement in antioxidant stability and the reduction of lipid oxidation were directly proportional to the OLP inclusion levels. Additionally, OLP influenced color parameters, increased hardness, and reduced cooking loss and shrinkage. These results demonstrate that olive leaf powder is an effective natural antioxidant and a promising alternative to synthetic additives in meat products.

KEYWORDS: Food preservation. Oxidation. Meat quality. Phenolic compounds. Shelf life.

RESUMO
Os hambúrgueres bovinos são produtos cárneos amplamente consumidos, porém, devido à sua composição, são altamente suscetíveis à oxidação lipídica, o que leva à deterioração da qualidade e à redução da vida de prateleira. Há uma tendência crescente de substituir antioxidantes sintéticos por alternativas naturais. As folhas de oliveira (*Olea europaea* L.), um subproduto agrícola da produção de oliveiras, são ricas em compostos bioativos com atividade antioxidante. Este estudo avaliou o potencial do pó de folha de oliveira (OLP) como aditivo antioxidante natural em hambúrgueres bovinos. Foram preparadas quatro formulações: um controle sem OLP e três tratamentos contendo 0,8%, 1,6% e 3,2% de OLP. Os hambúrgueres foram armazenados sob refrigeração (4 ± 1 °C) por 7 dias. Foram analisadas a atividade antioxidante pelos ensaios de sequestro de radicais ABTS e DPPH, a oxidação lipídica (TBARS), pH, cor, textura, perda por cocção, encolhimento e redução de diâmetro. As folhas de oliveira apresentaram elevados teores de compostos fenólicos e forte capacidade antioxidante. A melhoria na estabilidade antioxidante foi diretamente proporcional aos níveis de inclusão de OLP. Além disso, o OLP influenciou a oxidação lipídica, os parâmetros de cor, aumentou a dureza e reduziu a perda por cocção e o

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encolhimento. Esses resultados demonstram que o pó de folha de oliveira é um antioxidante natural eficaz e uma alternativa promissora aos aditivos sintéticos em produtos cárneos.

PALAVRAS-CHAVE: Compostos fenólicos. Oxidação. Preservação de alimentos. Qualidade da carne. Vida de prateleira.

RESUMEN
Las hamburguesas de res son productos cárnicos de amplio consumo, pero debido a su composición, son altamente susceptibles a la oxidación lipídica, lo que conlleva un deterioro de la calidad y una menor vida útil. Existe una tendencia creciente a sustituir los antioxidantes sintéticos por alternativas naturales. Las hojas de olivo (Olea europaea L.), un subproducto agrícola de la producción de aceitunas, son ricas en compuestos bioactivos con actividad antioxidante. Este estudio evaluó el potencial del polvo de hoja de olivo (OLP) como aditivo antioxidante natural en hamburguesas de res. Se prepararon cuatro formulaciones: un control sin OLP y tres tratamientos con 0,8 %, 1,6 % y 3,2 % de OLP. Las hamburguesas se almacenaron en refrigeración (4 ± 1 °C) durante 7 días. La actividad antioxidante se analizó mediante ensayos de eliminación de radicales ABTS y DPPH, oxidación lipídica (TBARS), pH, color, textura, pérdida por cocción, contracción y reducción del diámetro. Las hojas de olivo presentaron altos niveles de compuestos fenólicos y una fuerte capacidad antioxidante. La mejora de la estabilidad antioxidante fue directamente proporcional a los niveles de inclusión de polvo de hoja de olivo (OLP). Además, el OLP influyó en la oxidación lipídica, los parámetros de color, el aumento de la dureza y la reducción de las pérdidas y mermas por cocción. Estos resultados demuestran que el polvo de hoja de olivo es un antioxidante natural eficaz y una alternativa prometedora a los aditivos sintéticos en productos cárnicos.

PALABRAS CLAVE: Compuestos fenólicos. Oxidación. conservación de alimentos. Calidad de la carne. Vida útil.

INTRODUCTION

As one of the most appealing ready-to-serve foods, burgers—produced from beef, pork, or poultry—are widely consumed for their affordability and culinary convenience (López-Parra *et al.,* 2024; Patinho *et al.,* 2021). They are marketed in various formats, ranging from raw and frozen to semi-cooked and refrigerated, with formulations often incorporating added fats and diverse ingredients. Typically, these products feature a high lipid content, often reaching 20–30% *(Patinho et al.,* 2021).

Lipid oxidation is the main non-microbial cause of quality deterioration in meat and meat products and results in the degradation of essential fatty acids and vitamins and the formation of toxic compounds such as malondialdehyde (MDA). Oxidative reactions lead to undesirable sensory changes, including rancid flavor and odor (off-flavors and off-odors), directly affecting consumer acceptance and shelf life (Domínguez *et al.,* 2019).

The use of antioxidants is essential for meat products, as it controls oxidative processes by inhibiting oxidation reactions by interrupting free radical chain reactions and neutralizing reactive intermediates (López-Parra *et al.,* 2024). In the meat industry, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ascorbic acid, nitrates, and nitrites are commonly used to delay oxidation and preserve sensory quality (Ribeiro et al., 2019).

However, the frequent consumption of synthetic preservatives has been associated with potential health risks, including neurodegenerative diseases and cancer (Mafe; Büsselberg, 2025).

Consequently, there is growing interest in replacing synthetic antioxidants with natural alternatives that present lower toxicity and additional health benefits, such as antimicrobial, anti-inflammatory, neuroprotective, and anticancer properties (Babaoğlu *et al.,* 2022; López-Parra *et al.,* 2024).

The concept of *clean label* has become a major trend in the development of processed foods, reflecting the growing consumer demand for products with shorter, more transparent ingredient lists composed predominantly of natural substances, and free from synthetic additives such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Al-Rimawi; Tarawa; Elama, 2017). In this context, the preference for natural ingredients and plant-derived preservatives is closely linked to the demand for greater transparency and foods perceived as safer and healthier, thereby encouraging technological reformulation toward natural preservation strategies *(Cegiełka, Aneta, 2020; Inguglia et al.,* 2023). Moreover, the evolution of the *clean label* movement increasingly incorporates principles of sustainability and circular economy, promoting the valorization of agricultural by-products as functional ingredients. The use of plant-based residues with antioxidant properties, such as olive leaves, not only contributes to waste reduction but also adds functional value to food systems, aligning technological innovation with environmental and socio-economic sustainability goals.

Olive leaves (*Olea europaea* L.) are agricultural by-products generated in large quantities during olive cultivation and pruning processes, and they represent an underutilized biomass with significant biotechnological potential. These leaves are particularly rich in phenolic compounds, including secoiridoids such as oleuropein, phenolic alcohols such as hydroxytyrosol and tyrosol, as well as flavonoids and phenolic acids, which collectively exhibit strong antioxidant activity through radical scavenging, metal chelation, and inhibition of lipid peroxidation mechanisms (Difonzo *et al.,* 2021; Khemakhem *et al.,* 2018). The concentration and profile of these bioactive compounds may vary according to cultivar, climatic conditions, agronomic practices, and extraction methods, influencing their functional performance. Traditionally, olive leaves have been used in animal feed, cosmetics, and pharmaceutical formulations due to their antimicrobial, anti-inflammatory, and cardioprotective properties. More recently, however, increasing attention has been directed toward their application in the food industry, particularly as natural antioxidant additives, functional ingredients, and sustainable alternatives to synthetic preservatives *(Değirmencioğlu et al., 2020; Difonzo et al., 2021; Lazzaroli et al.,* 2023; Ribas *et al.,* 2023). Their incorporation into food systems aligns with current trends in clean-label product development and circular economy strategies, as it enables the valorization of agricultural residues while enhancing the technological and nutritional quality of processed foods.

Therefore, this study aims to evaluate the use of olive leaf powder as a natural antioxidant additive in beef burgers and to assess its effects on oxidative stability and physicochemical properties during refrigerated storage.

MATERIALS AND METHODS

Materials

Olive leaves from Arbosana cultivar tree were from the state of Paraná, Brazil after olive fruit harvesting for oil production to oil production. The leaves were sanitized, dried in a forced-air oven at 55 °C, ground, and sieved (60 *mesh*). The resulting powder was stored protected from light at room temperature. Beef and pork fat used for burger preparation were purchased from a local market in Maringá, Paraná, Brazil.

Folin–Ciocalteu reagent, sodium carbonate, gallic acid, ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

Determination of Bioactive Compounds in Olive Leaves

Olive leaf extracts were prepared using 100% methanol at a 1:100 (w/v) ratio. Samples were vortexed for 1 min, homogenized for 10 min, and centrifuged at 3000 rpm for 15 min. The supernatants were collected for analysis. For total phenolic compounds (TPC), an aliquot (125 µL) of extract was mixed with (125 µL) Folin-Ciocalteu solution (1:1 deionized water) and (2.250 mL) sodium carbonate (28 g/L), after homogenization by vortex, samples were incubated in the dark for 30 min. The reading was performed by spectrophotometer (EvolutionTM300) at 725 nm. Gallic acid was used for the standard curve and results were expressed in mg of gallic acid equivalent (GAE)/g of sample (Singleton; Rossi, 1965).

Antioxidant Activity of Olive Leaves

Antioxidant activity was evaluated using the percentage of ABTS and DPPH radical scavenging assay and iron reduction power (FRAP) method in OLP. The ABTS assay was performed by generating the ABTS+ radical cation through the reaction of 7 mM ABTS with 140 mM potassium persulfate, followed by a 16 h incubation in the dark at room temperature. Before use, the solution was diluted with ethanol to an absorbance of 0.70 ±0.02 at 734 nm. Subsequently, 1960 µL of the ABTS+ working solution was reacted with 40 µL of sample supernatant, and the absorbance was recorded at 734 nm after 6 min (Re et al., 1999). For the DPPH scavenging activity, 150 µL of sample extract was added to 2.85 mL of a 60 µM DPPH solution.

The mixture was incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 515 nm (Li *et al.,* 2009).

Ferric reducing power was assessed by extracting samples in methanol. Briefly, 250 µL of the extract was reacted with 1.25 mL of phosphate buffer (pH 7.0) and 1.25 mL of 1% potassium ferricyanide at 50 °C for 20 min. Following incubation and cooling, 1.25 mL of 10% trichloroacetic acid was added, and the solution was centrifuged (3000 rpm, 10 min). A 2.5 mL aliquot of the supernatant was then combined with 0.5 mL of $FeCl_3$, and the absorbance was recorded at 700 nm. The results were quantified using a gallic acid standard curve (0–300 mg/L) and expressed as mg gallic acid equivalents (GAE)/g of sample (Zhu *et al.,* 2002).

Proximate Composition of Olive Leaves

Moisture, crude protein, lipids, crude fiber, and ash contents were determined in dried samples (OLP) according to AOAC (2005). Carbohydrates were calculated by difference.

Beef Burgers preparation

Four burger formulations were developed. The control treatment was made from beef (82%), pork fat (7.5%), cold water (10%), and sodium chloride (0.5%), while the other treatments included increasing levels of OLP (0.8, 1.6, and 3.2%). To prepare the burgers, the meat tissue and fat adhering to the meat were manually removed, the meat was processed in an electric grinder, and the other ingredients were added in the following order: salt and olive powder and ice water, homogenized for 2 min then the fat was added and homogenized manually for 3 min. Subsequently, the burgers were shaped into 50 g units and stored under refrigeration at ±4 °C for up to 7 days of storage.

The burgers were cooked on an electric griddle until they reached an internal temperature of 70 °C, measured with a probe thermometer. After reaching this temperature, 3 min were timed. After cooking, they were cooled to room temperature for 30 min for subsequent analysis. The experiment was repeated three times, and the analyses were performed on three burgers of each treatment at 1, 4, and 7 days of refrigerated storage.

Physicochemical characterization

In raw burgers, the centesimal composition was determined after 4 days of storage; pH, antioxidant capacity and lipid oxidation were evaluated after 1, 4, and 7 days of refrigerated storage.

The chemical composition of dried samples was determined by analysis of moisture, ash content, crude protein by the Kjeldahl method, crude fiber by AOAC (2005) and total fat (Breil *et al.,* 2017). The total carbohydrates were determined by differences, subtracting from 100% the sum of moisture, crude protein, fat, crude fiber, and ash. The results were expressed on a dry matter basis.

The caloric value (kcal/100 g) was estimated using standard Atwater conversion factors based on the proximate composition of crude protein, lipids, and carbohydrates. Energy coefficients of approximately 4 kcal/g for proteins, 9 kcal/g for lipids, and 4 kcal/g for carbohydrates were applied, in accordance with the guidelines established in Caloric Value and Composition of Foods (1956). The pH was measured using a digital pH meter (Tecnopon, model mPA-210) following the methodology described by AOAC International (2005). Briefly, 5 g of sample were homogenized with 45 mL of distilled water, and the pH value was recorded after stabilization of the electrode.

In cooked burgers, colorimetric analyses and texture profile were performed after 1, 4, and 7 days of storage. The color was analyzed using a colorimeter (Minolta CR400) through the CIELab system (L^* (lightness), a^* (redness) and b^* (yellowness)) in accordance with the guidelines for meat color evaluation. Readings were taken in three different points, and the average of these readings was used for statistical analysis. The hardness (g) was measured on a CTIII texture analyzer with the TA4/1000 probe, a 10 g load, and a speed of 1 mm/s. Cooking loss (CL%) was determined by the weight of the burgers before and after the cooking process, and shrinkage (SH%) (El-Magoli; Laroia; Hansen, 1996). Diameter reduction (DR%) was performed using a digital caliper considering three diameter measurements at different points and calculated (Seabra *et al.,* 2002).

Antioxidant Capacity and Lipid Stability

The antioxidant capacity of the burgers was evaluated using the ABTS [2,2´-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging assay and the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) free radical method as previously described. Lipid oxidation was monitored by quantification of thiobarbituric acid reactive substances (TBARS) during a 7-day storage period. Extracts were prepared from the burger dilution (1:10 w/v) in an extracting solution (7.5% TCA, 0.1% gallic acid and 0.1% EDTA) followed by centrifugation (3000 rpm/10 min). The supernatant was collected and TBARS solution (562.5 µM HCl, 15% TCA and 1% thiobarbituric acid) was added (1:1 v/v). The solution was heated (100 °C for 15 min) and cooled for 5 min; the reading was in a spectrophotometer at 532 nm. Results were expressed as mg MDA/kg of sample (Ribas *et al.,* 2025).

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Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) using the SISVAR 5.6 software (UFLA, MG, Brazil). A factorial design was employed, considering OLP inclusion levels (0, 0.8, 1.6, and 3.2%) and storage time (1, 4, and 7 days) as fixed factors. Mean differences were assessed using Tukey's test at a 5% significance level ($p < 0.05$).

RESULTS AND DISCUSSION

Characterization of Olive Leaves

The physicochemical parameters of OLP obtained from Arbosana cultivar are summarized in Table 1. The total phenolic content (TPC) was 14.87 mg GAE/g, indicating a high concentration of bioactive compounds in the extract. This value is notably higher than those reported by Lorini *et al.* (2021), who observed TPC values ranging from 8.25 to 10.05 mg GAE/g in olive leaf cultivars such as Arbequina, Manzanilla, and Picual.

The higher TPC observed in the Arbosana cultivar can be primarily attributed to intrinsic genetic differences among olive genotypes, which directly regulate the biosynthesis and accumulation of phenolic compounds. It is well established that distinct cultivars exhibit specific metabolic profiles, resulting in significant variability in both the qualitative composition and quantitative concentration of secondary metabolites in olive leaves (OL) (Boulfia *et al.,* 2025). In addition to genetic factors, environmental conditions, agronomic practices, leaf maturity, and extraction procedures may also contribute to the observed differences (Pasković *et al.,* 2025).

Table 1. Physicochemical characterization of powdered (OLP) leaves of the 'Arbosana' cultivar (*Olea europaea* L.)

	OLP
Bioactive compounds	
TPC (mgEAG/g)	14.87 ± 0.55
Antioxidant activity	
DPPH (%)	90.16 ± 1.50
ABTS (%)	24.70 ± 2.51
FRAP (mgEAG/g)	3.23 ± 0.21
Proximate composition	
Moisture (%)	5.97 ± 0.05
Ash (%)	6.46 ± 0.46
Crude Protein (%)	12.73 ± 0.49
Crude Fibre (%)	18.40 ± 0.93
Lipids (%)	2.69 ± 0.06
Carbohydrates (%)	60.21 ± 0.11

Results are expressed as mean ± standard deviation. TPC: total polyphenols; EAG: gallic acid equivalent; DPPH: scavenging of the stable free radical 1,1-diphenyl-2-picrylhydrazyl; ABTS: scavenging of the radical 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid). FRAP: iron reducing power.

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Phenolic compounds constitute a broad and structurally diverse group of plant secondary metabolites widely recognized for their biological activities, particularly their antioxidant potential. In olive leaves (OL), these compounds are present in a complex phytochemical matrix including simple phenols, flavonoids (such as flavones, flavanones, flavonols, and flavan-3-ols), and secoiridoids. Among these, oleuropein is the most prevalent constituent and exhibits antioxidant effects through free-radical scavenging mechanisms (Ribas *et al.* 2023, Borjan *et al.* 2020).

The antioxidant activity of OLP was determined using different assays to obtain a comprehensive and representative assessment, as different analytical methods are based on distinct reaction mechanisms and may respond differently to the antioxidant profile of the samples (Lorini *et al.,* 2021; Oliveira *et al.,* 2025). In this context, radical scavenging assays and metal chelation capacity were employed to explore both hydrogen atom or electron transfer mechanisms and the ability to inhibit pro-oxidant metal ions (Shalaby; Shanab, 2013).

The results demonstrated a markedly higher scavenging capacity against the DPPH radical (90.16%), followed by ABTS radical scavenging activity (24.70%) and metal chelation capacity (3.23 mg GAE/g). The superior performance in the DPPH assay suggests a strong ability of the extract to donate hydrogen atoms or electrons to stabilize free radicals (Shalaby; Shanab, 2013). In contrast, the lower response observed in ABTS and metal chelation assays indicates that antioxidant effectiveness may vary depending on the radical species involved and the specific mechanism of action. These findings reinforce the importance of employing multiple analytical approaches to accurately characterize the antioxidant potential of olive leaf products.

The centesimal analysis confirms that OLP presented a predominance of carbohydrates (60%), followed by fiber (18.4%) and protein (12.7%). The high carbohydrate fraction likely reflects the presence of cellulose, hemicellulose, and other non-structural polysaccharides naturally occurring in olive leaf tissues.

The significant fiber content (18.4%) highlights the potential of OLP as a functional ingredient, particularly in the development of fiber-enriched food products especially for the development of plant-based meat analogues. Besides health benefits, fiber can contribute to water-holding capacity, texture modification, and structural stability in food matrices (Da Silva *et al.,* 2024). The protein content (12.7%) further enhances the nutritional value of OLP. However, proteins may interact with phenolic compounds, potentially influencing antioxidant activity and bioavailability.

Overall, this proximate composition suggests that OLP is not only a source of bioactive phenolic compounds with antioxidant activity, but also a nutritionally relevant material with potential applications in functional foods.

Proximate composition of Beef Burgers

Table 2 presents the centesimal characterization and caloric value of beef burgers formulated with different concentrations of olive leaf powder (OLP). In general, the incorporation of OLP did not promote significant changes in the composition of most of the parameters evaluated, indicating that the ingredient can be added without significantly compromising the nutritional matrix of the product.

The moisture content remained similar across treatments (T1–T4), ranging from 9.47 to 9.78%, with no significant differences. Similarly, the ash and protein content showed similar values across treatments (4.14–4.43% for ash and 63.37 to 65.79% for protein), indicating that the partial replacement of the formulation with OLP did not significantly alter the ash and protein fraction.

In contrast, the lipid content showed significant variation across treatments. Treatment T2 (0.8% OLP) exhibited the highest lipid content (5.97%), while T4 (3.2% OLP) showed the lowest value (4.34%). This progressive reduction in lipid content with higher OLP levels may be associated with the dilution effect of the lipid fraction and the higher proportion of fibrous components in T4 formulation.

Table 2. Centesimal characterization and caloric value of beef burgers formulated with different levels of olive leaf powder (OLP) during 7 days of refrigerated storage

Centesimal characterization of beef burgers (% na MS)				
	T1	T2	T3	T4
Moisture (%)	9.78 ± 0.14	9.47 ± 0.02	9.49 ± 0.43	9.76 ± 0.05
Ash (%)	4.37 ± 0.21	4.43 ± 0.15	4.15 ± 0.25	4.14 ± 0.18
Crude Protein (%)	63.37 ± 10.41	65.34 ± 2.66	65.75 ± 0.93	65.79 ± 1.53
Lipids (%)	4.90 ± 0.24^{B}	5.97 ± 0.14^{A}	4.81 ± 0.06^{B}	4.34 ± 0.20^{B}
Crude Fibre (%)	3.39 ± 1.03	4.43 ± 0.92	4.26 ± 0.15	6.06 ± 1.59
Carbohydrates (%)	18.51 ± 1.08	18.67 ± 1.66	20.45 ± 0.20	19.62 ± 0.52
Caloric value (kcal/100 g)	395.69 ± 0.15^{A}	395.82 ± 2.39^{A}	390.05 ± 0.90^{A}	377.21 ± 0.71^{B}

Means with different capital letters in the same column have a significant difference (P<0.05). Results are expressed as mean ± standard deviation. T1: 0%OLP; T2: 0.8%OLP; T3: 1.6%OLP; T4: 3.2%OLP.

Crude fiber content increased with the addition of OLP, particularly in T4, which showed the highest value (6.06%), directly reflecting the high fiber concentration of olive leaf powder. Carbohydrates showed a slight increase in treatments with OLP, especially in T3 (20.45%), which may be attributed to OL structural polysaccharides. A significant reduction in caloric value was observed in T4 (377.21 kcal/100 g) compared to the other treatments, indicating that higher levels of OLP may contribute to obtaining a product with lower energy density.

In general, the use of OLP in burger formulation increases fiber content and reduces caloric value, without compromising protein composition, highlighting the potential of OLP as a functional ingredient in meat products.

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pH

The evolution of pH values in control and OLP-supplemented burgers during 7 days of refrigerated storage is presented in Figure 1. On Day 1, no significant differences were observed among treatments (5.33–5.39). This suggests that OLP addition does not interfere with the intrinsic acidity of the meat matrix.

By Day 4, a moderate treatment-dependent effect began to emerge, reflecting the onset of biochemical and microbiological changes during storage. At the end of the storage period (Day 7), pH values increased in all treatments, ranging from 5.50 to 5.81. Treatment T3 showed the highest pH value (5.81 ± 0.34), significantly differing from the control (T1; 5.50 ± 0.28), whereas T2 and T4 presented intermediate values (5.59 ± 0.27 and 5.70 ± 0.33 for T2 and T4 respectively).

Similar pH values were reported in chicken burgers supplemented with 2% olive leaf powder, with initial values of 5.65 ± 0.02 on Day 0 and 5.77 ± 0.03 after 10 days of refrigerated storage (Ibrahim *et al.,* 2022).

The increase in pH during storage may be attributed to protein degradation and accumulation of basic nitrogenous compounds, such as ammonia and amines, resulting from microbial proliferation and/or endogenous enzymatic activity (Satarzadeh *et al.,* 2024). Despite this upward trend, the final pH values remained below 6.0, which is generally considered within the acceptable range for fresh meat products.

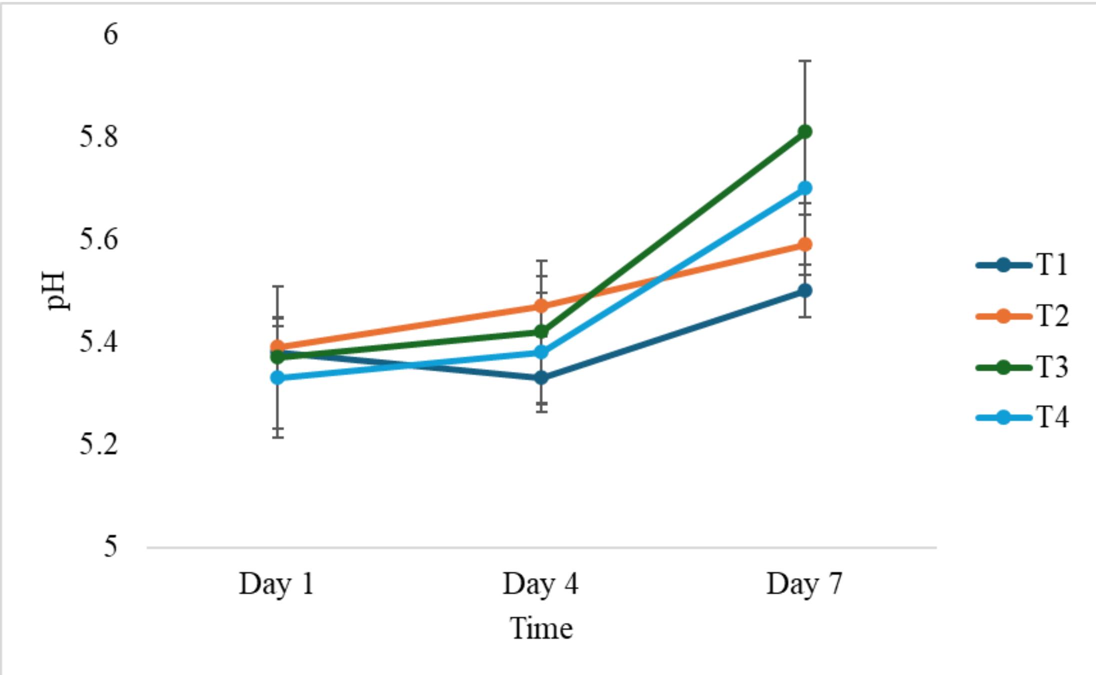

Figure 1. pH of beef burgers formulated with different levels of olive leaf powder (OLP) during 7 days of refrigerated storage. T1: 0%OLP; T2: 0.8%OLP; T3: 1.6%OLP; T4: 3.2%OLP.

Antioxidant activity and lipid oxidation

The antioxidant activity, determined by ABTS and DPPH radical scavenging assays, as well as the formation of secondary lipid oxidation products measured by TBARS, during 7 days of refrigerated storage is presented in Table 3. The incorporation of OLP significantly enhanced the antioxidant capacity of beef burgers compared to the control treatment.

Both ABTS and DPPH assays demonstrated a clear concentration-dependent effect, with antioxidant activity increasing proportionally to the level of OLP inclusion. This effect remained relatively stable throughout the storage period, indicating that the phenolic compounds present in OLP maintained their functional activity under refrigerated conditions. Among the assays, DPPH radical scavenging activity consistently showed higher values than ABTS in all treatments. A similar pattern was observed in the OLP raw material, reinforcing the role of its phenolic constituents in antioxidant activity.

Lipid oxidation increased over storage time in all treatments. However, burgers containing OLP showed lower TBARS values during 0 and 4 days of storage demonstrating the protective antioxidant effect of olive leaves. On day 7, differences were reduced, possibly due to oxygen exposure during storage.

Table 3. Antioxidant activity and lipid oxidation of beef burgers formulated with different levels of olive leaf powder (OLP) during 7 days of refrigerated storage

	Antioxidant activity		
	ABTS (%)		
	DAY 1	DAY 4	DAY 7
T1	6.94 ± 0.67^{Ca}	7.01 ± 0.29^{Da}	7.03 ± 0.57^{Ba}
T2	10.37 ± 1.97^{Ba}	9.58 ± 1.27^{Ca}	9.29 ± 0.97^{Ba}
T3	11.83 ± 2.20^{Ba}	11.85 ± 1.67^{Ba}	12.16 ± 2.27^{Aa}
T4	14.82 ± 1.63^{Aa}	13.72 ± 1.87^{Ab}	14.22 ± 1.97^{Aab}
	DPPH (%)		
T1	7.61 ± 0.90^{Ca}	8.54 ± 2.23^{Da}	8.51 ± 1.70^{a}
T2	28.44 ± 1.37^{Ba}	24.98 ± 1.31^{Cb}	26.19 ± 2.18^{Bab}
T3	30.67 ± 2.65^{Ba}	27.88 ± 0.58^{Bb}	28.61 ± 1.02^{Bab}
T4	37.75 ± 4.22^{Aa}	36.51 ± 1.47^{Aa}	35.16 ± 1.27^{Aa}
	TBARS mg MDA/kg		
T1	3.04 ± 0.60^{Aa}	3.87 ± 0.30^{Aa}	4.66 ± 1.44^{Aa}
T2	3.02 ± 0.96^{Ab}	3.01 ± 0.59^{Bb}	5.18 ± 0.86^{Aa}
T3	2.38 ± 0.50^{Bb}	3.02 ± 0.79^{Bb}	4.68 ± 0.73^{Aa}
T4	2.49 ± 0.31^{ABb}	2.87 ± 0.67^{Bb}	4.44 ± 0.56^{Aa}

Means with different uppercase letters in the same column have a significant difference; means with different lowercase letters in the same row have a significant difference (P<0.05). Results are expressed as mean ± standard deviation. T1: 0%OLP; T2: 0.8%OLP; T3: 1.6%OLP; T4: 3.2%OLP.

Meat deterioration is primarily associated with microbial proliferation and lipid oxidation, both of which contribute to the loss of nutritional value, sensory quality, and textural integrity (Oliveira *et al.,* 2025). Lipid oxidation leads to the generation of off-flavors, discoloration, and the degradation of essential fatty acids, thereby reducing product acceptability and shelf life (Amaral; Silva; Lannes, 2018).

Ground meat is particularly susceptible to oxidative deterioration due to its increased surface area, which enhances exposure to oxygen and accelerates oxidative reactions. Furthermore, the grinding process disrupts muscle cell structure, causing membrane rupture and the release of pro-oxidant components, such as iron ions from heme proteins. These ions can catalyze lipid oxidation through Fenton-type reactions, intensifying the formation of primary and secondary oxidation products (Ghaderi-Ghahfarokhi *et al.,* 2016). Consequently, meat products require effective technologies such as vacuum packaging, modified atmosphere or antioxidants as strategies to mitigate oxidative damage during storage (Amaral; Silva; Lannes, 2018).

According to Amaral *et al.,* (2018), the use of natural antioxidants, such as plant extracts, herbs, spices, and essential oils, has considerable potential in the meat industry as an alternative to synthetic additives. However, their large-scale application remains limited due to insufficient data regarding their efficacy, optimal dosage, stability during storage, and safety across different food matrices. Variability in raw material composition, extraction methods, and interactions with meat components further complicates standardization and industrial adoption.

By day 7 of refrigerated storage, TBARS values were similar among all treatments, indicating no significant differences in lipid oxidation between burgers with or without olive leaf powder (OLP). This may be attributed to the gradual depletion of phenolic antioxidants during storage. Although these compounds likely delayed oxidation initially, their effectiveness may have declined over time. Furthermore, lipid oxidation is influenced by multiple factors, including oxygen exposure and endogenous pro-oxidants, which may have outweighed the antioxidant effect of OLP during extended storage (Al-Shibli *et al.,* 2023). Some studies have shown that although phenolic-rich additives like OLP can reduce TBARS values in short-term storage or in specific conditions, their efficacy may plateau or diminish during extended storage without additional stabilization strategies (Al-Rimawi; Tarawa; Elama, 2017).

Similar to olive leaf powder (OLP), rosemary (*Rosmarinus officinalis*) extract has been widely investigated as a natural antioxidant in meat products due to its high content of phenolic diterpenes, particularly carnosic acid and carnosol, which effectively inhibit lipid peroxidation. Studies evaluating rosemary extract in frozen mixed hamburgers have demonstrated a significant reduction in TBARS values during prolonged storage, with antioxidant performance comparable to synthetic additives such as BHT, while maintaining acceptable sensory attributes (Borella *et al.,* 2019).

In this context, the present findings demonstrate that OLP acts as an effective natural antioxidant in beef burgers, significantly reducing lipid oxidation by 4 days.

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These results support the technological feasibility of incorporating OLP as a natural strategy to enhance shelf life and oxidative stability in meat products.

Color

Color is one of the most critical factors influencing consumer purchasing decisions *(Beya et al.,* 2025). The results of the instrumental color measurements are presented in Table 4.

Olive leaf addition reduced lightness (L*) and redness (a*) while increasing yellowness (b*), reflecting the presence of natural pigments such as chlorophylls and carotenoids. However, the storage time did not influence the color of the burgers after cooking. OLP incorporation may have influenced the optical properties of the meat matrix, possibly through interactions with muscle proteins and water retention, contributing to improved color stability and a visually lighter product.

Structural modifications in the meat matrix during thermal processing may have contributed to the reduction in L* values observed in cooked burgers. Heating promotes protein denaturation and myofibrillar shrinkage, resulting in the loss of free water and redistribution of moisture within the product (Khomola *et al.,* 2021). These changes affect light reflection on the surface, leading to decreased lightness (L*) and a darker visual appearance. Furthermore, high temperatures directly influence color stability, as thermal treatment can induce non-enzymatic browning reactions, such as the Maillard reaction, as well as the degradation of heme pigments, particularly myoglobin *(Saraiva et al.,* 2019). The conversion of myoglobin into denatured forms, such as hemichrome, contributes to changes in color parameters and reduced luminosity.

Table 4. Color of beef burgers formulated with different levels of olive leaf powder (OLP) during 7 days of refrigerated storage

	L*		
	DAY 1	DAY 4	DAY 7
T1	54.78 ± 2.39^{Aa}	55.03 ± 33.35^{Aa}	55.53 ± 2.90^{Aa}
T2	52.39 ± 2.77^{Ba}	51.78 ± 2.74^{Ba}	52.44 ± 2.28^{Ba}
T3	50.45 ± 2.91^{Ca}	50.57 ± 1.86^{Ba}	49.96 ± 1.70^{Ca}
T4	47.52 ± 1.81^{Da}	47.61 ± 1.43^{Ca}	47.33 ± 0.68^{Da}
	a*		
T1	6.75 ± 0.89^{Aa}	7.05 ± 0.94^{Aa}	8.45 ± 3.32^{Aa}
T2	3.16 ± 0.91^{Ba}	4.08 ± 0.49^{Ba}	4.47 ± 1.93^{Ba}
T3	2.29 ± 1.06^{Ca}	2.87 ± 0.40^{Ca}	3.00 ± 1.17^{BCa}
T4	1.21 ± 0.77^{Da}	1.48 ± 0.40^{Da}	1.86 ± 1.28^{Ca}
	b*		
T1	9.52 ± 0.40^{Da}	8.85 ± 0.76^{Da}	8.96 ± 0.56^{Da}
T2	10.40 ± 0.42^{Ca}	10.20 ± 0.81^{Ca}	10.64 ± 0.44^{Ca}
T3	11.46 ± 0.74^{Ba}	11.29 ± 0.92^{Ba}	11.57 ± 0.81^{Ba}
T4	12.94 ± 0.98^{Aa}	12.38 ± 1.34^{Aa}	12.71 ± 1.05^{Aa}

Means with different uppercase letters in the same column have a significant difference; means with different lowercase letters in the same row have a significant difference (P<0.05). Results are expressed as mean ± standard deviation. T1: 0%OLP; T2: 0.8%OLP; T3: 1.6%OLP; T4: 3.2%OLP.

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Texture and Cooking Properties

The effects of olive leaf powder (OLP) on hardness, cooking loss, shrinkage, and diameter reduction of beef burgers are presented in Table 5. Overall, texture and cooking performance were significantly influenced by OLP concentration, demonstrating a clear structuring effect of the ingredient within the meat matrix.

The incorporation of OLP enhanced structural rigidity, likely due to interactions between its dietary fiber fraction and myofibrillar proteins, resulting in the formation of a denser and more cohesive gel network during thermal processing. Plant-derived fibers are known to modify the microstructure of comminuted meat systems by increasing water immobilization, strengthening the heat-induced protein gel matrix, and improving structural integrity (El-Magoli; Laroia; Hansen, 1996; Seabra *et al.,* 2002; Talukder, 2013).

In the present study, hardness values generally increased during storage. On Day 7, T3 exhibited the highest hardness (9615 ± 2441 g), followed by T2 (8295 ± 1038 g) and T4 (7882 ± 2858 g), whereas the control (T1) showed significantly lower firmness (6389 ± 611 g). This progressive increase in hardness over time may be associated with the structural carbohydrates present in OLP, which can promote moisture redistribution and gradual protein network restructuring during refrigerated storage (Borella *et al.,* 2019).

Table 5 Texture profile (hardness), cooking loss, shrinkage, and diameter reduction of beef burgers formulated with different levels of olive leaf powder (OLP) during 7 days of refrigerated storage

	Hardness (g)		
	DAY 1	DAY 4	DAY 7
T1	5677 ± 1957^{ABa}	5710 ± 1682^{Ba}	6389 ± 611^{Ba}
T2	4855 ± 890^{Bb}	7002 ± 1542^{ABa}	8295 ± 1038^{ABa}
T3	7109 ± 3078^{ABa}	6480 ± 2493^{ABa}	9615 ± 2441^{ABa}
T4	8375 ± 926^{Aa}	8630 ± 2994^{Aa}	7882 ± 2858^{Aa}
	COOKING LOSS (%)		
T1	26.20 ± 5.33^{Aa}	25.40 ±1.31^{Aa}	25.57 ± 7.93^{Aa}
T2	25.39 ± 3.01^{ABa}	24.27 ± 2.57^{Aa}	21.90 ± 3.63^{Aa}
T3	22.50 ± 2.77^{BCa}	22.69 ± 3.84^{ABa}	22.72 ± 5.51^{Aa}
T4	19.70 ± 1.95^{Ca}	20.31 ± 2.16^{Ba}	17.20 ± 3.29^{Aa}
	SHRINKAGE (%)		
T1	14.20 ± 2.67^{Aa}	10.83 ± 4.03^{Aa}	11.21 ± 3.26^{Aa}
T2	11.08 ± 2.68^{ABa}	10.70 ± 1.98^{Aa}	7.97 ± 1.24^{ABa}
T3	7.92 ± 2.89^{Ba}	8.24 ± 2.53^{Aa}	7.11 ± 1.52^{Ba}
T4	8.99 ± 2.43^{Ba}	8.94 ± 2.74^{Aa}	7.21 ± 2.66^{Ba}
	DIAMETER REDUCTION (%)		
T1	17.64 ± 1.60^{Aa}	14.47 ± 3.57^{Aa}	14.87 ± 3.55^{Aa}
T2	14.51 ± 1.68^{Ba}	13.39 ± 2.71^{Aa}	12.35 ± 1.54^{ABa}
T3	11.44 ± 2.19^{Ca}	9.97 ± 3.00^{Aa}	10.08 ± 2.34^{Ba}
T4	11.71 ± 2.45^{Ca}	10.63 ± 3.11^{Aa}	9.88 ± 3.38^{Ba}

Means with different uppercase letters in the same column have a significant difference; means with different lowercase letters in the same row have a significant difference (P<0.05). Results are expressed as mean ± standard deviation. T1: 0%OLP; T2: 0.8%OLP; T3: 1.6%OLP; T4: 3.2%OLP.

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Interestingly, although T4 (3.2% OLP) presented high initial hardness on Day 1, its firmness did not increase proportionally throughout storage. This non-linear behavior suggests that excessive fiber incorporation may partially disrupt optimal protein–protein interactions, limiting further gel tightening and matrix compaction, as previously described in fiber-enriched meat products (Silva *et al.,* 2024).

Cooking loss decreased consistently with increasing OLP levels, demonstrating improved water retention capacity. On Day 1, T4 exhibited the lowest cooking loss (19.70 ± 1.95%), significantly lower than the control (26.20 ± 5.33%), and this reduction remained evident on Day 7 (17.20 ± 3.29%). The improved cooking yield can be attributed to the high fiber content of OLP, which enhances water-binding capacity and reduces exudate release during heating. Dietary fibers and phenolic-rich plant ingredients have been widely reported to improve water-holding capacity by entrapping water within the protein matrix and increasing viscosity, thereby limiting moisture migration during cooking (Talukder, 2013)**.**

Comparable technological effects have been observed in rabbit meat burgers enriched with oat bran fiber, where increasing fiber levels significantly reduced cooking loss and enhanced hardness due to improved structural cohesion and water immobilization within the myofibrillar network. These effects were attributed to the high β-glucan content of oat bran, which reinforces the heat-induced protein gel structure (Silva et al., 2024). Similarly, the incorporation of sweet basil (*Ocimum basilicum*) in chicken meatballs resulted in increased hardness at inclusion levels up to 3%, attributed to fiber–protein interactions and the strengthening of the thermal protein network (Patriani *et al.,* 2021).

Together, these findings indicate that the incorporation of fiber- and phenolic-rich plant materials, such as olive leaves, oat bran, and basil, consistently reinforces meat matrices through enhanced protein–fiber interactions, improved water retention, and stabilization of the heat-induced gel network. This structural reinforcement directly contributes to increased firmness, reduced cooking loss, and improved dimensional stability, highlighting the multifunctional technological role of olive leaf powder in meat systems.

CONCLUSIONS

The incorporation of olive leaf powder (OLP) into beef burgers significantly enhanced antioxidant activity and effectively reduced lipid oxidation during refrigerated storage, particularly within the first four days, demonstrating its protective effect against oxidative deterioration. In addition to its antioxidant functionality, OLP influenced key physicochemical parameters, including color stability, texture profile, cooking yield, and dimensional integrity, contributing to improved technological performance and product stability.

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Beyond its technological benefits, the use of olive leaf powder represents a promising strategy within the *clean label* framework, as it enables the partial replacement of synthetic antioxidants with a natural, plant-derived ingredient. Furthermore, the valorization of olive leaves—an abundant agricultural by-product of olive cultivation—aligns with principles of sustainability and circular economy, promoting waste reduction and adding functional value to agro-industrial residues. Therefore, OLP can be considered a multifunctional ingredient that simultaneously enhances product quality, supports clean-label reformulation, and contributes to more sustainable meat production systems.

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