



Potential of Artichoke Leaves to Preserve Beef Burgers' Quality Through Different Strategies: Direct Incorporation (Leaf Powder or Lyophilised Leaf Extract) or Covered with Antioxidant Bioactive Films

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Abstract

Artichoke leaves are a promising natural source of antioxidants. This study explores the valorisation of this by-product as a new ingredient to preserve beef burgers' quality through different strategies: directly mixed as leaf powder (ALP) or as lyophilised leaf extract (LE), and through the incorporation of ethanolic (EE) or lyophilised leaf extract into different bioactive films (made of gelatin (GF-EE/GF-LE) or alginate (AF-EE/AF-LE)). Films were characterised by FTIR, SEM, mechanical properties, and polyphenol release kinetics. The quality of burgers (stored at 4 °C) was evaluated during 7 days through lipid oxidation (Thiobarbituric Acid Reactive Substances, TBARS), metmyoglobin (MetMb) formation, colour deterioration, changes in pH, and microbial growth. Positive (C +, burger with commercial antioxidants) and negative (C –, burger meat) controls were also assessed. Burgers with ALP or LE showed the lowest TBARS values (0.037 and 0.080 mg MDA/kg meat), both comparable to C + (0.050 mg MDA/kg meat), whereas C – exhibited higher lipid oxidation (0.24 mg MDA/kg meat). ALP also reduced MetMb formation in burgers until the third day (~44.3%), but compromised their sensory characteristics (flavour and texture). In contrast, burgers covered with films showed higher MetMb (50.7–61.1%) and had better consumer acceptance. Gelatin films released more polyphenols (slope, 0.0042 and 0.0034 mg GAE/mL for GF-LE and GF-EE, respectively) than alginate films (slope, 0.0026 and 0.0029 mg GAE/mL for AF-LE and AF-EE, respectively), thus reducing lipid oxidation more effectively. These findings support the potential of artichoke leaves as a natural antioxidant source to replace synthetic additives in meat products.

Keywords Artichoke leaves · By-product · Meat quality · Antioxidant · Active packaging · Polyphenols

Introduction

Artichoke (*Cynara scolymus* L.) belongs to the Asteraceae family, which is an important crop of the Mediterranean diet (Gatto et al., 2013). Worldwide, in 2023, 1666 kt of artichokes were produced, of which 378 kt were produced in Italy, 200 kt in Spain, and 22 kt in France (FAO, 2024). From these large quantities, only the *capitulum*, or head, is consumed, which represents 30–40% of the plant, while the other parts (leaves, external bracts and stems) are discarded (Ayuso et al., 2024b; Llorach et al., 2002). These unexploited products represent ~60 to 70% of the plant (Ayuso et al., 2024b). Artichoke is widely recognised for its health benefits, due to its high content of bioactive compounds (Lattanzio et al., 2009; Pandino et al., 2013a, 2013b). Among others, the most

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abundant bioactive compounds that are described in artichoke and its by-products are phenolic compounds. These compounds can neutralise free radicals and play a crucial role in preventing food oxidation and deterioration (Biel et al., 2020; Ergezer & Serdaroğlu, 2018; Poljsak et al., 2021).

Meat is a highly perishable food product. Its short shelf-life is mainly due to its high moisture content, nutritious composition, and pH, which provide favourable conditions for microbial growth, lipid oxidation, or colour degradation. These factors compromise its quality and acceptance (Kaveh et al., 2023). Among various strategies, the use of preservative additives, such as antioxidants, has been widely employed. Simultaneously, growing consumer demand for natural ingredients is pushing the food industry towards 'clean label' preservation strategies (Yu et al., 2021). Therefore, some studies suggested incorporating antioxidant natural ingredients directly as powders or using their extracts (T. C. Lourenço et al., 2013; Yu et al., 2021; Zamuz et al., 2018). Ergezer and Serdaroğlu (2018) suggested that adding 27.3 mg/100 g artichoke external bracts extract was enough to reduce raw beef patties' lipid oxidation for 7 days, compared with the addition of a synthetic antioxidant (Butylated Hydroxytoluene, BHT). They also confirmed its antimicrobial activity, as 1000 ppm of lyophilised extract inhibited bacterial growth in beef patties (Ergezer et al., 2018). In this sense, an alternative approach to improve meat stability is the development of bioactive coatings or films capable of slowly releasing antioxidant compounds (Elhadeif et al., 2024; Tosati et al., 2017; Yu et al., 2021). When in contact with food, these bioactive films act as carriers and gradually release their active compounds, helping to prevent lipid and protein oxidation over time (Maniglia et al., 2015). Although the antioxidant potential of artichoke leaf extracts has been previously reported (Ayuso et al., 2024a; Karaduman et al., 2025), there are no studies that systematically compare different application strategies of artichoke crop leaves, such as direct incorporation and through delivery systems (films) for the preservation of beef burgers.

Therefore, given the potential of artichoke crop leaves and the importance of by-product valorisation, this study aimed to explore the potential of artichoke leaves, as a natural antioxidant ingredient in beef burgers to preserve their quality. For the first time, different application strategies were compared, including direct incorporation of artichoke leaf powder or lyophilised artichoke leaf extract, as well as the use of bioactive films (incorporating either ethanolic or lyophilised extract), placed above and below the burgers. This work contributes to by-product valorisation and sustainability, supporting its integration into clean-label strategies within the food industry.

Materials and Methods

Materials and Sample Codes

Chemicals were obtained from Merck (MerckKGaA, Darmstadt, Germany) or Sigma-Aldrich (St Louis, USA). Artichoke crop leaves (*Cynara scolymus*, Blanca de Tudela variety) were obtained from a local producer (41.3192330° N, 2.0402430° E, Baix Llobregat, Spain) and dried for 14 h at 50 °C. A total of three batches of ~3 kg each were collected at three different times. Each batch was ground with a food processor (Mambo 10,070, Cecotec, Valencia, Spain) and sieved (Filtira Vibracion, Barcelona, Spain) to obtain artichoke leaf powder (ALP) (particle size lower than 1 mm³). The powder was stored in sealed glass jars at −40 °C until it was used. After getting the three batches, they were mixed to obtain a representative initial sample, intended both for direct application in meat and for the extraction of phenolic compounds to obtain the leaf extract, and the sample was autoclaved (at 121 °C, 1.4 bar for 20 min) before use. Beef (neck cut) was obtained from a local butcher in Barcelona, Spain.

All the sample codes used throughout the manuscript are summarised in Table 1.

Ethanolic and Lyophilised Artichoke Leaves Extract Preparation

The polyphenol extraction from ALP was conducted following the method of El Hammadi et al. (2024b) with some modifications. Two grams of ALP were macerated with 20 mL of a 50% EtOH-H₂O solution (v/v) for 1 h, and centrifuged at 2830 g (Heraeus Multifuge 3 s, 3334 fixed rotor). The residue was re-extracted with 10 mL of 50% EtOH-H₂O, and the supernatants were combined to obtain the ethanolic artichoke leaf extract (EE). To obtain the lyophilised artichoke leaf extract (LE), the ethanol was evaporated using a rotary evaporator (Büchi Rotavapor R-200, Vacuum Controller V-800, New Castle, USA), and the remaining aqueous extract was freeze-dried (Unicryo MC2L, Biotech, Madrid, Spain). Three independent replicates of this procedure were conducted for the ALP and LE characterisation (Sections "Extraction of Free and Bound Polyphenol Fractions in Leaf Artichoke Powder" to "Determination of DPPH Radical-Scavenging Activity of Free Polyphenols").

Extraction of Free and Bound Polyphenol Fractions in Leaf Artichoke Powder

Free and bound polyphenols were determined as Zhang et al. (2022) with slight modifications. The free polyphenols extraction was performed as described in Section "Ethanolic and

Table 1 Sample codes and corresponding formulations for extracts, films, and burgers

Sample code	Definition
ALP	Artichoke leaf powder
Extracts	
EE	Ethanol extract, equivalent to free polyphenolic fraction
LE	Lyophilised artichoke leaf extract, obtained from EE freeze-drying
Films	
AF-EE	Alginate film containing EE (equivalent to 1% of ALP)
AF-LE	Alginate film containing LE (equivalent to 1% of ALP)
GF-EE	Gelatin film containing EE (equivalent to 1% of ALP)
GF-LE	Gelatin film containing LE (equivalent to 1% of ALP)
Burgers	
B-ALP	Burger with 1% of ALP
B-LE	Burger with LE, equivalent of 1% of ALP
B-AF-EE	Burger covered with AF-EE (top and bottom)
B-AF-LE	Burger covered with AF-LE (top and bottom)
B-GF-EE	Burger covered with GF-EE (top and bottom)
B-GF-LE	Burger covered with GF-LE (top and bottom)
C-	Burger without any additional ingredient or additive
C+	Burger with commercial mix

Lyophilised Artichoke Leaves Extract Preparation”. The residue was subjected to alkaline hydrolysis with 3 mL of NaOH (8 M) and stirred for 1 h at 50 °C. Subsequently, the pH was adjusted to pH 2 with hydrochloric acid (37%, w/v), and the bound phenolic compounds were extracted with ethyl acetate (3 times). The extracts were evaporated to dryness using a rotary evaporator, redissolved in 3 mL of EtOH:H₂O (50:50, v/v) and stored at −40 °C. The fractionation was performed in triplicate.

Determination of Polyphenol Content (PC) of Polyphenolic Fractions

For polyphenol content (free and bound polyphenol fractions) determination, the Folin-Ciocalteu technique was used, according to Ouerfelli et al. (2022). The absorbance was determined at 765 nm. The results were expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Determination of DPPH Radical-Scavenging Activity of Free Polyphenols

The ability to scavenge DPPH radicals was determined as described by Rahman et al. (2015). The absorbance was measured at 517 nm. Results are expressed in micromoles of Trolox equivalents per gram of dry weight (μM TE/g DW). In order to make a better comparison of the added ingredients in burgers, the commercial mix (added to the positive control) was also evaluated for its scavenging activity.

Film Preparation and Characterisation

Alginate and Gelatin Film Preparation

Alginate films were produced as described by Farias et al. (2021) with some modifications. In water, 1.5% alginate (Cimalgin 500, VINPAI, France) and 0.3% glycerol (w/w) were dissolved with either ethanolic or lyophilised extract (AF-EE and AF-LE) from artichoke leaves (obtained as described in Section “Ethanolic and Lyophilised Artichoke Leaves Extract Preparation”). The mixture was homogenised using an Ultra-Turrax (IKA T18 basic, Spain) and degassed under vacuum. Then, 25 g of the solution was poured into 110-mm-diameter Petri dishes and dried at 25 °C for 24 h. Dried films were sprayed with a crosslinking solution (~0.9 g) of calcium lactate (10 g/L) and dried at 25 °C. Finally, they were cut into 3.5-cm-diameter discs.

Gelatin films were prepared by dissolving 4% porcine gelatin and 0.8% sorbitol (w/v) in water with either ethanolic or lyophilised extract (GF-EE and GF-LE), and heated at 40 °C. The solution (25 g) was poured into 110-mm-diameter Petri dishes, dried (24 h, 25 °C), and cut into 3.5-cm-diameter discs.

The ethanolic or lyophilised extract was added for all the films to ensure each burger would be in contact with a 1% ALP equivalent (concentration choice follows the criteria described in burger preparation, Section “Burger Preparation and Characterisation”).

Active Film Characterisation

Fourier Transform Infrared Spectroscopy (FTIR) of Active Films FTIR spectrometer (L160000A, Spectrum Two, PerkinElmer, USA) was used from 650 to 4000 cm^{-1} . A total of 64 scans were taken for each sample with a resolution of 4 cm^{-1} . Spectral data were processed and normalised using OriginLab software (OriginLab Corporation, Northampton, MA, USA).

Mechanical Properties Mechanical tests were performed according to El Hammadi et al. (2024a) by using a Zwick Roell Texture machine (probe S/N 778312) (ZwickRoell, Ulm, Germany). Six tests were performed for each type of film. Young's modulus (E) was calculated from Eq. 1.

$$E = \frac{\sigma}{\epsilon} \quad (1)$$

where Young's modulus (E) is obtained by the tensile stress (σ) and strain (ϵ) in the linear fraction. From this curve, ultimate tensile strength (maximum stress reached before rupture) and elongation at rupture (ultimate tensile strain) were also obtained.

Scanning Electron Microscopy (SEM) Analysis The samples were cut (cubes of 2 mm) and mounted onto stubs and sputter-coated with carbon. The morphological analysis of films was performed by scanning electron microscopy (Nano Nova 230, FEI, Hillsboro, OR, USA), in high-vacuum mode using an accelerating voltage of 5 kV at 100 and $\times 1000$ magnification.

Migration Assay of Polyphenols from the Active Films Migration analysis was performed to evaluate the release of polyphenol compounds from active films to the burgers. For that, the films (3.5 cm^2) were submerged in 10 mL of ethanol 10% (v/v) that acted as the food simulant substitute for fresh meat, according to Commission Regulation (EU) No. 10/2011, of 14 January 2011, on Plastic Materials and Articles Intended to Come into Contact with Food (European Commission, 2011). Samples were stored for 24 h at 4 °C, and 200 μL of the simulant were taken every 10 min until 1 h, then every 1 h until 6 h and at 24 h. The amount of PC released was determined as described in Section “Determination of Polyphenol Content (PC) of Polyphenolic Fractions” and expressed as mg GAE/mL. All determinations were performed in duplicate.

Burger Preparation and Characterisation

Burger Preparation

Meat was purchased 2 days after slaughter and minced twice using a 4-mm grinder plate. Minced meat was stored

at 4 ± 1 °C until its use. Meat was then divided into four separate batches, and burgers (each one of 8 g) were formed with a 3.5-cm-diameter mould. One batch was formulated without any additional ingredient or additive to use for the negative control (C-) and for burgers that were covered with two (top and bottom) films (AF-EE, AF-LE, GF-EE and GF-LE) which contained LE or EE corresponding to the extractable fraction (free polyphenolic fraction) from 1% ALP. Another batch was mixed with 1% (the percentage recommended by the Company) of commercial mix of additives (corn starch, dextrose, sodium sulphite, sodium ascorbate, and trisodium citrate, ref.4409, Mane, Spain), to be used as the positive control (C+). The remaining two batches were formulated with 1% ALP and an equivalent amount of LE extracted from 1% ALP, respectively. All samples contained 1% of salt. The raw burgers were placed in plastic trays, using separate trays for each type of analysis. Each tray was tightly covered with plastic wrap (food-grade PVC film) and stored under refrigeration at 4 ± 1 °C, preserved from light. For pH, instrumental colour, metmyoglobin content, and DPPH assays, three independent burgers per treatment were analysed, whereas two independent burgers were used for microbiological analysis and TBARS determination.

Burger's Characterisation

Instrumental Colour and pH Variation Colour of the burgers was measured on the sample surface, at three different points. For burgers with films, the film was removed, momentarily. The tristimulus coordinates L^* , a^* , and b^* were determined using a spectrophotometer MiniScan XETM (Hunter Associates Laboratory Inc., Reston, VA, USA), with 22-mm aperture for 10 s. Results were calculated based on a 10° standard observer and D65 illuminant. Additionally, it was measured the colour differences (ΔE^*) of each sample each day, with respect to C+ formulation on day 0, by Eq. 2.

$$\Delta E^* = \sqrt{(L_s^* - L_{ref}^*)^2 + (a_s^* - a_{ref}^*)^2 + (b_s^* - b_{ref}^*)^2} \quad (2)$$

where subscript s indicates sample values corresponding to the day of storage and subscript ref indicates the reference value (coordinate C+ on day 0).

The pH of burgers was measured by a pH meter (YK-25, Yinmic, Shandong, China) according to Ivane et al. (2024). The measurements were carried out on days 0, 4, 5, 6, and 7, in triplicate.

Metmyoglobin (MetMb) Content MetMb was measured following the methodology of Villasante et al. (2020). One gram of meat was homogenised with 5 mL of 0.04 M

phosphate buffer (pH 6.8) using an Ultra-Turrax (IKA, Staufen, Germany) and centrifuged at 12,000 g for 10 min. The supernatant was analysed with the FLUOstar Omega

absorbance reader (BMG LABTECH, Ortenberg, Germany) at wavelengths of 525, 545, 565, and 572 nm. To calculate the percentage of MetMb, Eq. 3 was used.

$$\text{Metmyoglobin (\%)} = \left(-2.514 \times \left(\frac{A_{572}}{A_{525}} \right) + 0.777 \times \left(\frac{A_{565}}{A_{525}} \right) + 0.8 \times \left(\frac{A_{545}}{A_{525}} \right) + 1.098 \right) \times 100 \quad (3)$$

where A_n was the absorbance at wavelength n ($n = 572, 525, 545$ and 565 nm). The analysis was performed on days 0, 3, 4, 5, 6, and 7, in triplicate.

Scavenging Activity and Secondary Lipid Oxidation in Burgers

Scavenging activity was measured in burgers on days 0, 4, and 7 in triplicate. For that, meat from each experimental group was lyophilised, and 0.5 g samples were extracted with 5 mL of 50% EtOH:H₂O solution (v/v). The samples were centrifuged at 2830 × g for 10 min, and the supernatant was collected for the DPPH assay (measured as explained in Section “Determination of DPPH Radical-Scavenging Activity of Free Polyphenols”). Secondary lipid oxidation was determined on days 0, 3, 5, 6, and 7 in triplicate by measuring malondialdehyde (MDA) using the TBA method, forming a pink complex quantified spectrophotometrically at 532 nm against a calibration curve (5–200 μM MDA, $R^2 = 0.997$). The method employed was adapted from Vieira et al. (2021). To 1 g of sample, 0.5 mL of EDTA (0.3%) and 2.5 mL of trichloroacetic acid (10%) in HCl 0.25 M were added. The mixture was blended and filtered. Then, 1 mL of the filtrate was combined with 1 mL of 2-thiobarbituric acid (TBA, 0.375%), heated at 90 °C for 10 min and cooled down.

Aerobic Mesophilic and Psychrophilic Total Counts Aerobic mesophilic total counts of burgers were determined as described by Villasante et al. (2020) on days 0, 4, and 7. The inoculated Petri dishes were incubated at 37 °C for 48 h (ISO 4833–1, 2013), and results were expressed as log CFU/g. Aerobic psychrophilic count was performed as for mesophilic count and the Petri dishes were incubated at 6 °C for 10 days (ISO 17410, 2019). The analysis was performed in duplicate within the limits defined in legislation for minced meat (6.7 log CFU of mesophilic aerobic counts/g) (European Commission, 2005).

Sensory Evaluation An acceptability test and intention to purchase were conducted for the sensory evaluation of the products. The study was approved by the Ethics Committee for Research in Human Beings of the Universitat Politècnica de Catalunya (UPC). The evaluation involved

an untrained panel of twenty-nine consumers (10 females and 19 males, aged 20–60 years). The participants were selected based on their regular consumption of meat products. All samples were coded with three-digit numbers, and their presentation order within a session was randomised to minimise bias. A first evaluation was focused on raw burgers with the films (B-AF-EE and B-GF-EE), aiming to understand consumer perceptions of these products. Consumers assessed five attributes (appearance, colour, protection, safety, and hygiene) using a 9-point hedonic scale (‘dislike it very much’ to ‘like it very much’). A second sensory evaluation was focused on the sensory characteristics of cooked burgers (using a double-sided electric grill, similar to a sandwich press, for 5 min, removing films before cooking), which included five different samples: B-ALP, B-LE, B-AF-EE, and B-GF-EE. The burgers with films with lyophilised extract were excluded, as previous analyses showed no significant differences in the other studied parameters (lipid oxidation, MetMb, microbial growth and colour). In this case, the evaluated attributes included colour, texture, odour, flavour, appearance, and overall acceptability using a 9-point hedonic scale. Purchase intention was measured for both raw and cooked burgers, by asking whether they would buy the product, requiring a ‘yes’ or ‘no’ response.

Statistical Analysis

The experiment was conducted in three independent replicates. One-way ANOVAs were conducted to analyse the data, and a pairwise comparison test with Tukey’s post hoc test was employed. Normal distribution and the homogeneity of the variance were previously tested through the Shapiro–Wilk test. In order to evaluate the combined effects of sample (strategy carried out) and storage time, as well as their interaction, a two-way ANOVA was performed. The relationship between scavenging activity (DPPH assay) and lipid oxidation (TBARS) in burgers was evaluated using Pearson’s correlation coefficient. Statistical analysis was performed using Minitab Statistical Software 22®, with a significance level of $p < 0.05$.

Results and Discussion

Polyphenolic Fractions and Scavenging Activity of Artichoke Leaf Powder

Free polyphenols can be extracted using an organic solvent, such as ethanol, while bound polyphenols are covalently linked to cell wall polysaccharides, making them insoluble in these solvents (Krygier et al., 1982). In this study, both free and bound polyphenols were evaluated in ALP. LE represents the free polyphenol fraction, since it was obtained from the ethanolic extraction. ALP exhibited a much higher free polyphenol content (10.76 mg GAE/g DW) compared to bound polyphenols fraction (0.21 mg GAE/g DW, $p < 0.05$). Al-Subhi (2020) reported a similar concentration of free polyphenols (9.06 mg GAE/g DW) in artichokes leaves, whereas bound polyphenol content was higher (4.20 mg GAE/g DW) than those observed in the present study. These differences may be attributed to the variations in the extraction procedure (f.e., they used 80% ethanol solution and an extraction time of 20 min) among other factors. Indeed, previous research optimised free polyphenol extraction from artichoke waste, demonstrating that 50% ethanol and 90-min extraction at 60 °C were the optimal conditions (Lavecchia et al., 2019).

The free polyphenols fraction showed a DPPH-scavenging activity of 19.11 ± 0.04 $\mu\text{mol TE/g DW}$, while Biel et al. (2020) reported higher activity (261.9 $\mu\text{mol TE/g DW}$) in artichoke leaf extracts obtained with 70% methanol. These differences may result from climatic and geographic factors, as well as differences in harvest conditions and genotype (Lombardo et al., 2010; Pandino et al., 2013a, 2013b) affecting artichoke polyphenols profile and from using different extraction solvents, which alter the type and amount of polyphenols extracted (Giménez et al., 2022).

These results indicated that LE contained nearly all the extractable (without alkaline hydrolysis) polyphenols present in ALP, since the bound fraction represented only a minor proportion of the total. Therefore, LE can be considered a representative source of the polyphenols naturally present in artichoke leaves, supporting its selection for subsequent film and burger formulations.

On the other hand, the commercial mix used for the burger C+ showed a comparable scavenging activity (10.13 $\mu\text{mol TE/g DW}$) to those of the natural extracts probably due to the presence of sodium ascorbate in the commercial mix (Timoshnikov et al., 2020). This supports the potential of ALP and LE as natural alternatives to synthetic antioxidants.

Film Characterisation

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectra of all films are presented in Figure S1.

As observed, the spectral profiles differed according to the polymer type (alginate or gelatin), reflecting their distinct molecular structures. However, no relevant differences were observed between films containing different types of extract (EE and LE) within the same polymer matrix. Alginate films (Figure S1-a) showed two characteristic peaks at ~ 1600 and 1400 cm^{-1} , corresponding to asymmetric and symmetric stretching of carboxylate ($-\text{COO}^-$) groups (Bibi et al., 2023). The presence of glycerol was confirmed by bands at 945 cm^{-1} (O–H deformation) and 860 cm^{-1} (C–O symmetric stretching) (Kowalonek et al., 2024). Gelatin films (with ethanolic (GF-EE) and lyophilised (GF-LE) artichoke leaves extract) exhibited a similar spectral pattern. In contrast to the others, gelatin-based films (Figure S1-b) showed typical amide peaks at 3300, 1630, and 1545 cm^{-1} , corresponding to O–H stretching, amide I ($\text{C}=\text{O}$ and C–N stretching), and amide II (N–H bending), respectively (Dong et al., 2006; Taokaew et al., 2013; Villasante et al., 2020). The addition of EE or LE did not affect the characteristic molecular structure of the polymer matrix. This indicated that no covalent bonding occurred between the artichoke polyphenols present in the extracts and the polymers.

Mechanical Properties and Morphology of Films

Table 2 shows the results of the mechanical properties of films. As observed, films with the same base material (alginate or gelatine) showed similar mechanical properties, with

Table 2 Mechanical properties of alginate and gelatine films with either lyophilised or ethanolic extracts of artichoke leaves

Film	Parameter ¹		
	<i>E</i> (MPa)	σ (MPa)	ϵ (%)
AF-EE	348.67 ± 39.34^c	14.90 ± 1.36^c	15.00 ± 2.30^b
AF-LE	340.00 ± 46.24^c	12.63 ± 1.62^c	11.70 ± 2.57^b
GF-EE	960.50 ± 105.59^b	45.10 ± 1.70^b	29.60 ± 2.55^a
GF-LE	1970.00 ± 85.00^a	70.07 ± 16.64^a	7.35 ± 0.55^c

^{a–b}Mean values with different superscripts in a column are statistically different ($p < 0.05$). ¹Results are presented with the mean $\pm$ standard error. AF-EE, alginate film with ethanolic extract; AF-LE, alginate film with lyophilised extract; GF-EE, gelatin film with ethanolic extract; GF-LE, gelatin film with lyophilised extract. *E*, Young's modulus, σ , tensile stress at rupture; ϵ , ultimate tensile strain

slight variations depending on the extract (lyophilised or ethanolic).

Alginate films presented lower E (348.67–340.00 MPa) than gelatin ones (1970.00–960.50 MPa), indicating greater elasticity, while gelatin films were stiffer. This difference is likely due to the addition of glycerol in alginate formulations, which acts as a plasticizer by disrupting hydrogen bonds and reducing chain-to-chain interactions (Gao et al., 2019). Gelatin is obtained by collagen denaturation and loses its quaternary and tertiary structure during thermic processing. Upon cooling, chains realign and form helices, contributing to rigidity (Derkach et al., 2022). The porcine gelatin used typically shows high gel strength and stiffness (Said et al., 2023). On the other hand, alginate is a linear polysaccharide composed of β -D-mannuronic and α -L-guluronic acids. Its negatively charged carboxyl groups interact with Ca^{2+} , forming stable hydrogels through structures known as ‘egg-box’ ionic crosslinks (Cao et al., 2020). These structures are generally less rigid than those formed by gelatin (Draget, 2009; Hashemi et al., 2023; Parreidt et al., 2018). Gelatin films also had higher σ than alginate films, with GF-LE showing the highest σ ($p < 0.05$). On the contrary, in alginate films, calcium ions promote ionic crosslinking that restricts extensibility (lower ϵ) (El Hammadi et al., 2024a). Regarding the alginate films, no significant differences were observed between AF-EE and AF-LE indicating that the incorporation of either EE or LE did not significantly modify any of the evaluated mechanical parameters. This suggested that the type of extract did not substantially interfere with the integrity of the alginate matrix. On the contrary, in gelatin films, the type of extract significantly influenced all mechanical parameters. Regarding the Young’s modulus, GF-EE was more elastic than GF-LE, likely because ethanol disrupted collagen hydrogen bonds and reduced chain folding. In addition, the addition of LE, also resulted in higher tensile strength (σ), reflecting greater resistance to breakage. Regarding ϵ , GF-EE presented significantly higher values than GF-LE, suggesting that EE promoted a more extensible network, probably due to charge neutralisation process in gelatin when adding ethanol, which induces molecular unfolding and exposes aromatic amino acids such as tryptophan, tyrosine, and phenylalanine (Cen et al., 2021; Peng et al., 2022).

These results were in line with those observed in the scanning electron micrographs (Fig. 1). SEM images of films revealed that gelatin films exhibited smoother surfaces compared to alginate films, which showed a brittle structure with visible cracks. As seen in alginate films’ mechanical properties, the rupture force they withstand is low and the internal structure could have broken, probably caused by the methodology for film obtention (peeling from the Petri plate). Similar results were observed by Biao et al. (2019), who also found cracks in alginate films.

As seen in Fig. 1, in general, alginate films demonstrate a better dispersion and dissolution of the extract particles, as no light-coloured particles are distinguished in SEM images. Among alginate films, AF-LE exhibited a rougher and more irregular structure, likely due to worse particle dispersion. There may be lower solubilisation efficiency when the extract is added in lyophilised form compared to its incorporation as an ethanolic solution, which may promote a more heterogeneous distribution within the matrix. However, despite these microstructural differences, no significant changes are observed in the mechanical properties of these films (Table 2). In gelatin films, particle dispersion improved when the ethanol extract was added (since the agglomeration of light particles was lower) compared to films with lyophilised extract incorporated. This was likely due to the formation of insoluble polyphenol particles within the biopolymer matrix during ethanol evaporation. In this case, lower solubilisation of polyphenols has an observable effect on the mechanical properties of the films (Table 2).

Polyphenol Content and Release Assay of Active Films

The release kinetics of polyphenols for each film are presented in Fig. 2.

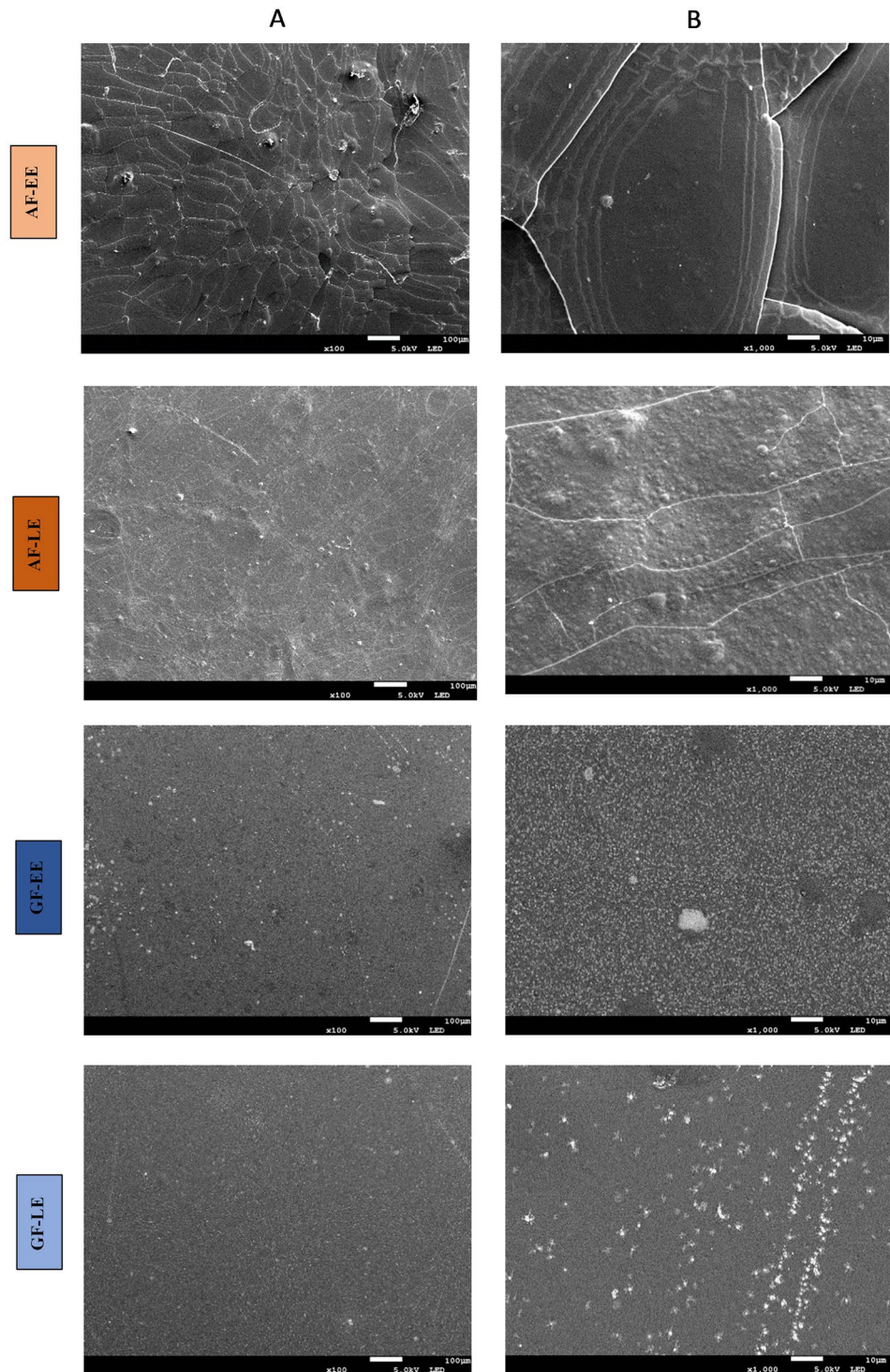
All films exhibited similar release kinetics. As observed in Fig. 2, the release rate (higher slope) is significantly higher for gelatin films compared to alginate films ($p < 0.05$), indicating faster migration of polyphenols from the film to the simulant (ethanol 10%). This is likely due to the poorer dissolution of the polyphenol particles in the gelatin films, as observed in the SEM images (Section “Mechanical Properties and Morphology of Films”). In this study, all films reached equilibrium within the first hour. Similarly, in a study with gelatin films which incorporate tea polyphenol solution (2 mg/mL), the release reached equilibrium within the first hour (Kuai et al., 2020). Polyphenol migration in alginate films (AF-EE and AF-LE) had similar rates (0.0026 and 0.0029 mg GAE/mL), regardless of whether the extract was incorporated in lyophilised or ethanolic form. In contrast, for gelatin films, the release rate was higher when the extract was lyophilised (GF-LE), likely due to the poorer dissolution of polyphenol particles (see Fig. 1, Section “Mechanical Properties and Morphology of Films”). Although the films exhibited different release rates, the final concentration of polyphenols at equilibrium was comparable ($p > 0.05$), ranging from 0.028 to 0.033 mg GAE/mL.

Beef Burger Quality Assessment During Storage

Microbiological Counts

Meat is one of the most perishable foods, and refrigeration temperatures are commonly applied to delay spoilage (Swanson, 2011). However, psychrotrophic bacteria, such as *Pseudomonas*

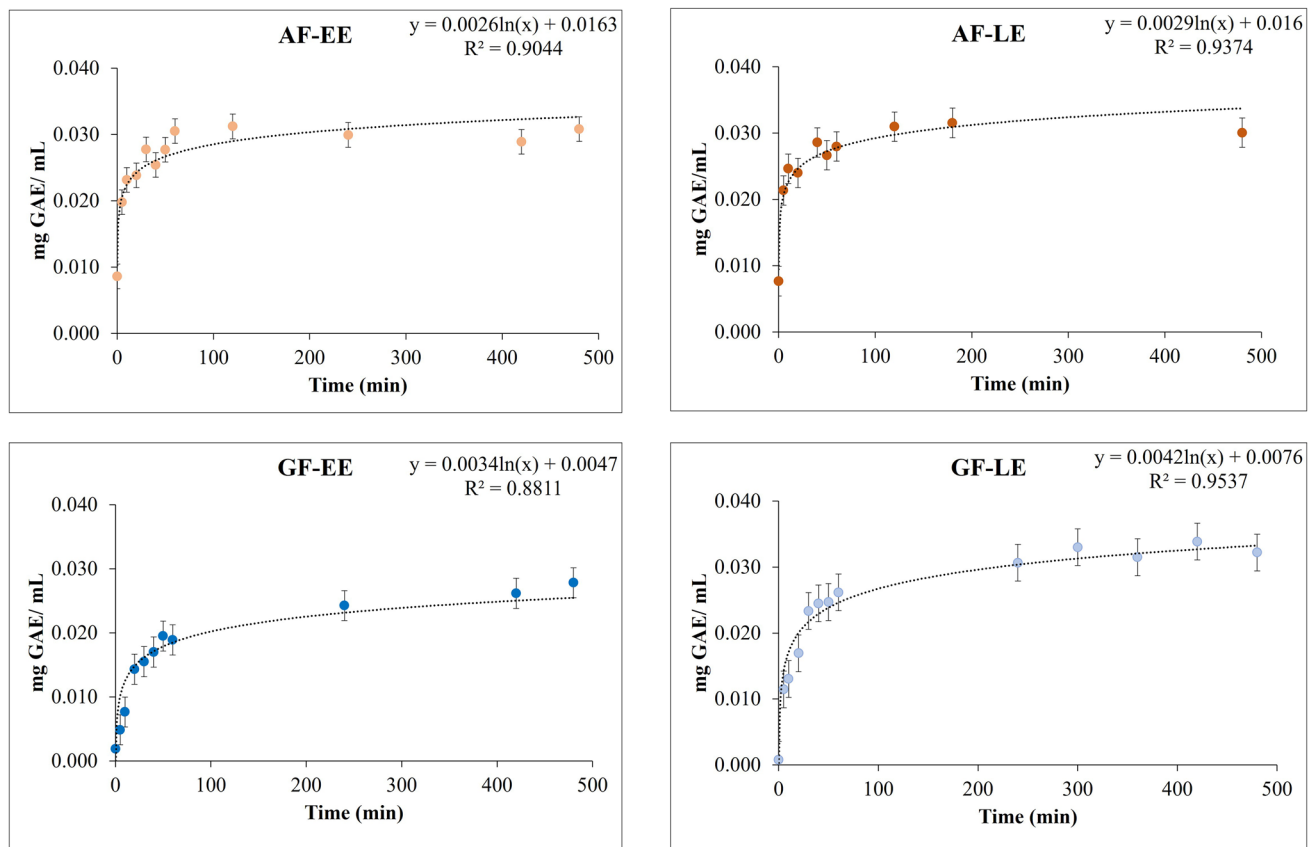
Fig. 1 Scanning electron micrographs (SEM) of the surfaces of alginate and gelatin films with either lyophilised or ethanolic extracts of artichoke leaves. **A** $\times 100$ magnification; **B** $\times 1000$ magnification



AF-EE: alginate film with ethanolic extract; **AF-LE:** alginate film with lyophilised extract; **GF-EE:** gelatin film with ethanolic extract; **GF-LE:** gelatin film with lyophilised extract

spp., which proliferate at low temperatures, can impact meat preservation (Ercolini et al., 2009). Table 3 shows mesophilic and psychophilic counts for the different burgers over time. Both factors, sample and time of storage, significantly determined the microbiological counts, as did the interaction sample $\times$ time

($p < 0.05$), for both mesophilic and psychophilic counts. On day 0, all burgers showed a similar total aerobic mesophilic count ($p > 0.05$), ranging from 3.17 to 3.26 log CFU/g, indicating that the addition of these ingredients may not compromise the initial microbial counts of burgers. Mesophilic counts increased during



AF-EE: alginate film with ethanolic extract; **AF-LE:** alginate film with lyophilised extract;
GF-EE: gelatin film with ethanolic extract; **GF-LE:** gelatin film with lyophilised extract

Fig. 2 Release kinetics of polyphenols from alginate and gelatin films with either lyophilised or ethanolic extracts of artichoke leaves

storage but remained below the legal limit of 6.7 log CFU/g for all the burgers after 7 days (European Commission, 2005). At day 7, C+ showed the lowest mesophilic count (4.06 log CFU/g) and therefore exhibited the greatest antimicrobial potential of the commercial mix present in this sample. Along with C+, B-LE achieved similar values of mesophilic count (4.21 CFU/g, $p > 0.05$), which corresponded to the lowest values of the burgers formulated with artichoke leaf extract or powder and were not significantly different from B-ALP (4.3 CFU/g). These findings align with Ergezer et al. (2018), who reported that 1000 ppm of artichoke bract lyophilised extract reduced aerobic bacteria in beef patties by half after 7 days, suggesting that the extracts have antimicrobial potential.

C – in our study exhibited the highest ($p > 0.05$) microbial load (4.77 log CFU/g) on day 7. However, no significant differences were observed between C – and burgers with gelatin films (B-GF-EE and B-GF-LE, with 4.70 and 4.75 log CFU/g, respectively). In contrast, alginate film treatments (B-AF-EE and B-AF-LE) significantly reduced microbial loads (4.58 and 4.51 log CFU/g, respectively). Psychrotrophic counts were substantially higher on day 7 than mesophilic counts, aligning

with the optimal growth temperature of psychrotrophs, around 15 °C, compared to the mesophiles (30–40 °C) (Heidt et al., 2008). Psychrotrophic growth followed a trend similar to mesophilic bacteria. B-ALP and B-LE burgers had the lowest bacterial counts (6.46 and 6.49 CFU/g) with no significant differences observed compared with C+ (6.14 CFU/g). This was consistent with Ergezer et al. (2018), who reported a slight increase in psychrotrophic bacteria but observed similar reductions in bacterial growth when a 1000 ppm artichoke bract lyophilised extract was added (6.43 CFU/g on day 7). C – showed the highest psychrotrophic counts on day 7, though these did not significantly differ from burgers with gelatin films (B-GF-EE and B-GF-LE). In contrast, burgers with alginate film demonstrated a significant reduction of microbial counts, by nearly one logarithmic unit compared to C –. Villasante et al. (2020) previously described similar results with films containing 15% of pecan shell extract that slightly reduced bacterial growth in beef patties after 7 days. The antimicrobial activity of the artichoke leaf extracts could have contributed to the results observed. Furthermore, the film might limit oxygen transfer to the burger, thereby inhibiting bacterial proliferation.

Table 3 Mesophilic and psychrophilic counts for the different burgers

Microbiological results (log CFU/g) ¹			
Sample	Day 0	Day 4	Day 7
Mesophilic count			
B-ALP	3.17 ± 0.14 ^{Ac}	3.56 ± 0.02 ^{CDb}	4.30 ± 0.02 ^{CDa}
B-LE	3.21 ± 0.08 ^{Ac}	3.51 ± 0.01 ^{DEb}	4.21 ± 0.02 ^{DEa}
AF-EE	3.26 ± 0.01 ^{Ac}	3.61 ± 0.01 ^{BCb}	4.51 ± 0.01 ^{BCa}
AF-LE	3.26 ± 0.01 ^{Ac}	3.61 ± 0.02 ^{BCb}	4.58 ± 0.04 ^{ABCa}
GF-EE	3.26 ± 0.01 ^{Ac}	3.65 ± 0.01 ^{Bb}	4.70 ± 0.02 ^{ABa}
GF-LE	3.26 ± 0.01 ^{Ac}	3.73 ± 0.01 ^{Ab}	4.75 ± 0.01 ^{Aa}
C-	3.26 ± 0.01 ^{Ac}	3.75 ± 0.01 ^{Ab}	4.77 ± 0.01 ^{Aa}
C+	3.22 ± 0.07 ^{Ac}	3.46 ± 0.02 ^{Eb}	4.06 ± 0.14 ^{Ea}
Psychrophilic count			
B-ALP	3.14 ± 0.01 ^{Ac}	5.44 ± 0.04 ^{BCb}	6.46 ± 0.02 ^{CDa}
B-LE	3.15 ± 0.04 ^{Ac}	5.18 ± 0.05 ^{Cb}	6.49 ± 0.16 ^{CDa}
AF-EE	3.15 ± 0.07 ^{Ac}	5.48 ± 0.18 ^{ABCb}	6.8 ± 0.11 ^{BCa}
AF-LE	3.15 ± 0.07 ^{Ac}	5.18 ± 0.06 ^{Cb}	6.66 ± 0.02 ^{Ca}
GF-EE	3.15 ± 0.07 ^{Ac}	5.71 ± 0.07 ^{ABb}	7.12 ± 0.07 ^{ABa}
GF-LE	3.15 ± 0.07 ^{Ac}	5.54 ± 0.03 ^{ABb}	7.39 ± 0.07 ^{Aa}
C-	3.15 ± 0.07 ^{Ac}	5.77 ± 0.01 ^{Ab}	7.20 ± 0.05 ^{Aa}
C+	3.08 ± 0.09 ^{Ac}	4.47 ± 0.04 ^{Db}	6.14 ± 0.16 ^{Da}

^{a-b}Mean values with different superscripts within the same formulation but at different times are statistically different; ^{A-E}mean values with different superscripts on the same day but from different formulations are statistically different ($p < 0.05$); ¹values were expressed as the mean ± standard error. *B-ALP*, burgers with artichoke leaf powder; *B-LE*, burger with lyophilised extract; *B-AF-EE*, burgers with alginate film with ethanolic extract; *B-AF-LE*, burgers with alginate film with lyophilised extract; *B-GF-EE*, burgers with gelatin film with ethanolic extract; *B-GF-LE*, burgers with gelatin film with lyophilised extract; *C+*, positive control; *C-*, negative control

In fact, although oxygen is good for maintaining the colour of the meat, in modified atmosphere packaging, the oxygen content is reduced and carbon dioxide is added to prevent bacterial growth (Jakobsen & Bertelsen, 2000).

pH, MetMb Content, and Colour Changes in Burgers

Variations in pH can cause biochemical changes in meat, which might influence myoglobin (Mb) stability and meat colour (Sørheim & Høy, 2013). After their production, all samples exhibited a similar value of pH ($p < 0.05$), which ranged from 5.52 to 5.61 (Table 4). During the first 4 days, the pH value decreased slightly, probably from the microbiological proliferation of acid-producing bacteria in burgers (Majdinasab et al., 2020). Subsequently, on day 5 the pH increased in all samples, potentially due to the microbial deproteinization of the meat that can cause metabolites such as amines and ammonia arising the pH of the meat (Cortez-Vega et al., 2012; Manzoor & Ahmad, 2024). Similarly, Elhadeef et al. (2024) and Manzoor and Ahmad (2024) also reported a pH increase when films containing anti-oxidant extracts were added, attributing this effect to protein degradation during storage. Moreover, a similar trend in the pH evolution was observed by Ergezer et al. (2018) when added artichoke bract aqueous extract (500 and 1000 ppm) to beef patties.

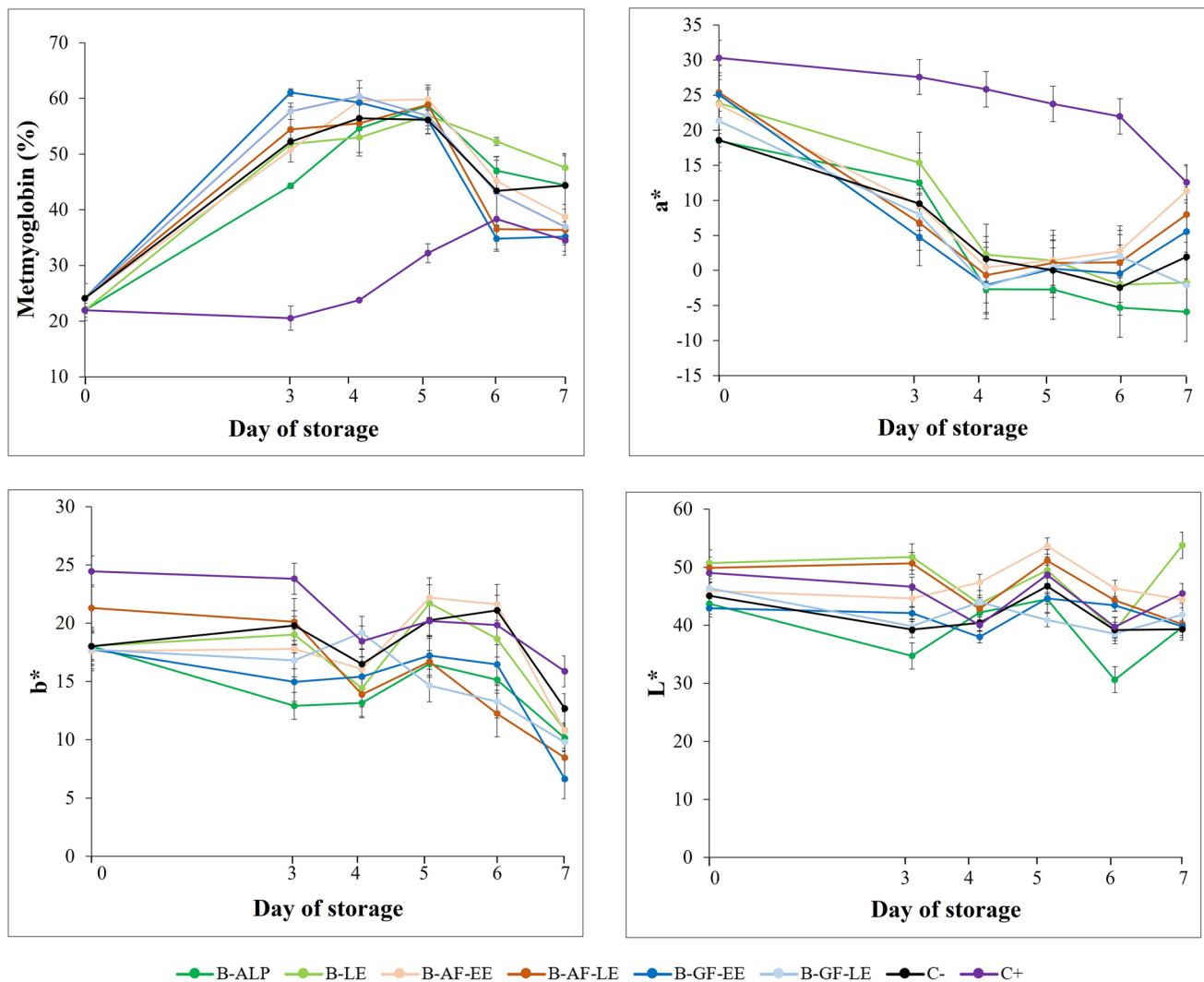
The results of MetMb for the different samples during storage are presented in Fig. 3. MetMb and colour depended on the sample and time of storage, as well as its interaction sample and time ($p < 0.05$).

MetMb levels increased on day 3 for all samples, except for *C+*, which increased ($p < 0.05$) only by day 5. However, within day 3, *B-ALP* showed the lowest MetMb content (44.29%) although there was no significant difference with *B-LE*. The lower MetMb formation observed in burgers containing ALP or LE may be attributed to their higher and

Table 4. PH values for the different burgers for 7 days at 4 °C

Sample ¹	Day 0	Day 4	Day 5	Day 6	Day 7
B-ALP	5.61 ± 0.02 ^{Ab}	5.53 ± 0.02 ^{ABb}	5.95 ± 0.4 ^{Aab}	5.90 ± 0.03 ^{CDab}	6.42 ± 0.06 ^{Aa}
B-LE	5.58 ± 0.03 ^{Ab}	5.51 ± 0.01 ^{ABb}	5.99 ± 0.5 ^{Aab}	5.92 ± 0.04 ^{Cab}	6.47 ± 0.06 ^{Aa}
B-AF-EE	5.57 ± 0.00 ^{Ad}	5.51 ± 0.03 ^{ABd}	5.83 ± 0.03 ^{Ac}	6.15 ± 0.05 ^{Bb}	6.36 ± 0.04 ^{Aa}
B-AF-LE	5.52 ± 0.05 ^{Ab}	5.47 ± 0.02 ^{ABab}	5.95 ± 0.4 ^{Aab}	5.80 ± 0.03 ^{CDab}	6.16 ± 0.05 ^{Ba}
B-GF-EE	5.56 ± 0.06 ^{Ad}	5.44 ± 0.01 ^{Be}	5.99 ± 0.04 ^{Ab}	5.86 ± 0.02 ^{CDc}	6.43 ± 0.04 ^{Aa}
B-GF-LE	5.60 ± 0.02 ^{Ad}	5.50 ± 0.09 ^{ABd}	5.99 ± 0.04 ^{Ac}	6.33 ± 0.05 ^{Ab}	6.50 ± 0.05 ^{Aa}
C-	5.60 ± 0.01 ^{Ab}	5.40 ± 0.00 ^{Bc}	5.63 ± 0.02 ^{Ab}	5.62 ± 0.03 ^{Eb}	6.17 ± 0.08 ^{Ba}
C+	5.60 ± 0.03 ^{Abc}	5.58 ± 0.04 ^{Ac}	5.60 ± 0.03 ^{Abc}	5.75 ± 0.09 ^{DEab}	5.82 ± 0.05 ^{Ca}

^{a-b}Mean values with different superscripts within the same treatment but at different times are statistically different; ^{A-E}mean values with different superscripts on the same day but from different treatments are statistically different ($p < 0.05$); ¹values are expressed as the mean ± standard error. *B-ALP*, burgers with artichoke leaf powder; *B-LE*, burgers with lyophilised extract; *B-AF-EE*, burgers with alginate film with ethanolic extract; *B-AF-LE*, burgers with alginate film with lyophilised extract; *B-GF-EE*, burgers with gelatin film with ethanolic extract; *B-GF-LE*, burgers with gelatin film with lyophilised extract; *C+*, positive control; *C-*, negative control



B-ALP: Burgers with artichoke leaf powder; **B-LE:** Burger with lyophilised extract; **B-AF-EE:** Burgers with alginate film with ethanolic extract; **B-AF-LE:** Burgers with alginate film with lyophilised extract; **B-GF-EE:** Burgers with gelatin film with ethanolic extract; **B-GF-LE:** Burgers with gelatin film with lyophilised extract; **C+:** Positive control (with commercial mix of additives); **C-:** Negative control (Burger without any additional ingredient or additive).

Fig. 3 Metmyoglobin content and changes in instrumental colour for all burgers for 7 days of storage at 4 °C

faster active compounds availability, associated with their polyphenol content and scavenging capacity (Section “Polyphenolic Fractions and Scavenging Activity of Artichoke Leaf Powder”). In contrast, burgers with films exhibited higher MetMb levels by day 3, with the B-GF-EE showing the highest MetMb content (61.06%). However, no significant differences were observed between gelatin and alginate films, nor between ethanolic and lyophilised extracts within the film matrix. This indicated that neither the polymer matrix nor the extract type significantly influenced MetMb formation. It is important to note that burgers containing films showed overall higher MetMb values than those with direct extract incorporation, likely due to the gradual release

of polyphenols from the polymer matrices, which may delay their protective effect against Mb oxidation. The effectiveness of these antioxidants can be understood in the context of Mb oxidation mechanisms. Mb is a heme protein in muscle that binds oxygen, and it is the major factor responsible for meat colour (Suman & Joseph, 2013). MetMb is one of the four redox forms of Mb (Mancini & Hunt, 2005). The formation of MetMb occurs due to the oxidation of the ferrous (Fe^{2+}) form into a ferric state (Fe^{3+}), leading to a brownish colour of meat (Suman & Joseph, 2013). When 60% or more of the Mb converts to MetMb, the meat develops a noticeable brown colour that can be detected by the eye (Inai et al., 2014). The formation of MetMb is often associated with the growth

of spoilage bacteria, particularly species of *Pseudomonas* (Abdallah et al., 1999; Chan et al., 1998). Polyphenols can limit MetMb formation by their scavenging activity and their antimicrobial activity. Oxygen availability and pH also play a crucial role in Mb redox stability. Films can reduce oxygen availability on the surface of burgers and promote reduction reactions. Jakobsen and Bertelsen (2000) found optimal meat colour is maintained at an oxygen content of 55–80%. As observed in Fig. 3, MetMb remains stable on days 4 and 5 and decreases on days 6 and 7, except in C+. This decrease may relate to the microbial activity of certain bacteria such as *Pseudomonas fragi* that can reduce MetMb to deoxymyoglobin, influencing colour during spoilage (Motoyama et al., 2010). It is well known that myoglobin is a pH-dependent reaction. Gutzke and Trout (2002) reported faster Mb oxidation at a pH of 5.5, while higher pH levels (6.0–6.5) significantly slow the oxidation process. Moreover, MetMb reductase activity by bacteria is highest at pH 6.5 (Hagler et al., 1979). This aligns with the pH results (Table 2), as MetMb production occurred fast until days 3–4, when the pH ranged from 5.52 to 5.58, and subsequently declined as the pH began to increase from day 4 onwards. This would be also related to the fact that in burgers with films, which had greater bacterial growth, MetMb content decreased more on day 7. In another study where 5 and 10% artichoke bracts extracts were added, MetMb was reduced, being almost half (30%) when 10% extract was added compared to the control group (60%) at 10 days (Demir & Ağaoğlu, 2021). These results may suggest that higher concentrations may be required to observe greater effects in MetMb reduction.

Colour is one of the most important parameters of the visual appearance of meat. It is influenced by biochemical changes, including Mb oxidation. The colour results are shown in Fig. 3, and they were in line with the MetMb results. L^* (lightness) was slightly higher in B-ALP, consistent with Demir and Ağaoğlu (2021) due to darker artichoke leaf powder pigments. Moreover, a^* (redness) is a key indicator of meat colour and its decrease is in line with Mb oxidation (Demir & Ağaoğlu, 2021). On day 0, a^* values of all the burgers ranged from 20.23 to 30.30. B-ALP and B-LE had lower a^* values throughout the experiment, likely due to the green colour of B-ALP and B-LE, which gave the meat a browner colour. Until day 6, a^* values decreased, but increased again on day 7 for all the samples except for C+, reflecting the MetMb trend (Fig. 3), which initially increased and then decreased ($p < 0.05$). In contrast, C+, a^* values content showed a decrease over time, as the MetMb just increased. On day 7, a^* increased for burgers with films and the negative control. The b^* (yellowness) values remained relatively stable across all treatments until day 7, when a decrease on b^* was observed in all samples. This, along with the increase in a^* , suggests a purplish colour in the meat. This may be attributed to the conversion of MetMb

into deoxymyoglobin by bacteria activity which is consistent with the considerable increase in psychrotrophic counts previously observed (Section “Microbiological Counts”). When added artichoke extract in minced meat, b^* values also decreased with time for Demir and Ağaoğlu (2021).

The total colour difference (ΔE^*) is a widely used parameter to quantify overall colour change between two samples, integrating variations in L^* , a^* , b^* (Saluena et al., 2019). In the present study, the total colour difference (ΔE^*) of samples was calculated using the C+ (day 0) as a reference and the rest of the samples for each day were compared with this reference to obtain each ΔE^* . ΔE^* of samples ranged between 8.26 and 14.03 on day 0, indicating notable initial colour differences among samples with C+ (day 0). In the commercial mix, used in the positive control (C+) compounds such as sodium ascorbate, sodium sulfite, and sodium citrate are known for their antioxidant properties that also contribute to colour preservation (Hood, 1975; Stivarius et al., 2002). By day 7, ΔE^* values increased significantly for all the samples. While C+ (at day 7) exhibited the lowest ΔE^* (20.19), the other samples showed higher values, between 24.70 and 40.20. Among them, burgers coated with alginate films showed the smallest differences ($\Delta E^* = 24.70$ – 29.09), whereas the B-ALP exhibited the highest colour variation (~ 40.20) probably due to the green pigmentation of the ALP. Notably, the incorporation of powdered extract (B-LE) mitigated this effect, leading to a substantial reduction in ΔE^* values ($\Delta E^* \sim 35.43$).

Secondary Lipid Oxidation and Scavenging Activity in Burgers

Lipid oxidation is a major cause of meat deterioration, affecting colour, smell, flavour, texture, and nutritional value (Geng et al., 2023). It occurs via a free-radical chain reaction in three phases: initiation, propagation, and termination (Geng et al., 2023). Hydroperoxides (ROOH) are primary products that decompose into secondary compounds, such as aldehydes, ketones, and alcohols (Pokorny et al., 2021). In this sense, TBARS evaluates the formation of aldehydes (malondialdehyde) present in meat.

Lipid oxidation depended on the sample and time of storage, as well as its interaction, sample $\times$ time ($p < 0.05$). In general, in this study, TBARS values increased ($p < 0.05$) for all samples during storage (Fig. 4A). C– and burgers with films exhibited higher TBARS values than those formulated with ALP (B-ALP) and LE (B-LE) and the commercial mixture of additives (C+) over time. ALP showed a stronger antioxidant effect over time than LE. On day 5, TBARS values increased significantly in B-LE (0.08 mg MDA/kg), while the increase in B-ALP was much smaller (0.035 mg MDA/kg), indicating fewer secondary lipid oxidation reactions in burgers containing

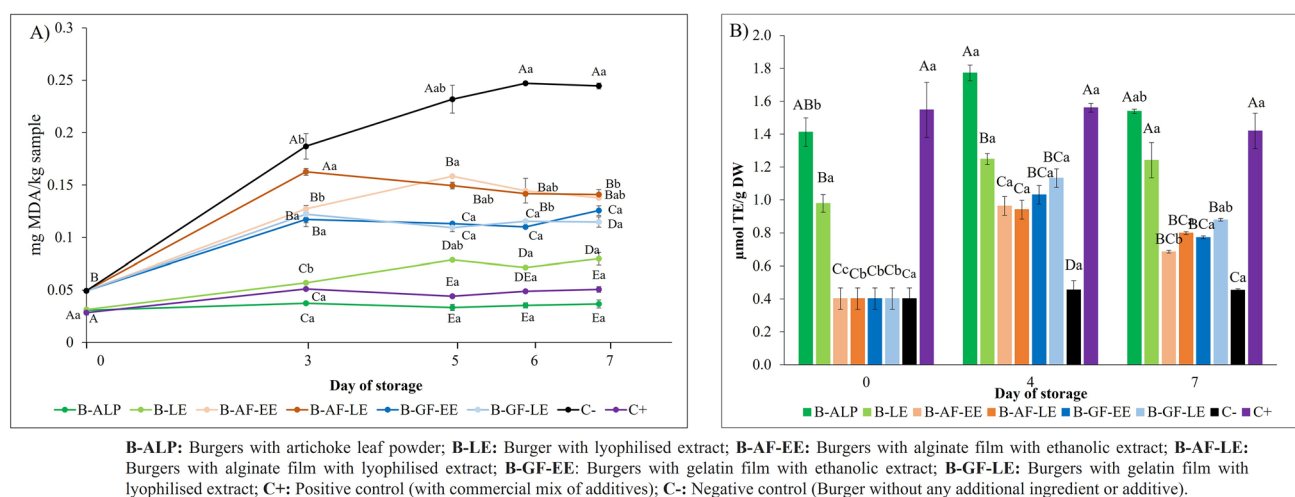


Fig. 4 TBARS values (A) and scavenging activity for radical DPPH (B) for the different samples of burgers during storage at 4 °C

ALP. This difference is probably attributed to the polyphenol composition of B-ALP and B-LE. B-LE contained only the soluble polyphenol fraction obtained through a simple extraction process (Section “Film Preparation and Characterisation”), whereas B-ALP includes both soluble polyphenols and insoluble, matrix-bound polyphenols (Section “Extraction of Free and Bound Polyphenol Fractions in Leaf Artichoke Powder”), which can also contribute to antioxidant activity. Additionally, the matrix effect could play a role. In B-ALP, the polyphenols may be better protected inside the matrix, enhancing their stability and activity. Moreover, the gradual release of matrix-bound polyphenols during storage or microbial activity in the meat could further prolong their antioxidant effect, as has been described before in living organisms (Cömert & Gökmen, 2017; Shahidi & Yeo, 2016).

By day 7, B-ALP and C+ maintained the lowest TBARS values, with no significant differences between them. This result highlights the antioxidant potential of ALP, which achieved a lipid oxidation level similar to that of the synthetic antioxidant in C+, demonstrating its potential as a natural alternative. These findings align with Ergezer et al. (2018), who reported that 1000 ppm of artichoke bract aqueous extract reduced TBARS values in beef patties threefold compared to the C-. In their study, TBARS values increased from 0.38 to 0.58 mg MDA/kg in the beef patty with 1000 ppm of artichoke bract lyophilised extract from day 0 to day 7, while C- rose from 0.38 to 1.26 mg MDA/kg. Similarly, in another study, burgers treated with artichoke extract had lower TBARS values (0.72 mg MDA/kg) than those treated with a synthetic antioxidant butylated hydroxytoluene (BHT) (0.92 mg MDA/kg) (Ergezer & Serdaroğlu, 2018).

TBARS values were higher for burgers with films, probably due to the lower amount of polyphenols released from

the films. However, these values remained lower than those observed for C-. Burgers with gelatin films showed lower TBARS than with alginate films, possibly due to higher polyphenol release (Section “Polyphenol Content and Release Assay of Active Films”), regardless of extract type (ethanolic or lyophilised). Several authors have previously demonstrated that bioactive films enriched with natural antioxidant extracts can effectively limit lipid oxidation in food systems (Elhadef et al., 2024; S. C. Lourenço et al., 2020; Villasante et al., 2020; Yaghoubi et al., 2024). Overall, artichoke leaves have demonstrated a strong antioxidant potential, especially when used as a powder (B-ALP).

Scavenging activity determined by the DPPH radical for all the raw burgers over time is presented in Fig. 4B. The scavenging activity taking place in the burgers aligns with the TBARS results. B-ALP exhibited the highest scavenging activity over time. B-ALP values were comparable to C+ and were followed by B-LE ($p < 0.05$). Burgers with films showed lower activity on day 0 due to slow polyphenol release, which increased by day 4 and declined by day 7 as polyphenols were degraded. Burgers with gelatin films on day 4 exhibited higher scavenging activity than those with alginate films, consistent with the faster release of polyphenols observed for gelatin-based films (Section “Polyphenol Content and Release Assay of Active Films”) and the lower TBARS values. C- also displayed measurable scavenging activity, which could be attributed to peptides with intrinsic antioxidant capacity that can reduce oxidative stress in meat systems (Grassi et al., 2023; Serpen et al., 2012). Several authors have previously studied the scavenging activity of artichoke extract and artichoke bract extract in meat (Demir & Ağaoğlu, 2021; Ergezer & Serdaroğlu, 2018; Ergezer et al., 2018). However, this is the first study that evaluates the scavenging activity of artichoke leaves (powder or

extract) or artichoke leaf extract incorporated in bioactive films taking place in meat.

The lipid oxidation of the burgers was closely linked to their scavenging activity, although this relationship evolved during storage. On day 0, no significant correlation was observed ($p > 0.05$), likely due to the initial induction period of lipid oxidation. However, as storage progressed, on day 4, a negative correlation was observed ($r = -0.973$, $p < 0.05$), indicating that the higher the DPPH radical scavenging activity, the more limited was the formation of TBARS. By day 7, the correlation remained significant but weakened ($r = -0.813$, $p < 0.05$). This was probably due to the degradation of polyphenols over time.

Sensorial Evaluation

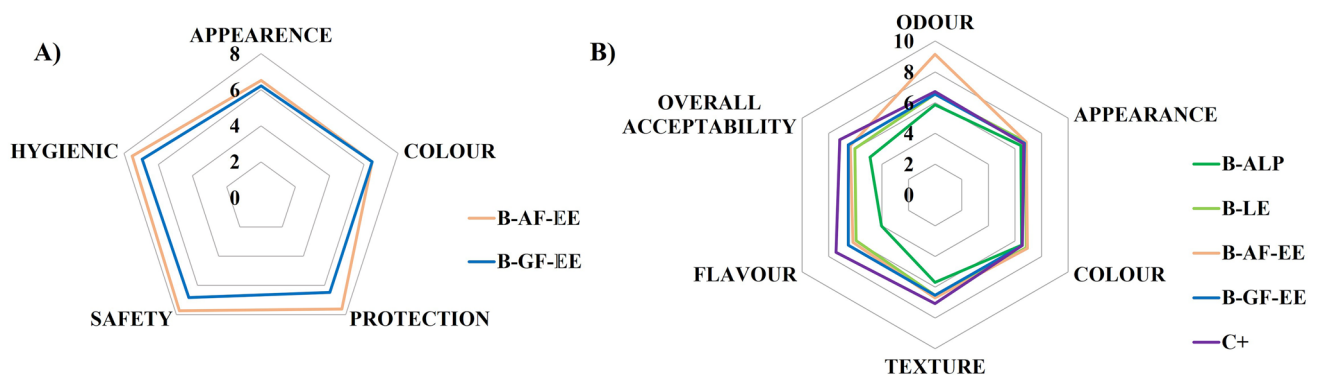
Figure 5 presents the results obtained in both tests (acceptability tests with raw and cooked burgers).

Figure 5A shows the sensory evaluation of raw burgers with protective films. Among the assessed attributes, only protection and safety differed significantly ($p < 0.05$). B-AF-EE scored higher for both protection (7.2 ± 0.9) and safety (7.7 ± 1.1) compared to B-GF-EE (6.5 ± 1.7 and 6.8 ± 1.5 , respectively). Moreover, raw B-AF-EE demonstrated a higher consumer purchase intention (85.7%) compared to B-GF-EE (75.0%), consistent with the sensory results. This suggested that alginate may be purchased more due to a more favourable perception of protection and safety in raw burgers. Both films achieved high purchase intention values, indicating great consumer acceptance of burgers coated with bioactive films and supporting their potential as an effective bioactive coating for fresh meat products.

Regarding the cooked samples, among the evaluated attributes, only texture and flavour showed

significant differences ($p < 0.05$) (Fig. 5B). The texture of C+ received the highest score (7.1 ± 1.3), while B-ALP scored the lowest (5.69 ± 1.5). No significant differences ($p > 0.05$) were observed among the other samples. Similarly, flavour was rated highest for the C+, followed by B-GF-EE. The B-GF-EE did not differ significantly from B-AF-EE and B-LE. Cooked B-ALP received the lowest score for flavour, likely due to the bitterness of ALP. These results differed slightly with the findings from a previous study in which artichoke (florete) powder was added to meatballs (Gedrovica & Karklina, 2013). In this study, the incorporation of small amounts of Jerusalem artichoke powder improved the texture and flavour of meatballs, while higher additions led to a grainier and tougher texture. Although the powder negatively affected colour and appearance, flavour acceptability increased (Gedrovica & Karklina, 2013). These effects were likely influenced by the presence of other ingredients, such as bread and onion, which may have masked some sensory changes and could represent a strategy to improve the acceptability of artichoke incorporated in burgers. Similarly, Ayuso et al. (2024a) reported no sensory differences when artichoke extracts were added to fat-replaced burgers, likely because olive oil masked undesirable flavours. Colour was the only sensory attribute that did not differ significantly among samples, which can be considered a positive result. Despite the relatively high instrumental colour differences (ΔE^*) observed at day 0 in comparison with C+ (8.26–14.03), these differences did not negatively affect consumers' colour acceptability scores.

Regarding the purchase intention of cooked burgers, C+ exhibited the highest purchase intention, with 86.2% of participants indicating they would buy it. This was followed by B-GF-EE (purchase intention of 72.4%)



B-ALP: Burgers with artichoke leaf powder; **B-LE:** Burger with lyophilised extract; **B-AF-EE:** Burgers with alginate film with ethanolic extract; **B-GF-EE:** Burgers with gelatin film with ethanolic extract; **C+:** Positive control (with commercial mix of additives)

Fig. 5 Acceptability test of raw (A) and cooked (B) burgers

and B-AF-EE and B-LE, with similar purchase intention (55.2%). In contrast, B-ALP received the lowest purchase intention (24.14%). The texture and flavour evaluations might explain these results as these attributes received the lowest scores among the tested samples. Overall, although ALP negatively affected flavour and texture perception, the use of bioactive films containing EE maintained good sensory acceptance and high purchase intention.

Conclusions

Artichoke leaves are rich in phenolic compounds with high antioxidant activity, making them a promising functional ingredient for the formulation of meat products with improved quality properties. The direct incorporation of ALP into beef burgers was the most effective strategy in significantly reducing lipid oxidation, followed by B-LE. Bioactive films incorporating ethanolic and lyophilised extracts of artichoke leaves also exhibited antioxidant effects, with gelatin-based films (B-GF-EE/B-GF-LE) being more effective than alginate-based ones (B-AF-EE/B-AF-LE), likely due to a higher release of bioactive compounds from the films. MetMb content increased by day 3 for all the samples except the positive control, with the B-ALP showing the lowest MetMb content and thus, maintaining meat colour. From a sensory perspective, the use of ALP slightly negatively affected texture and flavour, reducing consumer acceptability. However, bioactive films better preserved the organoleptic properties of burgers and maintained a favourable consumer purchase intention. These findings support the potential of artichoke leaves as a natural alternative to synthetic antioxidants in meat products, either as a direct ingredient or as an extract incorporated in active films. Overall, this study demonstrates the potential of artichoke crop leaves as a natural antioxidant source for beef burgers when applied through different strategies, including direct incorporation and active packaging systems. Future research should focus on determining the minimum effective concentration of ALP or LE that ensures both oxidative protection and consumer acceptability, evaluating the effectiveness of these extracts in other meat matrices, and optimising film formulations to enhance the controlled release of polyphenols, particularly during the early stages of oxidation.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11947-026-04316-y>.

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Author Contribution I.C.-T. and M.P.A. were responsible for conceptualization; M.M.-N. and S.-C.P. performed data curation; M.M.-N., S.-C.P., and J.V. conducted the formal analysis. M.M.-N. prepared all figures and tables; M.P.A. was responsible for funding acquisition and resources; M.M.-N. and J.V. developed the methodology; M.P.A. and I.C.-T. carried out the validation; and I.C.-T. performed the initial visualization. The original draft was written by M.M.-N. with support from S.-C.P., while I.C.-T. and M.P.A. handled review and editing. All authors have read and reviewed the manuscript.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations During the preparation of this work the authors used Gemini and ChatGPT to improve the readability and language of the manuscript. After using these tools, the authors reviewed and edited the content to ensure accuracy and take full responsibility for the final text.

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