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Development of bioactive edible coatings enriched with extracts of celery and banana inflorescence for the preservation of persimmons

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ABSTRACT

Edible coatings enriched with bioactive compounds have emerged as promising alternatives to synthetic preservatives, aligning with sustainability goals and offering solutions for the conservation of perishable fruits. This study aimed to develop and evaluate the efficacy of edible coatings formulated with celery (*Apium graveolens* L.) and banana inflorescence (*Musa acuminata* var. "Dwarf Cavendish") extracts, known for their antioxidant properties, in extending the shelf life of persimmons (*Diospyros kaki* L. var. Giombo). The extraction of phenolic compounds and flavonoids from both botanical sources was optimized using Response Surface Methodology (RSM), and the extracts were incorporated into agar-based edible coatings. Physicochemical parameters such as color (L*, a*, b*), pH, total phenolics, antioxidant activity (DPPH, FRAP), and total carotenoids were analyzed throughout storage at both refrigerated and room temperatures. The coatings exhibited moderate but significant effects on persimmon preservation, particularly in maintaining lightness (L*). Celery extract-based coatings showed 61.0 ± 2.1 at day 10 under refrigeration, compared to 55.0 ± 3.4 in banana inflorescence formulations. Over the storage period, total phenolics in coated samples degraded at a slower rate (0.3 ± 0.2 mg GAE/g in refrigerated conditions), while the antioxidant activity remained higher (DPPH EC50 = 80 ± 12 μg/mL for celery coatings at day 10). Color degradation was evident across all treatments, but coatings with celery extract were more effective in reducing changes in b (yellow-blue coordinate)**, maintaining a b value of 51 ± 6 at day 10, whereas uncoated samples decreased to 47 ± 10. These findings suggest that while the tested coatings provide some level of preservation, further optimization is required to enhance their efficacy, particularly in improving barrier properties and bioactive compound stability. Future research should focus on refining formulations by incorporating additional stabilizers and optimizing temperature conditions to enhance the preservation and safety aspects of coated fruits, ensuring their viability for commercial applications in the food industry.

1. Introduction

The use of edible coatings enriched with bioactive compounds extracted from plants represents a promising approach to extending the shelf life and preserving the quality of perishable food products. These coatings have gained prominence due to their potential to replace synthetic preservatives and reduce agricultural waste, aligning with the principles of the circular economy and environmental sustainability (Al Aboody, 2021; Kooti & Daraei, 2017).

Edible coatings have emerged as a promising solution, offering an alternative to conventional plastic packaging and chemical preservatives. Edible coatings consist of thin, consumable layers applied

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directly to the surface of food items, preserving quality and extending shelf life (Gupta et al., 2024; Perez-Vazquez et al., 2023). However, the selection of active ingredients is crucial for their effectiveness. In this study, celery and banana inflorescence extracts were chosen as sources of phenolic and antioxidant compounds, which possess antimicrobial and preservative properties but have not yet been widely explored in coatings for persimmons. These coatings are particularly beneficial for perishable foods, such as fruits and vegetables, as they help reduce microbial contamination, oxidation, and moisture loss (Perez-Vazquez et al., 2023). When formulating edible coatings, it is essential to consider barrier properties, such as oxygen and carbon dioxide permeability, while ensuring transparency and sensory neutrality, so they do not alter the taste, aroma, or appearance of the food (Gupta et al., 2024; Karnwal et al., 2025). Although they may have some limitations, such as less-than-ideal mechanical properties or limited antimicrobial efficacy, edible coatings continue to advance (Karnwal et al., 2025; Martins et al., 2024). The incorporation of natural additives, such as antimicrobials and antioxidants, can enhance their functionality and stability. In addition to being biodegradable, these coatings can be made from food industry by-products, promoting the circular economy and contributing to sustainability by reducing food waste and dependence on plastic packaging (Martins et al., 2024; Vanaraj et al., 2024).

The persimmon (*Diospyros kaki* L. var. Giombo) is a highly perishable fruit rich in bioactive compounds, including flavan-3-ols (catechins and proanthocyanidins), phenolic acids, and carotenoids (Jung et al., 2005; Lee et al., 2012). These compounds contribute to its antioxidant properties, which play a crucial role in reducing oxidative stress and preserving the quality of the fruit. However, the high concentration of soluble condensed tannins in astringent persimmons can influence sensory attributes, making their stabilization during storage essential.

(Serrano et al., 2009). The application of bioactive edible coatings may help mitigate these issues by maintaining the fruit's physicochemical properties and extending its shelf life under different storage conditions (Karnwal et al., 2025; Vanaraj et al., 2024).

In this study, edible coatings were enriched with bioactive extracts from celery stem (*Apium graveolens*) and banana inflorescence (*Musa* spp.), two plant sources known for their content of phenolic compounds. Celery is abundant in flavonoids such as apigenin and luteolin, which exhibit antimicrobial and antioxidant properties (Rana et al., 2022; Momin & Nair, 2001). Banana inflorescence, often considered an agro-industrial byproduct, contains sterols, triterpenes, and polyphenols with potential anti-hyperglycemic and anti-inflammatory effects (Liyanage et al., 2016; Ramu et al., 2017). The incorporation of these bioactive extracts into edible coatings offers a sustainable approach to fruit preservation while reducing food waste and promoting the use of natural preservatives (Karnwal et al., 2025; Panyayong & Srikaeo, 2022).

Despite the increasing interest in edible coatings, studies evaluating the incorporation of celery and banana inflorescence extracts for persimmon preservation remain scarce. Previous research has primarily focused on coatings with chitosan, alginate, or essential oils (Han et al., 2018; Ribeiro et al., 2021), but there is a lack of studies exploring the synergistic effects of phenolic-rich botanical extracts in maintaining the quality of persimmons.

The objective of this study was to develop edible coatings enriched with optimized celery and banana inflorescence phenolics extracts and apply them on persimmons, focusing on evaluating their influence on the physicochemical stability of the fruits during storage. Special attention was given to maintaining color, structural integrity, and quality under different storage temperatures, as well as analyzing antioxidant properties, total solids, and carotenoid retention. Additionally, Linear Discriminant Analysis (LDA) was used to compare treatments and identify preservation patterns, providing a more precise evaluation of the coatings' effects on fruit stability. By exploring these factors, this study aims to offer innovative alternatives for the food industry, focusing on the preservation and quality of perishable foods.

2. Methodology

2.1. Materials

The edible films were produced using agar-agar (vegetable gelatin, Mercearia e Bomboniere Towa Ltda, São Paulo, SP, (23°33′17"S 46°38′06"W), glycerin, and the plants celery (*Apium graveolens* L.) from Estevão Luís Salvador, Lda, a company located in Portugal, more precisely in Almargem do Bispo, Sintra (38°52′19"N 9°15′36"W). Banana inflorescence *Musa acuminata* var. "Dwarf Cavendish" sourced from Madeira Island, Portugal (32°45′24.6"N 17°01′01.9"W), and persimmons (*Diospyros kaki* L. var. Giombo) from the São Manuel Experimental Farm of the São Paulo State University (UNESP), São Paulo, Brazil (22°46′09"S 48°34′18"W). Acetonitrile of HPLC grade was from Lab-Scan (Lisbon, Portugal). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Phenolic standards and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma (St. Louis, MO, USA). Sodium acetate trihydrate, glacial acetic acid P.A., hydrochloric acid P.A., ferric chloride hexahydrate, ferrous sulfate heptahydrate, TPTZ (2,4,6-Tris (2-pyridyl)-*s*-triazine) were from Sigma Aldrich. Folin reagent was from Panreac (Barcelona, Spain), and sodium carbonate, monobasic, and dibasic potassium phosphate were from Merck Millipore (Darmstadt, Germany). Sodium acetate and potassium chloride were obtained from Thermo Scientific (Waltham, MA, USA). Ethanol and all other chemicals were obtained from Sigma. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA). Bacteria and fungi were obtained from Frilabo (Porto, Portugal). The cells were purchased from the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH (Inhoffenstraße 7B 38,124 Braunschweig Science Campus Braunschweig-Süd, Germany), except for the macrophages, which were purchased from European Collection of Authenticated Cell Cultures - ECACC (Culture Collections UK Health Security Agency, Porton Down, Salisbury, SP4 0JG, UK).

2.2. Experimental design for extraction optimization

A five-level central composite design (CCD) integrated with response surface methodology (RSM) was used to optimize the extraction of phenolic compounds from celery (*Apium graveolens*) and banana inflorescence (*Musa acuminata* "Dwarf Cavendish"). The coded and natural values of the independent variables, X1 (extraction time, t, in minutes), X2 (sonication power, P, in %), and X3 (solvent proportion, S, in % ethanol, *v*/v), are detailed in **Table 5** (supplementary material). The CCD design shown in **Table 6** (supplementary material) consisted of 17 experimental points, including 3 replicated center points to estimate pure error and axial points to ensure rotatability, where rotatability guarantees uniform prediction variance at all points equidistant from the center. This design, created using MATLAB R2023a (v.9.14, MathWorks, Inc., Natick, MA, USA), was constructed with an alpha (α) value of 1.00, representing the axial distance that maintains rotatability in the experimental space. Experimental runs were randomized to mitigate the influence of uncontrolled variability on the observed responses, thereby improving the reliability of the results. For this optimization study, celery stem and banana inflorescence (purple leaves) were used. For each optimization, 1 g of sample was weighed in 100 mL of ethanolic solution according to the optimization points (solvent: 0, 50, and 100 %), as presented in **Table 5** (supplementary material).

2.2.1. Response criteria and mathematical modeling

The response criteria, including luteolin derivative (Y1), chrysoeriol derivative (Y2), apigenin derivative (Y3), total flavonoids (TF, Y4), and total phenolic content (TPC, Y5) in celery, as well as *p*-coumaroyl-acetyl-sucrose (Y1), *p*-coumaroyl-di-acetyl-sucrose isomers (Y2), rutin (Y3), *p*-coumaroyl-tetra-acetyl-sucrose isomers (Y4), total flavonoids (TF, Y5), total phenolic acids (TPA, Y6), and total phenolic content (TPC, Y7) in

banana inflorescence, were evaluated to optimize the extraction process and maximize the recovery of bioactive compounds. The individual compounds were chosen based on their highest abundance in each species. Mathematical modeling was performed using response surface methodology (RSM) with second-order polynomial equations:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_i X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j \quad (1)$$

Where Y represents the dependent variable (response) to be modeled, b_0 is the constant coefficient, and b_i, b_{ii}, and b_{ij} denote the regression coefficients for the linear, quadratic, and interaction effects, respectively. The variables X_i and X_j represent the independent variables (extraction time, temperature, and solvent concentration), while n indicates the total number of variables in the study ($n = 3$).

2.2.2. Model fitting and statistical analysis

The model fitting, coefficient estimation, and statistical analyses were performed using MATLAB R2023a v.9.14 (MathWorks, Inc., Natick, Massachusetts, USA). The significance of the regression coefficients was assessed through analysis of variance (ANOVA), with a confidence level of 95 % ($\alpha = 0.05$). The coefficient of determination (R^2) and the adjusted coefficient of determination (R^2_{adj}) were used to evaluate the adequacy of the polynomial models in describing the responses, representing the proportion of response variability explained by the model.

Only statistically significant terms ($p < 0.05$) were included in the final models. Term significance was determined based on F-values, while the lack-of-fit-test was used to verify the model's suitability for describing the functional relationship between the independent variables and the observed responses. A non-significant lack-of-fit result ($p > 0.05$) indicated that the model was appropriate for predicting the responses.

Additionally, MATLAB was used to generate response surface plots illustrating the influence of the independent variables on the evaluated responses. These plots facilitated the visualization of combined variable effects and assisted in identifying optimal conditions for maximizing the extraction of bioactive compounds from celery leaves and banana inflorescences.

2.3. Preparation of samples and extracts

The samples were obtained as previously described in section 2.1. After collection, the plants were carefully washed with distilled water to remove impurities, frozen, and subsequently dried using a freeze-drying (Coolvacuum Technologies, S.L.; Freeze dryer - model: Triepic −55; year: TPC23035 / 2024; Electric supply: 3600 W; 230VAC (II + T); 50 Hz; Maximum pressure: 24Bar; Granollers, Barcelona, Spain). The process involved the preparation of phenolic extracts, which were obtained from the dried samples under the conditions described in **Table 5** (supplementary material), according to the optimized extraction parameters.

2.4. Phenolic compounds analysis

The analysis of the phenolic compounds in each species was performed by initially dissolving the samples in an EtOH:H_2O solution (20:80, *v*/v) to a final concentration of 10 mg/mL and filtered using a Clarify-Ny 25 mm syringe filter (de Oliveira et al., 2024b). The analysis was conducted using an LC-DAD-ESI/MS^n system (Dionex Ultimate 3000 UPLC; Thermo Scientific, San Jose, CA, USA) equipped with a diode array detector (DAD) set at wavelengths of 280, 330, and 370 nm, and an electrospray ionization mass spectrometer (Linear Ion Trap LTQ XL, ThermoFinnigan, San Jose, CA, USA) operating in negative mode. Compound identification was performed by comparing chromatographic parameters with those reported in the literature and, when available, with commercial standards, including apigenin-6-C-glucoside, caffeic acid, chlorogenic acid, hesperetin, luteolin-7-*O*-glucoside, naringenin, quercetin-3-*O*-rutinoside, rosmarinic acid, rutin and coumaric acid. Calibration curves were established from the area of the peaks recorded at 280 (benzoic acids, dihydroflavones), 330 (hydroxycinnamoyl derivatives), 370 nm (flavones and flavonols) and 330 nm (for coumaric derivatives). For banana inflorescence, the calibration curves used were y = 504,847× – 45,869 for *p*-coumaric acid and hydroxycinnamoyl derivatives, and y = 96,307× - 5711.4 for rutin and flavones and flavonols. For celery, the calibration curves were y = 504,847× – 45,869 for *p*-coumaric acid and hydroxycinnamoyl derivatives, and y = 13,586× – 7949.7 for luteolin-6-C-glucoside and apigenin-7-*O*-glucoside. The results were expressed in mg/g of extract.

2.5. Bioactivity measurements

The total phenolic content in the ethanolic extracts was determined using a modified Folin-Ciocalteu method. Samples were mixed (500 µL) with Folin-Ciocalteu reagent (2.5 mL), sodium carbonate (2 mL) then incubated in the dark for 90 min. Absorbance was measured at 725 nm, and gallic acid was used to construct the standard curve (70 % ethanol y = 5.8096× - 0.0508; R^2 = 0.999). Results, expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract, represent the mean ± standard deviation of triplicate analyses (Singleton & Rossi, 1965).

Antioxidant activity was evaluated using dried extracts prepared as stock solutions in water at a concentration of 10 mg/mL and subsequently diluted. For the DPPH radical scavenging assay, extract solutions (270 µg/mL) were mixed with DPPH solution (6×10^{-5} M) and kept in the dark for 30 min. Activity was measured by DPPH discoloration at 517 nm. Reducing power was determined by the ability to convert Fe^{3+} to Fe^{2+}, also read at 517 nm. Lipid peroxidation inhibition was assessed by measuring the reduction of thiobarbituric acid reactive substances (TBARS) in porcine brain homogenates, with absorbance measured at 532 nm. Results were expressed as EC50 values, representing the concentration required to achieve 50 % antioxidant activity or an absorbance of 0.5 in the reducing power assay. Trolox was used as a positive control.

Cellular antioxidant activity was evaluated using RAW 264.7 murine macrophage cells cultured in DMEM medium at 37 °C with 5 % CO_2. After preparation and centrifugation, the cells were adjusted to a density of 70,000 cells/mL. Aliquots were incubated for 48 h in black clear-bottom microplates. Subsequently, the cells were washed, treated with different extract concentrations (32.5–2000 µM) for 1 h, washed again, and exposed to an AAPH solution (600 µM). Fluorescence was measured every 5 min for 1 h, and results were expressed as the percentage inhibition of the oxidation reaction (de Oliveira et al., 2024a).

The cytotoxic activity of the extracts was tested against four tumor cell lines (gastric adenocarcinoma - AGS, breast carcinoma - MCF-7, colorectal adenocarcinoma - Caco-2, non-small cell lung carcinoma - NCI-H460) and a non-tumor PLP2 cell line (non-tumor cell line from porcine liver), using the sulforhodamine B method. Cells were treated with extracts at a maximum concentration of 400 µg/mL, and growth inhibition (GI_{50}) was determined based on absorbance at 540 nm, using ellipticin as a positive control as described by de Oliveira et al. (2023). Anti-inflammatory activity was evaluated using the RAW 264.7 macrophage cell line cultured in DMEM medium. The extracts' ability to inhibit nitric oxide (NO) production was measured as described by de Oliveira et al. (2024b), with results expressed as EC_{50} values (µg/mL). Dexamethasone (50 µM) served as the positive control.

The antibacterial potential of the extracts was evaluated against pathogenic bacteria, including Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella Typhimurium* ATCC 13311, *Enterobacter cloacae* ATCC 35030, *Pseudomonas aeruginosa* ATCC 9027, *Yersinia enterocolitica* ATCC 8610) and Gram-positive (*Staphylococcus aureus*atcc 11632, *Bacillus cereus* clinical isolate, and *Listeria monocytogenes* NCTC 7973), using the minimum inhibitory concentration (MIC) method through a

colorimetric assay in microplates. The extracts were dissolved in 5 % DMSO and diluted in TSB to obtain concentrations ranging from 10 to 0.03125 mg/mL. The inoculum was standardized to 1.5×10^5 CFU/mL, and the plates were incubated at 37 °C for 24 h. MIC was determined by the absence of color change after the addition of INT, while the minimum bactericidal concentration (MBC) was assessed by plating on blood agar and incubating for 24 h at 37 °C. The same methodology was applied for the films using the disk diffusion method on Mueller-Hinton Agar. Positive and negative controls were included in all assays (de Oliveira et al., 2024a).

For antifungal activity, *Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404), cultured on malt agar and incubated at 25 °C for 72 h. Spore suspensions were standardized to 1.0×10^5 spores/mL. Extract activity was tested using the microdilution method to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), expressed in mg/mL. The commercial fungicide ketoconazole was used as a positive control. The same methodology was applied to the films using the disk diffusion method on malt agar, followed by incubation at 26 °C for 72 h (de Oliveira et al., 2024b).

2.6. Persimmon samples

Persimmons (*Diospyros kaki* L. var. Giombo) for the application of edible coatings, were sourced from the São Manuel Experimental Farm of the São Paulo State University (UNESP), São Paulo, Brazil (22°46′09"S 48°34′18"W). They were harvested at commercial ripeness (visually by the uniformity of the orange-reddish color of the skin). Before the application of the edible coating, the persimmons were sanitized and dried at room temperature.

2.7. Production and application of edible coatings

2.7.1. Production of edible films

The edible films were produced based on the methodology described by Garcia, Osiro, Vanin, Yoshida, & de Carvalho (2022), using agar-agar (vegetable gelatin, Mercearia e Bomboniere Towa Ltda., São Paulo, Brazil, 23°33′17"S 46°38′06"W) as the polymer and glycerol (Synth, São Paulo, Brazil) as the plasticizer. Initially, 4 g of agar-agar (2 g/100 g of the filmogenic solution) were dissolved in boiling water (100 °C) on a heating plate (IKA, C-MAG HS 7), under mechanical stirring (IKA, RW 20) at 600 rpm for 10 min. Subsequently, 0.8 g of glycerol (0.4 g/100 g of the film-forming solution) was added as the plasticizer, followed by homogenization for an additional 2 min. After the addition of the plasticizer, the filmogenic solution was cooled to 40 °C. At this stage, phenolic extracts in dry powder of celery (5 g, 10 g, and 15 g/100 g of film-forming solution) and banana inflorescence (5 g/100 g of film-forming solution), previously obtained in the conditions optimized for the maximum recovery of bioactive phenolic compounds (optimal points, Fig. 1), were incorporated separately as detailed in section 3.1. After the addition of the phenolic extracts, the mixture was homogenized on a heating plate without heating at 600 rpm for 5 min to ensure the complete homogenization of the extracts in the film-forming solution. The films intended for subsequent analyses (without application to fruit) were dried in an oven at 30 °C for 12 h and then stored in a desiccator (NaBr solution, 58 % RH) to maintain their stability until further analysis. Meanwhile, the films intended for application were applied immediately after the 5-min homogenization of the film-forming solution and extract.

2.8. Application of edible films

The edible films were applied by spraying onto persimmons using different treatments: control (without the edible coating), agar-agar combined with banana inflorescence extract (5 g/100 g of film-forming solution), and agar-agar combined with celery extract at three concentrations (5 g, 10 g, and 15 g/100 g of film-forming solution). After application, the fruits were stored under two distinct conditions: ambient temperature (25 °C to 30 °C, with relative humidity of 45 % to 50 %) and refrigeration (8 °C to 10 °C, with relative humidity of 65 % to 70 %) during the analysis period, which corresponded to 10 consecutive days, analyzing all parameters at T0, T5 and T10. The temperature and

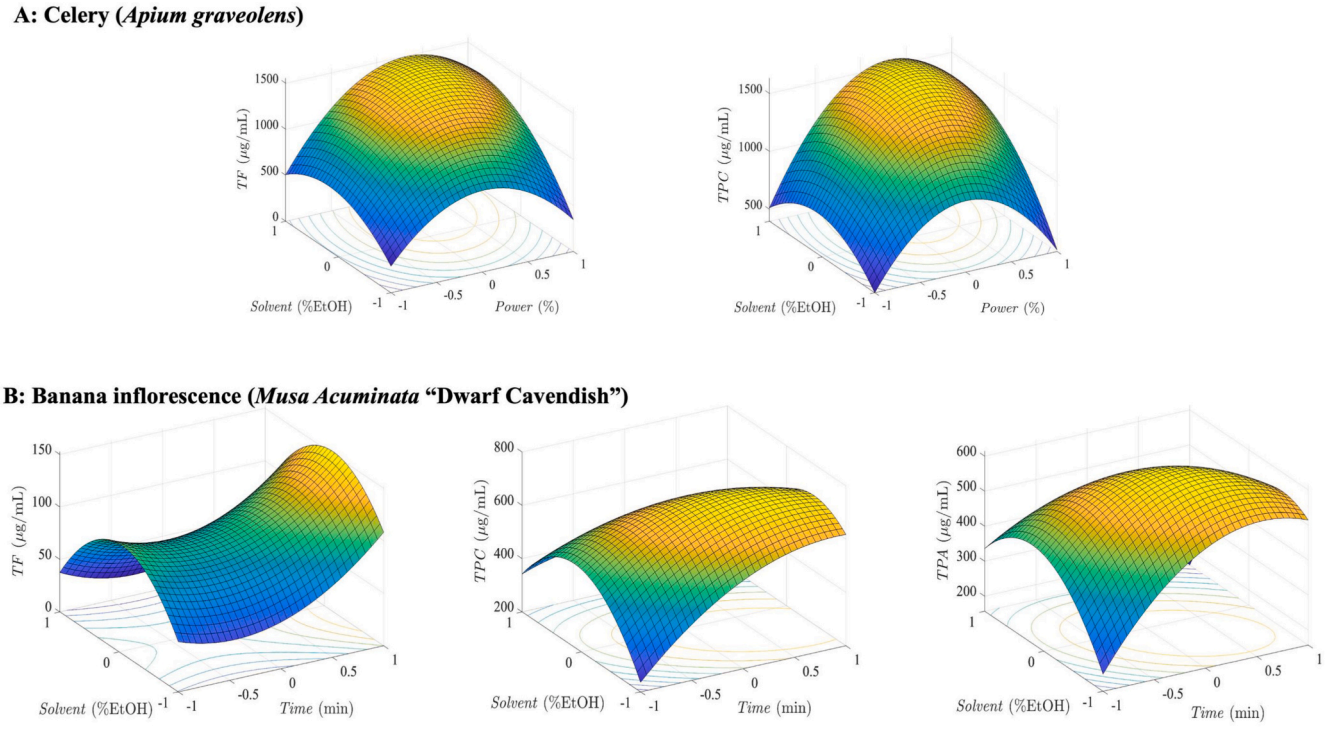

Fig. 1. Response surface graphs for the combined effects of the independent variables time, ultrasonic power and solvent ratio on the phenolic contents obtained from celery (A) and banana inflorescence (B).

relative humidity of the persimmons were monitored in two test environments throughout the analysis period using thermohygrometer.

2.9. Physicochemical analyses of fruits

To analyze color, a Konica Minolta Chroma Meter CR-400 (Chiyoda, Tokyo, Japan) was used, equipped with a D65 illuminant, in accordance with the International Commission on Illumination (CIE) standards. The instrument featured an 8 mm aperture and a 10° observation angle. The L* (lightness, representing the scale from dark to light); a* (chromaticity ranging from green (−) to red (+)); b* (chromaticity ranging from blue (−) to yellow (+)); and c* (color saturation, where values closer to 0 indicate less intensity and values near 60 indicate higher intensity) coordinates were used. The total color difference (ΔE) between each sample and the control was calculated using the formula proposed by Pathare, Opara, & Al-Said (2013):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

The pH of the samples was determined using a potentiometer integrated into a titrator (Hanna Instruments HI 902, Rhode Island, EUA). Prior to measurement, the samples were homogenized in distilled water, and the electrode was then immersed to obtain accurate readings. The device was calibrated with standard buffer solutions at pH 4.01 and 7.00 to guarantee precise and reliable measurements.

The total acidity was assessed based on the procedure described by Zanebon & Pascuer (2005). A 10 mL aliquot of the sample was diluted in 100 mL of distilled water, and its pH was measured. The solution was then titrated with 0.1 M sodium hydroxide until the pH reached a value between 8.2 and 8.4. The acidity was calculated using the formula:

$$\text{Acidity (molar solution per 100 mL)} = AV \times f \times M \times 100$$

where:

- VV = Volume of 0.1 M sodium hydroxide used (in mL),
- ff = Correction factor for the 0.1 M sodium hydroxide solution,
- MM = Molarity of the sodium hydroxide solution (0.1 M),
- AA = Sample mass (in mL).

The determination of total solids was carried out following the methodology described by Zanebon & Pascuer (2005). A small portion of the homogenized sample was placed directly into the device. The analysis was performed using a Digital Brix Refractometer (Handheld Electronic Saccharimete, Arizona, EUA), which provided the °Brix value corresponding to the total solids content.

2.10. Bioactive analyses of the fruits and films

For the analyses of total phenolics, antioxidant activity (FRAP and DPPH), and total carotenoids, samples (5 g), obtained from three persimmon fruits per treatment, were homogenized and subjected to ultrasound-assisted extraction (sonication power, in %, 30 % = 150 W, ultrasonic system - CY-500, Optic Ivymen System, BCN, Spain) in 15 mL of 70 % ethanol solution (solvent proportion, in % ethanol, *v/v*) for 30 min (extraction time, in minutes) at 30 °C. The resulting filtrate was centrifuged at 10,000 ×*g* for 15 min at 4 °C (ThermoFisher scientific, Zweigniederlassung Osterode Hanz, Germany) and subsequently filtered using Whatman n° 4 filter paper (chmlab group, Barcelona, Spain) for subsequent analyses (Du et al., 2009; Pu et al., 2013). The methods for the analysis of total phenolics, antioxidant activity to DPPH, as well as antibacterial and antifungal activity have been described above (section 2.5).

The FRAP (Ferric Reducing Antioxidant Power) assay was carried out based on a modified method by (Benzie & Strain, 1996), using freshly prepared reagents. The FRAP reagent was composed of acetate buffer, TPZ solution, and ferric chloride in a 10:1:1 ratio. For the assay, 100 μL of extract, 3 mL of FRAP reagent, and 300 μL of water were mixed and incubated at 37 °C for 30 min. Absorbance was measured at 593 nm. Antioxidant activity was determined by the reduction of Fe^{3+} to Fe^{2+}, indicated by the increase in absorbance. A 1000 μM Trolox solution was used to generate the standard curve, which was expressed as $\mathbf{y} = 0.0458\times$ - 0.1446 ($R^2 = 0.999$).

Total carotenoids were extracted in 15 mL of diethyl ether and measured at 445 nm, as described by Rodriguez-Amaya (2001).

2.11. Statistical analysis

The mean and standard deviation were used to express all the data in the study. Using SPSS Software, version 25, a two-way ANOVA was performed using type III sums of squares. The two variables, storage time (ST) and preservative type (PT), were treated as independent variables in this multivariate general linear model. This allows the effects of each variable to be examined separately and offers more insight into how each element contributed to the result. When available, the estimated marginal means (EMM) plots were used to draw some general conclusions and tendencies if a significant interaction ($p > 0.05$) between the two components was observed. If a significant interaction was not found ($p < 0.05$), each component was then categorised independently using Tamhane's T2 test for non-homoscedastic samples, and a Tukey's multiple comparison test for homoscedastic means. Levene's test was used to assess homoscedasticity. The linear discriminant analysis (LDA), it was also done using the above-described statistical software, with a Mahalanobis and stepwise methods using an F-value of 3.84 for entry and 2.71 for removal with a leave-one-out classification, allowing to visualize the co-variance within groups.

3. Results and discussion

The results demonstrated that edible coatings enriched with celery and banana inflorescence extracts influenced persimmon preservation differently. Celery coatings exhibited superior effects in maintaining lightness (L) and reducing carotenoid degradation, while banana inflorescence-based coatings showed moderate antioxidant stability. Throughout storage, phenolic content degradation was delayed in coated samples, suggesting enhanced bioactive retention compared to the control. The following sections provide a detailed discussion of these findings.

3.1. Optimization of the extraction of bioactive compounds

3.1.1. Analysis of experimental results from CCCD

The experimental results obtained from the 17-run central composite design (CCD) for the optimization of ultrasound-assisted extraction (UAE) of bioactive compounds from celery and banana inflorescence are presented in **Table 6** (supplementary material). **Table 6,** experimental results obtained under the extraction conditions defined in the central composite design (CCD) matrix for the ultrasound-assisted extraction of celery and banana inflorescence, expressed in μg/mL. The natural values of the independent variables X1 (time), X2 (power) and X3 (solvent proportion) are presented in **Table 5** (supplementary material).

In the celery extracts, the luteolin derivative (Y1) concentration varied from 45.63 μg/mL (run 5) to 273.11 μg/mL (run 9). The highest yields of chrysoeriol derivatives (Y2, 309.61 μg/mL) and apigenin derivatives (Y3, 1248.71 μg/mL) were observed in runs 10 and 16, respectively, both of which used moderate to high extraction times and power with balanced solvent proportions. The total flavonoids (TF, Y4) and total phenolic compounds (TPC, Y5) in celery extracts reached maximum values of 1616.32 μg/mL and 1701.01 μg/mL, respectively, under balanced extraction conditions (run 16).

In the banana inflorescence extracts, the concentration of *p*-coumaroyl-acetyl-sucrose (Y1) ranged from 17.46 μg/mL (run 8: high time and power with high solvent proportion) to 56.93 μg/mL (run 11). The

highest concentrations of rutin (Y3, 185.37 μg/mL) and *p*-coumaroyl-tetra-acetyl-sucrose isomers (Y4, 267.94 μg/mL) were achieved in run 16, which used balanced extraction parameters. Total flavonoids (TF, Y5) peaked at 107.76 μg/mL in run 7 (moderate extraction time and solvent proportion with high power), while total phenolic acids (TPA, Y6) reached 562.28 μg/mL in run 12. The total phenolic compounds (TPC, Y7) content varied from 214.1 μg/mL (run 2) to 650.13 μg/mL (run 17), with balanced extraction conditions consistently yielding higher values.

Extreme solvent proportions, whether too high or too low, negatively influenced the extraction of bioactive compounds in both matrices, likely due to inadequate solubilization or compound degradation. Moderate extraction conditions optimized the recovery of bioactive compounds across the response variables in most of the cases.

3.1.2. Analysis of theoretical response surface models

In this study, second-order polynomial models were developed to predict the effects of independent variables on bioactive compounds extracted from celery and banana inflorescence. The experimental response values were fitted to the general polynomial model shown in Eq. (1), and specific mathematical models were derived for each response. Only statistically significant coefficients at the 95 % confidence level ($\alpha = 0.05$) were retained in the final models to represent the relative influence of each independent variable on the responses. Non-significant terms were excluded to simplify the equations and enhance predictive accuracy.

The regression analysis, analysis of variance (ANOVA), and optimized extraction conditions are summarized in Table 7 (Supplementary Material). Response surfaces illustrating the effect of the extraction variables on the recovery of the main phenolic classes in celery and banana inflorescence are shown in Fig. 1. The models demonstrated excellent fits, with high coefficients of determination ($R^2 > 0.92$ for most responses), indicating that a significant proportion of the response variability was explained by the models. The high adjusted R^2 values further validated the models' robustness. Additionally, the *p*-values for all models were statistically significant ($p < 0.05$), confirming that the independent variables and their interactions had meaningful effects on extraction efficiency.

The coefficients in the derived mathematical models describe the effects of independent variables and their interactions on each response variable. Larger parametric values (regardless of sign) indicate a stronger influence of the respective variable, while for interactions, a positive sign represents a synergistic effect, and a negative sign indicates antagonism between variables.

For celery, the solvent ratio (X3) was the most influential factor for the extraction of flavonoids and total phenolics, with a strong positive linear effect as indicated by the high magnitude of the linear coefficient (b_3). This result suggests that increasing the solvent proportion enhances the extraction of these compounds. However, the quadratic term of the solvent ratio (b_{33}) was negative, indicating a decline in yields beyond optimal levels, likely due to dilution effects in excessive solvent volumes. For phenolic acids (TPA), ultrasonic power (X2) had a significant positive linear effect, while its quadratic term was negative, emphasizing the importance of moderate ultrasonic power to maximize yields without degrading sensitive compounds.

In banana inflorescence, time (X1) had the most pronounced effect on total phenolic content, with a strong positive linear influence, underscoring the role of prolonged extraction. However, the quadratic term for time (b_{11}) was negative, reflecting potential compound degradation at excessively long durations. For phenolic acids, ultrasonic power (X2) exhibited a positive linear effect, but its quadratic term (b_{22}) was negative, highlighting the need for careful control of sonication intensity to avoid instability. For flavonoids, the solvent ratio (X3) played a critical role, showing a positive linear effect alongside significant negative quadratic interactions with time (b_{13}), illustrating the complex balance required between these factors for optimal recovery.

The optimal extraction conditions for TPC, TPA, and TF were derived from the response surface models and are presented in Supplementary Material (**Table 7**). For celery, the highest TPC yield was achieved with 82.47 min extraction time, 56.93 W ultrasonic power, and a solvent ratio of 74.32 %. Similarly, the optimal TF yield occurred under slightly longer extraction time (90 min) with 57.48 W ultrasonic power and 76.99 % solvent ratio. For TPA, moderate ultrasonic power (68.7 W) and 90 min of extraction with a balanced solvent ratio (67 %) maximized yields.

For the banana inflorescence, the maximum TPC and TF were observed at high ultrasonic power (80 W), moderate extraction time (61.78–90 min), and solvent ratios between 36.79 % and 42.84 %. For TPA, a similar trend was observed, with optimal conditions requiring 80 W ultrasonic power and a solvent ratio of 34.52 %, highlighting the consistency in factors influencing phenolic acid recovery.

3.1.3. Assessment of phenolic content and bioactivity of the optimized extracts

The phenolic contents and antioxidant activities of the (celery) and banana inflorescence extracts obtained under the optimal extraction conditions are shown in Table 1. For celery, the values of total phenolics (TPC) and total flavonoids (TF) were remarkably high, reaching approximately 3181 μg/mL and 3092 μg/mL, respectively. The evaluation of antioxidant activity using the DPPH method demonstrated notable performance, with EC_{50} values around 8.6 μg/mL. The TPC and TF values for the extract of banana inflorescence were lower compared to celery, yet still relevant, with maximum values of 1595.6 μg/mL and 668.2 μg/mL, respectively. The antioxidant activity, measured by DPPH, was also weaker in banana inflorescence (14.2 μg/mL) than in celery, suggesting that the bioactive compounds present in this latter extract possess a greater ability to neutralize free radicals. The lower EC_{50} values obtained for celery coatings indicate a stronger antioxidant activity, which may have contributed to delaying oxidative degradation of the persimmon during storage. This suggests that the phenolic compounds present in celery extracts played a key role in maintaining fruit stability. This difference could also be attributed to the higher concentration of phenolic compounds in the celery extract, as observed in the total phenolic content analysis. Statistically, the *t*-tests and ANOVA confirmed significant differences between the values obtained, with *p*-values less than 0.05 for almost all analyzed parameters. This reinforces that the optimal extraction conditions maximize the yield and antioxidant efficacy of the phenolic and flavonoid compounds present in the extracts.

The **Table 8** (Supplementary Material) shows that the prepared extracts from banana inflorescence and celery exhibited promising antimicrobial activity, particularly against *Yersinia enterocolitica* (MIC of 2.5 mg/mL and MBC of 5 mg/mL). Additionally, they exhibited a bacteriostatic effect against *E. coli*, *Enterobacter cloacae*, *P. aeruginosa*, and *S. enterocolitica* (MIC of 10 mg/mL). Nevertheless, their efficacy was inferior to that of the synthetic antibiotics that were evaluated. Furthermore, both extracts exhibited minimal anti-inflammatory and antifungal activity under the tested conditions, were non-toxic to non-tumor cells (PLP2) at concentrations exceeding 400 μg/mL and exhibited no cytotoxic activity against tumor cells.

3.2. Evaluation of the edible films

3.2.1. Antibacterial activity

The results of the disk diffusion tests showed that the Agar+Celery films exhibited concentration-dependent antimicrobial activity, being more effective against Gram-positive bacteria and *Escherichia coli* (Gram-negative). Notably, *Bacillus cereus* showed increasing inhibition zones with higher celery concentrations, reaching 2.3 mm at 15 g of dried celery extract podwer *Listeria monocytogenes* was inhibited only at the highest concentration (1.1 mm), while *Staphylococcus aureus* demonstrated a progressive increase in activity (0.8–1.3 mm).

Table 1
Phenolic content and antioxidant activities of the extracts obtained at optimal points.

	TPA	TF	TPC	TBARS EC_{50} (μg/mL)	DPPH EC_{50} (μg/mL)	FRAP (μg/mL)	Total Phenolics (Gallic acid Eq/μg sample)
Apium graveolens	89.1 ± 0.2	3090.9 ± 1.4	3180 ± 1	4523 ± 301	1081 ± 106	5338 ± 383	8.6 ± 0.1
Banana Inflorescence	933 ± 1	663 ± 5	1596 ± 5	671 ± 26	725 ± 124	1374 ± 750	14.5 ± 0.3
t-statistic	−24.6	−5.71	−5.51	8.12	−5.91	−10.82	−3.91
p-value (t-test)	< 0.0001	< 0.0001	< 0.0001	0.0012	< 0.0001	< 0.0001	0.0007
ANOVA F-statistic (one-way)	452.46	32.68	30.27	12.58	34.14	117.89	12.73
ANOVA *p*-value	< 0.0001	0.0015	0.0019	0.0153	0.0011	< 0.0001	0.0148

Among the gram-negative bacteria, *E. coli* showed the highest inhibition (2.0 mm) with 15 g dried of celery, while *Enterobacter cloacae* and *Salmonella enterocolitica* exhibited moderate inhibition. *Pseudomonas aeruginosa* and *Yersinia enterocolitica* were resistant to the celery-based films. The Agar+Banana films demonstrated more limited activity but were still effective against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus*, with inhibition zones ranging from 1.2 to 1.8 mm (Table 2).

The microbiological results from the present study confirm the effectiveness of incorporating celery and banana inflorescence extracts into agar-based films for antimicrobial activity. Based on the polyphenol content classification proposed by Vasco et al. (2008), both extracts exhibit moderate to high levels of phenolic compounds, a characteristic often linked to antimicrobial properties. Thus, Tajkarimi et al. (2010), noted that phenolic compounds derived from plant byproducts are crucial in creating barriers to prevent pathogenic microorganism proliferation.

The agar+celery films demonstrated superior inhibitory effects against Gram-positive bacteria, with *Bacillus cereus* showing the largest inhibition zone, while Gram-negative bacteria like *Escherichia coli* were moderately affected. These results echo the findings of Verdeguer et al. (2020), who reported significant antimicrobial activity from natural extracts (Essential Oils to *Satureja montana* and *Mentha longifolia*) against post-harvest fungi and bacteria. It is also relevant to note that the antimicrobial effects in the present study were concentration-dependent, a factor also observed in the studies of Ahmed et al. (2022), who emphasized the need for optimal concentrations of active agents in edible coatings for effective microbial control.

The minimal inhibitory concentrations (MICs) of celery and banana inflorescence extracts, although higher than synthetic antibiotics, underscore their potential as sustainable alternatives. This aligns with the observations of Moradi et al. (2021), who emphasized the importance of MIC evaluations in designing effective antimicrobial films. The limited antifungal activity against *Aspergillus* strains found in the study is consistent with the findings of Aloui and Khwaldia (2016), suggesting that while the extracts are more effective against bacteria, further optimization may enhance their antifungal properties.

The combination of these extracts with agar resulted in films that not only provided a physical barrier but also exhibited functional properties, reducing microbial proliferation and contributing to food preservation. As Ahmed et al. (2022) and Ribeiro et al. (2021) suggested, such edible coatings act as multifunctional layers, combining barrier properties with the active release of antimicrobial agents. This dual function is critical for prolonging the shelf life of perishable fruits, as demonstrated by the extracts' ability to extend the shelf life of fruits at room temperature and under refrigeration.

3.2.2. Evaluation of the coating effects on persimmons

For the determination of the physical-chemical profile, as stated in the Statistical analysis section, a 2-way analysis of variance (ANOVA) was applied, allowing for an individual assessment of each parameter, and at the same time the assessment of the interaction among them. In this way, Tables 3 and 4 are divided in four sections, corresponding the two bigger ones to the temperatures of storage, namely room and fresh temperature. The subsections between these two correspond to the 2-way ANOVA, being the upper part for the storage time (ST), and the lower part the preservative type (PT) used in the coating. For each tested ST, all tested PTs are included, and for each tested PT, all STs are included. This representation allows for the aforementioned individual assessment of each parameter, which means that the standard deviation should not be regarded as the accuracy of the analysis, but rather a range of values for the non-fixed parameter (PT and ST). If between these parameters a significant interaction is detected, by presenting a *p*-value of PT × ST lower than 0.05, no multiple comparisons can be extracted, which means that both parameters significantly contributed for the changes, only allowing tendencies to be obtained from the Estimated Marginal Means (EMM) plots. Conversely, if the *p*-value of CT × ST is higher than 0.05, each parameter is analyzed and classified individually.

Table 3 shows the color coordinates, (L*, a* and b*), the total color (ΔE), the pH and acidity of the films, both at room and fresh temperatures. It can be seen that there was a significant interaction between ST and PT (*p*-value <0.05), hindering any individual classifications.

Table 2
Antibacterial activity of agar-based films by disk diffusion method.

Bacteria	Agar	Agar+Banana (5 g)	Agar+Celery (5 g)	Agar+Celery (10 g)	Agar+Celery (15 g)
	MIC (mm)				
Gram-negative bacteria					
Enterobacter cloacae	-	1.2	-	1.1	1.4
Escherichia coli	-	1.8	1.7	1.8	2
Pseudomonas aeruginosa	-	1.2	0	-	-
Salmonella enterocolitica	-	1.2	1	1.1	1.2
Yersinia enterocolitica	-	-	-	-	-
Gram-positive bacteria					
Bacillus cereus	-	1.2	1.3	1.8	2.3
Listeria monocytogenes	-	-	-	-	1.1
Staphylococcus aureus	-	1.2	0.8	1.1	1.3

MIC- minimal inhibitory concentration (mm).

Table 3
Color coordinates, total color, pH and acidity index of the persimmon stored in fresh conditions and room temperature.

		L*	a*	b*	pH	Acidity
Fresh Temperature						
Storage Time (ST)	T1	59 ± 4	21 ± 8	51 ± 6	6.47 ± 0.04	0.17 ± 0.04
	T5	57 ± 6	18 ± 8	47 ± 10	6.2 ± 0.8	0.3 ± 0.1
	T10	54 ± 7	22 ± 9	41 ± 13	6.71 ± 0.06	0.36 ± 0.02
p-value ($n = 18$)	Post-hoc Test	<0.001	0.222	<0.001	<0.001	<0.001
Preservative Type (PT)	Control	46 ± 5	26 ± 6	33 ± 9	6 ± 1	0.30 ± 0.06
	Agar	57 ± 3	29 ± 6	39 ± 9	6.54 ± 0.9	0.30 ± 0.08
	Agar+Banana	55 ± 5	18 ± 4	42 ± 8	6.5 ± 0.1	0.25 ± 0.09
	Agar+Celery (5 g)	61 ± 2	17 ± 6	54 ± 2	6.60 ± 0.07	0.29 ± 0.11
	Agar+Celery (10 g)	60 ± 1	13 ± 3	55 ± 2	6.62 ± 0.16	0.3 ± 0.1
	Agar+Celery (15 g)	61 ± 4	22 ± 11	55 ± 6	6.6 ± 0.1	0.2 ± 0.1
p-value ($n = 36$)	Post-hoc Test	<0.001	0.002	<0.001	<0.001	0.001
ST×PT ($n = 108$)	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001
Room Temperature						
Storage Time (ST)	T1	60 ± 5	20 ± 7	49 ± 8	6.46 ± 0.08	0.19 ± 0.04
	T5	55 ± 9	22 ± 5	35 ± 8	6.64 ± 0.07	0.32 ± 0.05
	T10	54 ± 4	19 ± 4	34 ± 8	6.60 ± 0.09	0.32 ± 0.04
p-value (n = 18)	Students t-Test	<0.001	<0.001	<0.001	<0.001	<0.001
Preservative Type (PT)	Control	52 ± 5	25 ± 4	31 ± 6	6.6 ± 0.1	0.32 ± 0.07
	Agar	54 ± 12	24 ± 4	36 ± 7	6.64 ± 0.03	0.31 ± 0.06
	Agar+Banana	59 ± 3	23 ± 3	46 ± 9	6.5 ± 0.1	0.28 ± 0.05
	Agar+Celery (5 g)	62 ± 1	15 ± 6	44 ± 8	6.51 ± 0.05	0.25 ± 0.07
	Agar+Celery (10 g)	56 ± 6	17 ± 4	41 ± 12	6.6 ± 0.1	0.2 ± 0.1
	Agar+Celery (15 g)	57 ± 5	18 ± 3	39 ± 12	6.56 ± 0.09	0.26 ± 0.06
p-value (n = 36)	TT/TT2T	<0.001	<0.001	0.001	<0.001	<0.001
ST×PT (n = 108)	*p*-value	0.007	0.001	<0.001	0.003	<0.001

T1(time 1) to T10 (time ten) refer to the days of storage.

Table 4
Color coordinates, total color, pH and acidity index of the persimmon stored in fresh conditions and room temperature.

		Total Solids	Total Phenols	DPPH	FRAP	Total Carotenoids
Fresh Temperature						
Storage Time (ST)	T1	22 ± 2	0.4 ± 0.1	94 ± 1	551 ± 195	7 ± 4
	T5	20 ± 1	0.2 ± 0.0	70 ± 18	143 ± 69	8 ± 3
	T10	21 ± 1	0.2 ± 0.0	87 ± 3	199 ± 25	4 ± 2
p-value ($n = 18$)	Students *t*-Test	<0.001	<0.001	<0.001	<0.001	<0.001
Preservative Type (PT)	Control	22 ± 1	0.22 ± 0.10	91 ± 3	230 ± 129	7 ± 2
	Agar	21 ± 2	0.24 ± 0.09	82 ± 15	240 ± 168	4.5 ± 0.2
	Agar+Banana	20 ± 1	0.3 ± 0.2	74 ± 26	238 ± 105	2.9 ± 0.8
	Agar+Celery (5 g)	22 ± 1	0.3 ± 0.2	89 ± 2	296 ± 219	6 ± 3
	Agar+Celery (10 g)	20 ± 1	0.3 ± 0.2	80 ± 12	400 ± 310	9 ± 5
	Agar+Celery (15 g)	21 ± 3	0.3 ± 0.2	85 ± 8	382 ± 257	8 ± 4
p-value ($n = 36$)	Tukey's test	<0.001	<0.001	<0.001	<0.001	<0.001
ST×PT (n = 108)	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001
Room Temperature						
Storage Time (ST)	T1	21 ± 1	3 ± 1	94.5 ± 0.6	336 ± 206	6 ± 2
	T5	21 ± 1	2 ± 1	84 ± 6	218 ± 226	6 ± 3
	T10	22 ± 3	0.9 ± 0.0	91 ± 1	197 ± 24	7 ± 1
p-value (n = 18)	Students t-Test	<0.001	<0.0001	<0.001	<0.001	<0.001
Preservative Type (PT)	Control	24 ± 3	0.7 ± 0.4	87 ± 7	133 ± 29	6.9 ± 0.1
	Agar	21 ± 2	2 ± 2	91 ± 3	168 ± 63	7.12 ± 0.07
	Agar+Banana	22 ± 1	2 ± 2	91 ± 3	241 ± 103	7.1 ± 0.3
	Agar+Celery (5 g)	20 ± 1	1 ± 1	86 ± 8	234 ± 122	7.9 ± 0.9
	Agar+Celery (10 g)	21 ± 1	0.8 ± 0.4	91 ± 3	175 ± 12	4 ± 2
	Agar+Celery (15 g)	20 ± 2	3 ± 2	93 ± 2	551 ± 250	4 ± 2
p-value (n = 36)	Tukey's test	<0.001	<0.001	<0.001	<0.001	<0.001
ST×PT (n = 108)	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001

Considering a* (red-greenness) coordinates of the freshly stored samples, through the partial ETA squared (results not shown), it was possible to consider that for this color coordinate, the treatment showed a much bigger influence than the storage time. For the other parameters, some conclusions could be extracted from the EMM plots (Fig. 2). The slight variations in pH across treatments suggest that coatings did not

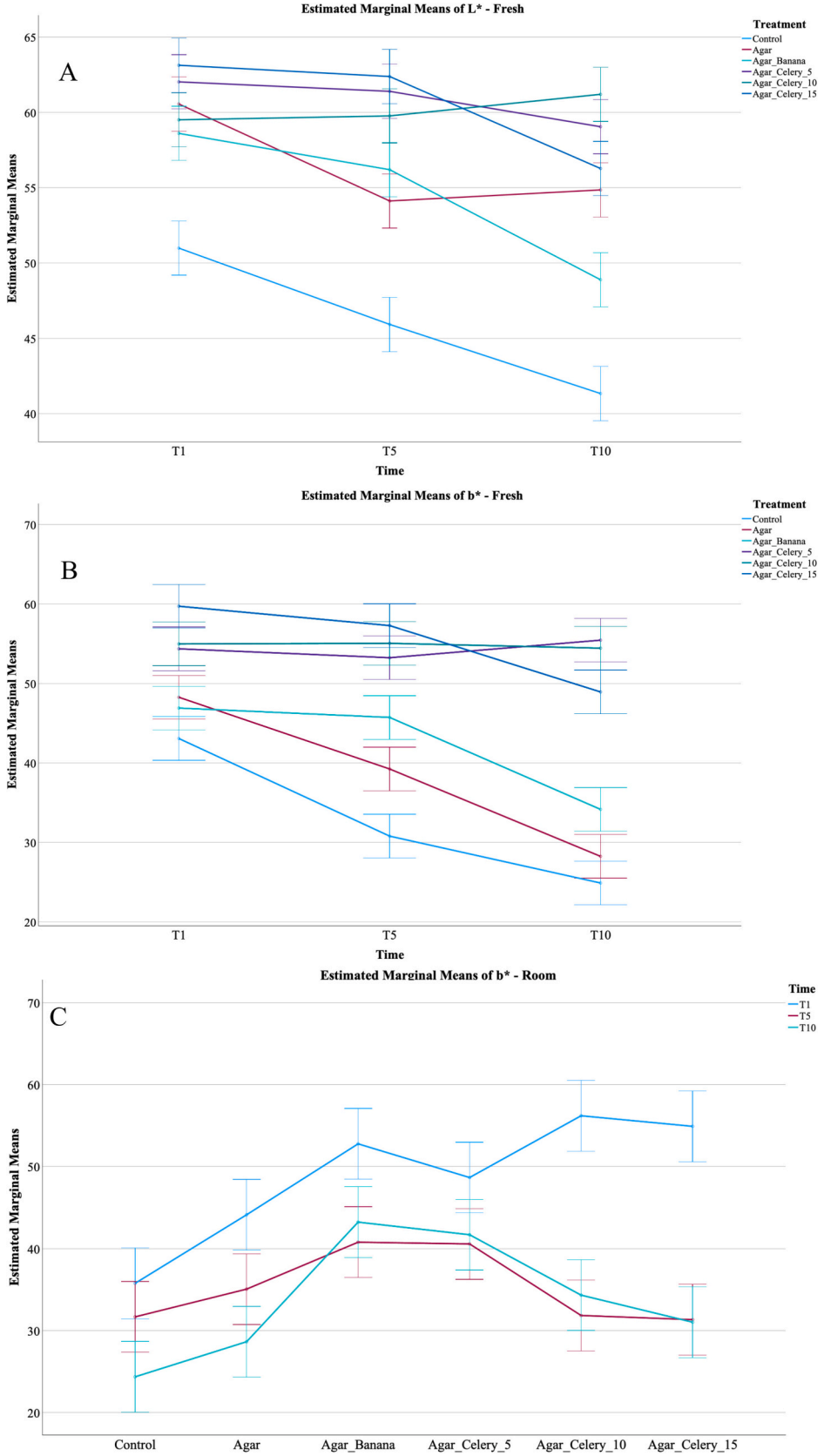

Fig. 2. Estimated marginal means plot for A - L* (lightness), B – b* (yellow-blueness) for fresh stored samples, and C – b* for room temperature samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significantly alter the natural acidity of persimmons. However, a more stable pH may contribute to preserving phenolic compounds, reducing their degradation during storage.

Several tendencies can be extracted from Fig. 2. For L*, (Fig. 2A), it can be seen that the control sample and banana darkened over time, while the samples with celery maintained their lightness. In terms of yellow-blueness (Fig. 2B), once again the control, banana and agar samples reduced the values, showing a tendency for the blue color, while the samples with celery maintained the color at T0. Finally, in Fig. 2C, corresponding to the room temperature samples, the yellow-blueness component decreased over time in all samples. The correlation between color retention and antioxidant activity suggests that coatings with higher phenolic content were more effective in minimizing oxidative degradation. Samples coated with celery extract showed higher L values, which align with their superior antioxidant properties, indicating a protective effect against enzymatic oxidation. In this case, the EMM plot does not show the passage of time from left to right, but rather from top to bottom. Thus, it is still possible to see that the samples coated with celery showed a more intense yellow (higher values) at T1 than at T10 and T15, while the samples with banana and celery at 5 g were the most yellow at days 5 and 10.

In Table 6, the antioxidant, bioactive properties and total solids of the persimmon are shown, namely the DPPH and FRAP values, total carotenoids and total phenols. A significant interaction was found for ST and PT, not allowing for classifications. Still, through the partial ETA squared it could be concluded that for the total carotenoids, the storage time showed higher influence in the reduction of these bioactives than the different incorporations, while for a* (blue greenness), the treatment showed greater influence.

Some general conclusions could also be extracted from the EMM plots, shown in Fig. 3. Regarding DPPH results it is clear that over time the samples with celery at 5 and 10 g increased the antioxidant activity from T1 to T5 (lower values reveal a lower concentration to inhibit 50 % of DPPH radicals). Overall, even at T10 the samples with these amounts of celery were the ones with the higher antioxidant activity, while all others were very similar to the control samples, which was, over the whole span of the experiment that with the least antioxidant activity.

Fig. 4, in the top section, shows the true color of the samples, converting the L*, a* and b* coordinates to RGB. This Figure follows the same principle as the 2-way ANOVA in Table 5 and 6, in each circle of the storage time, all preservative samples are included and, in each preservative type, all storage times are also included to represent the final. Over time, the freshly stored samples tended to darken, with a clear difference between T1 and T10. Considering each type of coating, the celery films showed the lightest tone, which was corroborated with the b* variation over time (EMM plot), while the control sample was the darkest, being the most similar that with only agar. Regarding the cold storage samples, the darkening from T1 to T5 was less pronounced, reducing the color loss from T5 to T10. As for the different films, the differences among samples were much more subtle, with only the celery at 5 g being the most differentiated in terms of lightness. The control and agar samples were once again the most similar. Storage temperature played a crucial role in coating performance. Refrigerated conditions helped preserve the bioactive compounds for longer periods, while samples stored at room temperature showed faster degradation. This highlights the importance of optimizing both coating formulation and storage conditions to maximize fruit preservation.

3.2.3. *Linear discriminant analysis*

A linear discriminant analysis (LDA) was performed for storage time and treatment (type of coating) for both room and freshly stored samples, as well as one including both storage temperatures. The aim was to ascertain whether the samples showed differences depending on the storage temperatures, as well as the possible effect of the treatments on the analyzed parameters.

Fig. 5 shows the LDA plots for freshly stored samples, where Fig. 5A refers to the classification according to the type of preservation treatment and Fig. 5B to the classification for time of storage. In Fig. 5A, the two functions (1 and 2) accounted for 83.1 % of the variance, with function one being responsible for 67.4 % and function 2 for 15.7 %. Overall, five functions accounted for 100 % of variance. Of the 12 variables (analyzed parameters), the three best predictors, with the highest discriminating ability were a*, L* and total carotenoids. In terms of clustering, the samples coated with celery are clearly separated from the other ones, indicating that celery protected samples showed higher differences in relation to the control sample. On the other hand, the agar+banana samples were the closest ones to the control,

Only two functions were necessary to account for 100 % of the variance for the classification of samples in Fig. 5B. The first function was responsible for 75.7 % and the second one for 24.3 %. The three best predictors were acidity, DPPH antioxidant activity and pH values. By analyzing the figure, a clear discrimination of the three storage times is visible, showing a much bigger difference between T1 and T5 (the function accounts for 75.7 % of variance), than between these two and T10.

Fig. 6 shows the LDA plots for the samples stored at room

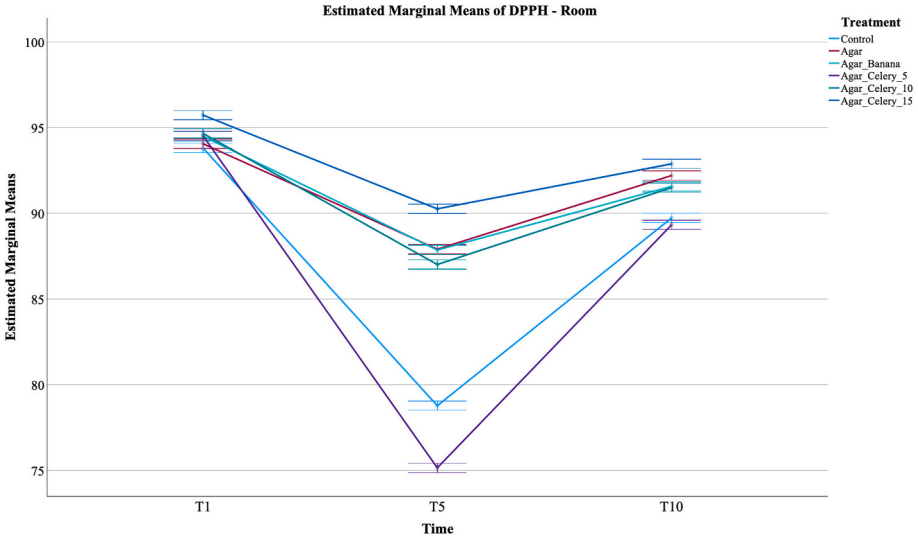

Fig. 3. Estimated marginal means plot for antioxidant activity assessed through the DPPH assay in room stored samples.

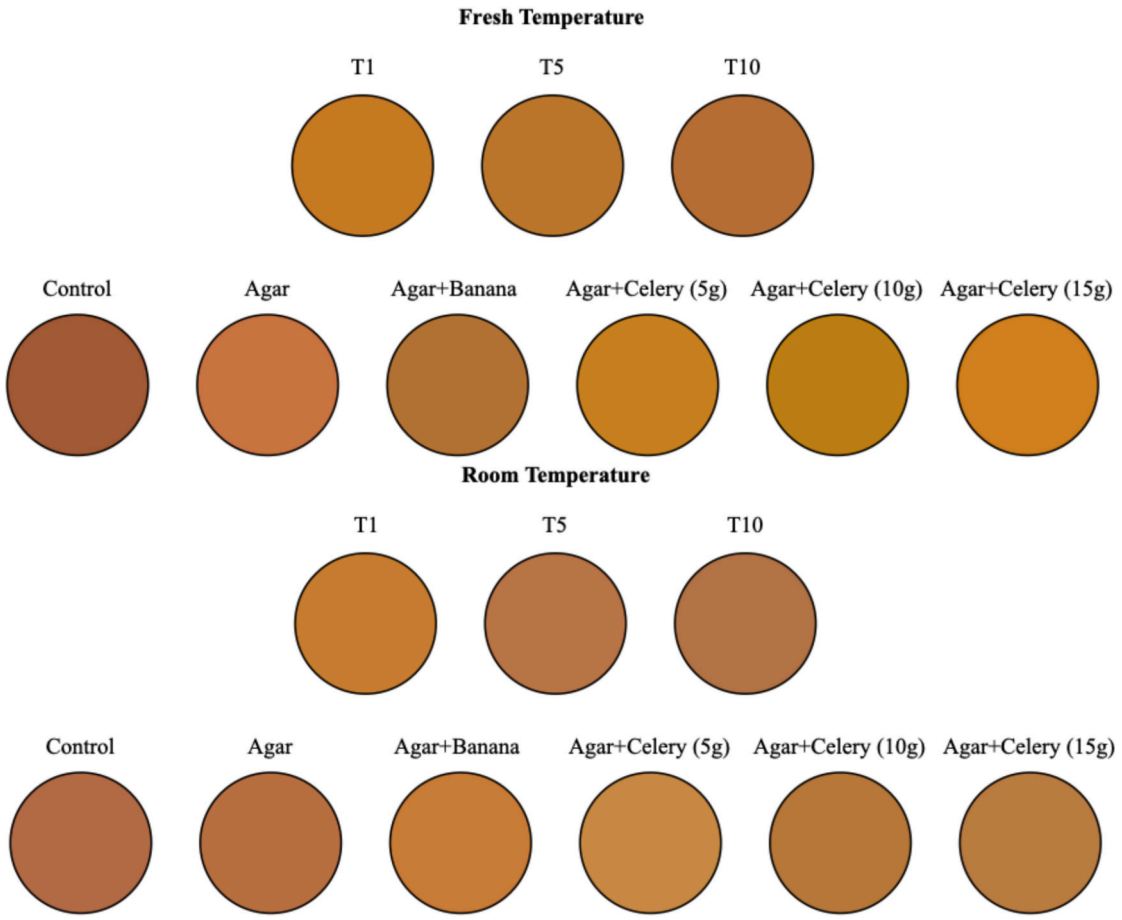

Fig. 4. Top section - conversion of L*, a* b* coordinates to RGB for interpretation of the difference among the persimmon for fresh and room temperature. In each storage time circle, all preservative colors are combined, and in each preservative circle, all storage times are included. Bottom section - ΔE (color differences between the coated samples and the control.

temperature. In Fig. 6A, the plot shows the samples discriminated by treatment. The model needed 5 functions to account for 100 % of the variance, in which function1 was responsible for 66.9 % and function 2 for 18.2 %. The best three predictors of the variance were b*, DPPH antioxidant activity and pH values. Comparing the predictors to the ones of the freshly stored samples, in the storage at room temperature only b* was the best discriminator, while for the fresh samples both a* and L* were the best. This reveals that samples stored at lower temperatures tend to better maintain the color. In Fig. 6B, showing the plot for the room temperature stored samples discriminated by storage time, two functions accounted for 100 % of variance. The first function was responsible for 77.1 % and the second for 22.9 %. In this case, the three best discriminators were DPPH, Total Phenols and pH values. Similar to the freshly stored samples, DPPH and pH values were also the best predictors. Analyzing the figures, the clusters in Fig. 6 are closer to the centroid group than in Fig. 5, revealing lower variance within each of the tree storage times. Nevertheless, as in Fig. 5 T1 and T5 are more distant from each other than T10.

Fig. 7 shows once again LDA plots, in this case for both storage times together, with A showing the discrimination by treatment and B by storage time. This plot allows to check the discrimination between the two temperatures for the same treatment and storage time, allowing for more consistent conclusions on the effect of the three variables (i.e., temperature, treatment and storage time). In Fig. 7A, 11 functions were necessary to describe 100 % of variance, in which function 1 was responsible for 95.3 % and function 2 for 2.3 %. In this case, function 1 was responsible for almost all the variation, being discrimination from left to right large than from top to down. The best three predictors were b*, Total Solids and Total carotenoids. Interestingly, the yellow-blue coordinate (b*) allowed to discriminate between the samples, meaning that the color is one of the best ways to tell apart each sample. Analyzing the plot in the Fig. 7A, the clustering of room and fresh samples is quite notorious. Although the vertical variance represented only about 2 % of the total, there was still a high difference between the control and agar+celery 15 g sample stored at room temperature, while agar, banana and celery 15 g were clustered together. Regarding samples stored in fresh, all samples were somewhat clustered, although the biggest difference was found between the control sample and celery 10. This is a clear indicator that temperature had an influence on all analyzed parameters.

Fig. 7B shows the discriminant plot according to the storage time for both samples stored at room and fresh temperatures. This model also needed only two functions to account for 100 % of the variance, in which the first function described 72 % and the second 27.5 %. The three best predictors were Total Phenols, DPPH values and Acidity. Interestingly, regarding the storage time none of the color coordinates had a high discriminant influence, indicating that color changes were prevalent in all samples, and that, when considering samples stored at fresh and room temperature together, the main discrimination was made according to phenolics and antioxidant activity, as well as acidity. The analysis of the results suggests that the antioxidant activity, evaluated through DPPH assay and total phenolics are related to the presence of natural extracts incorporated into the films, while the increase in acidity can be associated with acidification processes resulting from microbial growth, particularly in samples stored at room temperature, where these conditions would favor microorganism proliferation. These observations

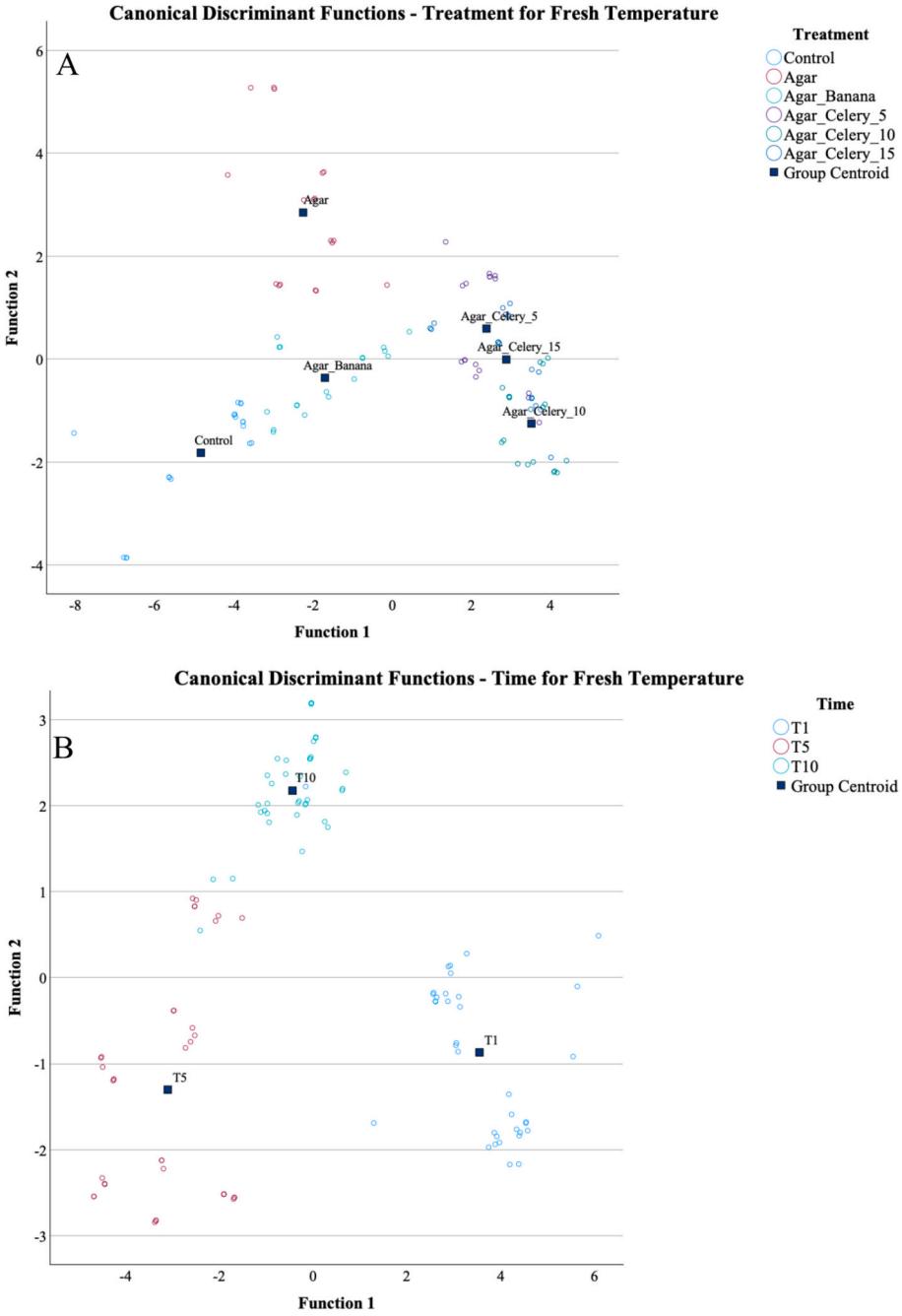

Fig. 5. LDA plots for freshly stored samples for a –type of coating and b – storage time.

highlight the importance of assessing not only the bioactive compounds but also the microbiological stability of the films during storage.

Extracts from plants are often added to packaging products and incorporated into films. The phenolic compounds found in plants, especially polyphenols and flavonoids, can work as antimicrobials and antioxidants. Active packaging containing phytochemicals prevents food contamination and decay without affecting foods and altering their nutritional value (Zhang et al., 2021). Thus, the selection of DPPH results, total phenolics, and acidity as discriminants demonstrates the influence of natural extracts and storage conditions on the analyzed parameters, but further microbiological confirmation is required to confirm the acidification as due to microbial growth (Siddiqui et al., 2023). Analyzing Fig. 7B, a clear separation of the three storage times is observed, which could be attributed to the high variability of the analyzed parameters. Overall, this plot, due to higher scattering does not allow for important conclusions, thus showing the importance of analyzing the samples individually in terms of storage temperature.

The films were applied to the fruits at the onset of ripening. The agar+banana and agar+celery treatments were able to extend the shelf life of fruits that were stored at ambient temperature by up to 15 days. However, the statistical analysis of these samples was restricted to 10 days due to the fact that the control and the treatment containing only agar deteriorated before this period. These refrigerated samples were therefore included solely as a reference to assess the effects of cold storage. These results underscore the importance of both the type of films and, primarily, temperature in extending the shelf life of fresh fruits.

In addition to temperature, the linear discriminant analysis confirmed that color was the most relevant and differentiating factor for monitoring the condition of the fruits during storage. The chromatic

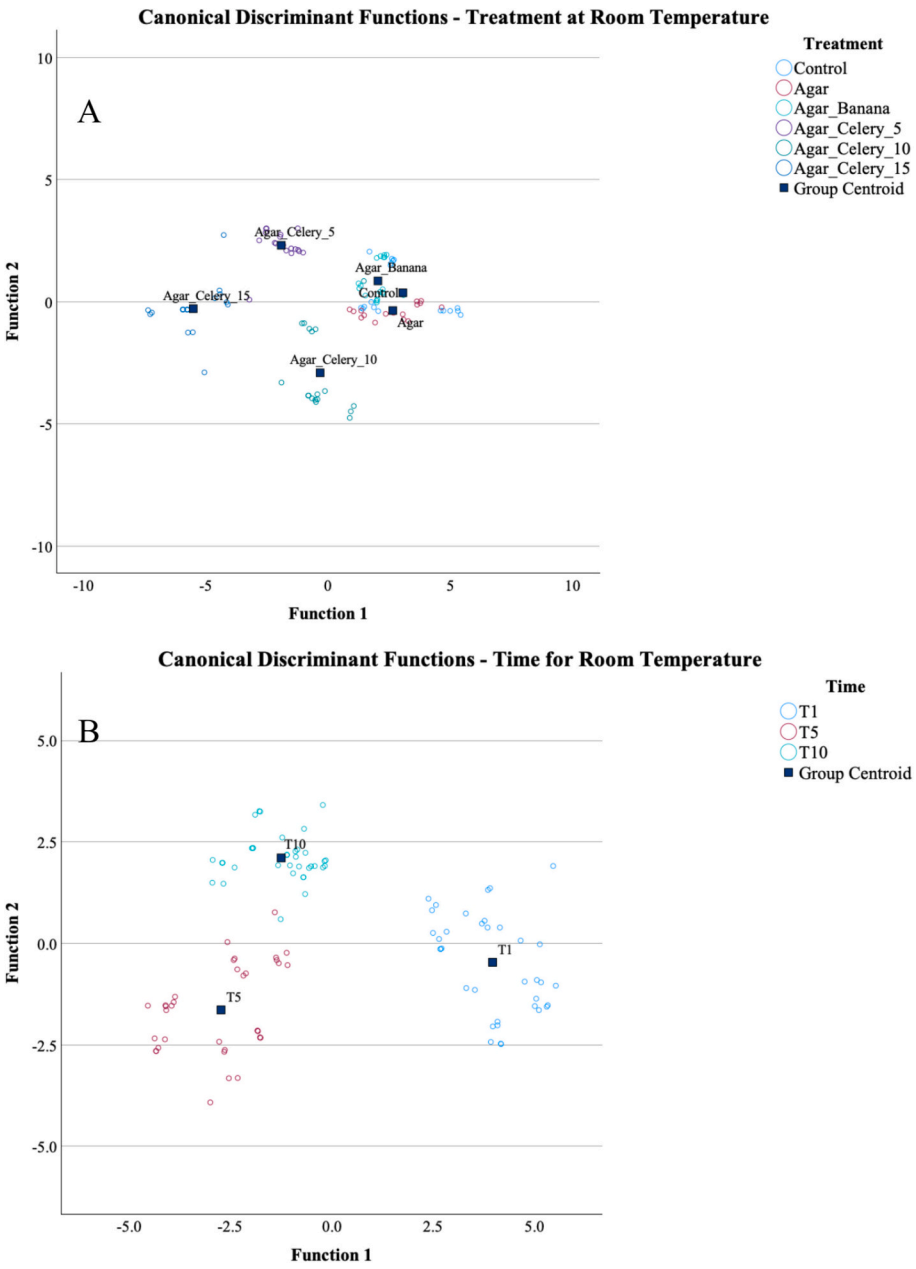

Fig. 6. LDA plots for room temperature stored samples for a –type of coating, and b – storage time.

coordinates b* (yellow-blue), L* (lightness), and a* (red-green) emerged as the main descriptors, demonstrating that visual changes were crucial for assessing treatments and identifying the effects of storage conditions.

Temperature had a significant impact on the evolution of the fruits during storage. For fruits kept at room temperature, progressive darkening and a marked reduction in yellow tones were observed, particularly in the control and *agar*-only treatments, which reached a shelf life of only 10 days. Conversely, the agar+celery and agar+banana treatments delayed these changes, extending the shelf life to up to 15 days. Nevertheless, these samples still exhibited significant degradation compared to fruits stored under refrigeration.

For fruits stored under refrigeration, the agar+celery treatments showed greater effectiveness in preserving lightness and yellow intensity, attributes often associated with consumer perception of freshness. The LDA confirmed that, under these conditions, variables such as b*, L*, and total carotenoids were determining for differentiating the samples. The agar+celery treatments exhibited greater visual stability, whereas the controls and fruits treated with lower concentrations of extracts showed more pronounced degradation.

The combined analysis of samples stored under refrigeration and at room temperature reinforced that the b* coordinate was the most consistent descriptor, indicating that color changes were the primary visual indicator for distinguishing the groups. In addition, functional variables such as antioxidant capacity (DPPH) and total phenol content emerged as important discriminators, particularly in samples treated with the plant extracts, reflecting the moderate contribution of these compounds to preserving bioactive properties.

The whole of these findings demonstrates that, as expected, temperature was the most critical factor in preserving the samples, directly influencing the maintenance of their sensory and bioactive properties. Color, in turn, stood out as the most affected and relevant characteristic for differentiating the treatments and storage conditions. These results highlight the importance of combined strategies, such as rigorous temperature control and the use of optimized formulations of films with

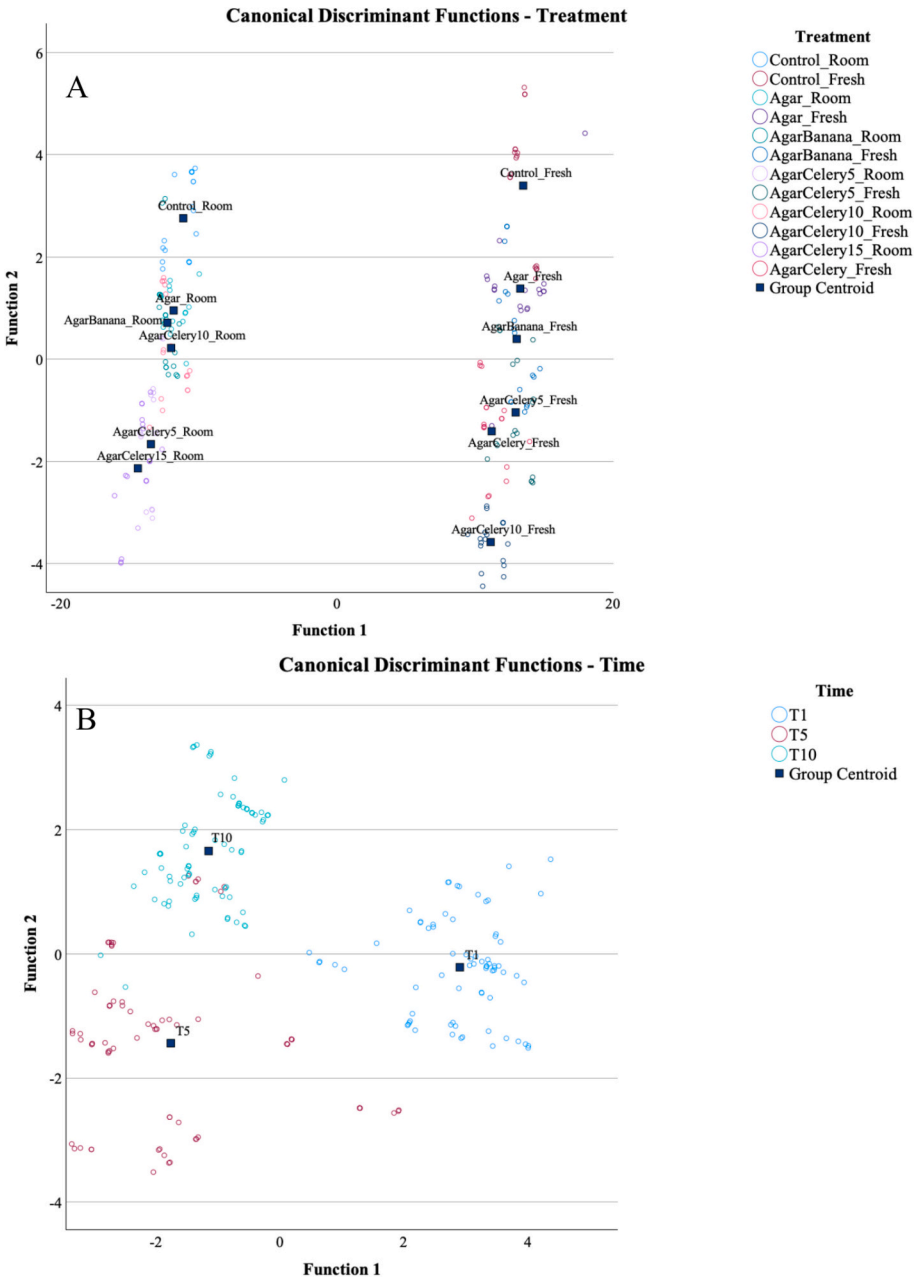

Fig. 7. LDA plots for both fresh and room temperature stored samples for a –type of coating, and B – storage time.

natural extracts, to maximize the visual and functional conservation of fresh fruits.

As in the present study, where color attributes (b* and L*) were identified as key discriminant descriptors, Mohammed et al. (2024) emphasized the relevance of yellow coloration for consumer acceptance of *Hilali* dates. The rapid darkening associated with climacteric ripening was mitigated by complementary conservation methods, such as coatings, highlighting color as a critical quality attribute. Moreover, studies such as that by Ogurlu, Kucuker, Aglar, & Kizgin Ozcengiz (2024) on pears demonstrated that modified atmosphere applications and compounds like putrescine effectively delayed color changes and ripening, further reinforcing the importance of visual control as a quality parameter.

The use of plant extracts in coatings has also been explored to protect against spoilage caused by fruit diseases. Thus, Verdeguer et al. (2020) showed that essential oils from *Satureja montana* inhibited up to 90 % of fungal development in tomatoes and persimmons. Similarly, Yang et al. (2024) utilized a neem essential oil emulsion in biodegradable films, reducing disease incidence in tomatoes by more than 70 %. These observations support that natural extracts can not only enhance antioxidant properties, but also act as antimicrobial barriers, as also pointed to in the present study.

The use of agro-industrial byproducts, such as banana inflorescence and celery, as coatings represents an innovative and underexplored approach to food preservation. Studies such as that by Jorge et al. (2023) demonstrated the potential of food residues, including orange and pumpkin peels, for creating sustainable films, while Moreno-Ricardo et al. (2024) utilized collagen extracted from fish residues to develop biodegradable films. In connection with other studies, the present work advances in the field by exploring celery and banana inflorescence extracts as components of edible coatings, contributing to innovation and sustainability in fresh fruit conservation. The obtained results demonstrated that color was an essential attribute for distinguishing treatments, and that refrigeration combined with coatings can be a suitable

strategy to extend the shelf life of fruits, mitigating sensory and functional changes.

4. Conclusions

The application of edible coatings enriched with celery and banana inflorescence extracts demonstrated a modest, but significant, impact on improving the preservation of persimmons, without meaningfully modifying the physicochemical and bioactive properties of the fruits. The celery-based coatings were particularly effective in maintaining lightness (L), with values of 61.0 ± 2.1 at day 10 under refrigeration, compared to 55.0 ± 3.4 for banana inflorescence coatings. Additionally, the total phenolic content degraded at a slower rate in coated samples, with a final concentration of 0.3 ± 0.2 mg GAE/g in refrigerated conditions, while antioxidant activity remained significantly higher (DPPH EC_{50} = 80 ± 12 μg/mL for celery coatings at day 10). The two-way ANOVA indicated that the interaction between storage time and coating type did not result in substantial variations, except for specific parameters such as color. Actually, among the key descriptors evaluated, color showed to be a key factor for distinguishing treatments. The maintenance of lightness (L*) was more evident in coatings containing celery extracts, particularly at concentrations of 10 g and 15 g, while coatings with banana inflorescence showed a lower capacity to preserve the initial color of the persimmons. Temperature played a central role in the degradation of color parameters and bioactive properties, with refrigeration conditions proving effective in mitigating losses. Edible coatings combined with refrigeration demonstrated greater efficiency in extending the shelf life of persimmons although only films containing extracts with antioxidant properties showed the ability to preserve the sensory characteristics of the fruits during the assayed 15 days of storage, highlighting the importance of these compounds in coating formulations. Linear Discriminant Analysis (LDA) confirmed that the main variance descriptors, such as lightness (L*), pH and antioxidant activity measured by the DPPH method, were consistent across treatments. However, none of the coatings provided robust preservation, as noticeable reductions in total solids and total phenolics were observed over time, regardless of the formulation used.

The antimicrobial properties of celery and banana inflorescence extracts, combined with their incorporation into agar-based films, provide an innovative approach to fruit preservation. These findings highlight the potential of agro-industrial byproducts as sustainable sources of bioactive compounds, in line with the growing demand for environmentally friendly food preservation strategies. The results also reinforce the importance of combining active packaging technologies with proper storage conditions, as noted in the broader literature, to maximize the shelf life and safety of fresh products.

In summary, the coatings that were tested did not substantially enhance the preservation of the physicochemical and bioactive properties of persimmons. However, they did not induce substantial adverse changes in these characteristics and contributed to the reduction of color degradation. These results can be used as a foundation for future optimizations, with the objective of improving the functionality of these coatings as sustainable alternatives for the food industry. In order to optimize the preservative potential of these materials, additional research is necessary, such as modifications to extract concentrations and polymer combinations. Future research should focus on the incorporation of additional stabilizers, modifications in film composition, and optimization of storage conditions to enhance both the preservation and safety of coated fruits. These advancements will be crucial for ensuring the viability of bioactive edible coatings for commercial applications in the food industry.

Author contribution

I. Oliveira: investigation, writing– original draft; T. Silveira: investigation; V. Garcia: Project administration, methodology, investigation; P. Santos: Project administration, methodology, investigation; G. Lima: Project administration, methodology, investigation; *L. lima*: investigation; C. Santos-Buelga: writing-review and editing, supervision; L. Barros: writing-review and editing, supervision, project administration; S. Heleno: project administration, writing-reviewing and editing, supervision, conceptualization; M. Carocho: conceptualization, writing original draft, writing-review and editing.

CRediT authorship contribution statement

Izamara de Oliveira: Writing – original draft, Investigation. **Tayse F.F. da Silveira:** Investigation. **Vitor Augusto dos Santos Garcia:** Project administration, Methodology, Investigation. **Pricila Veiga-Santos:** Project administration, Methodology, Investigation. **Giuseppina Pace Pereira Lima:** Project administration, Methodology, Investigation. **Laíres A. Lima:** Investigation. **Celestino Santos-Buelga:** Writing – review & editing, Supervision. **Lillian Barros:** Writing – review & editing, Supervision, Project administration. **Sandrina A. Heleno:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Márcio Carocho:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2025.116608.

Data availability

No data was used for the research described in the article.

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