



# Development and Characterization of Gelatin/CMC-based Oral Films Incorporating *Crescentia cujete* L. Extracts for Bioactive Delivery

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## Abstract

Oral films (OFs) are versatile platforms that combine efficacy, ease of administration, and adsorption. The plant *Crescentia cujete* L. has shown bioactivity for respiratory applications. In this study, fruit pulp extracts obtained under different conditions (ripening stage, pulp pretreatment, and extraction method) were incorporated into gelatin/carboxymethyl cellulose (CMC) matrices to evaluate their influence on film properties. Extracts from freeze-dried pulp using the Soxhlet showed the highest flavonoid ( $8.7 \pm 0.8$  mg quercetin/g extract) and phenolic content ( $28.4 \pm 2.4$  mg gallic acid/g extract), strong antioxidant capacity against the ABTS<sup>•+</sup> radical ( $EC_{50} = 19.0 \pm 0.2$  µg/mL). The agar diffusion method confirmed antibacterial activity against the oral bacterium *S. mutans*. Films with 0–7% extract were produced by casting and analyzed by physicochemical, morphological, thermal, mechanical, and FTIR methods. Increasing extract concentration modified visual appearance ( $b^*$  parameter), phenolic content ( $7.8 \pm 0.5$  mg GA/g), contact angle ( $\sim 16^\circ$ ), and disintegration time ( $\sim 29$  s), while thickness ( $0.05 \pm 0.01$  mm), moisture content ( $11.9 \pm 0.9\%$ ), and antibacterial activity remained unchanged. SEM showed homogeneous morphology, and the extract addition decreased the melting temperature ( $\sim 14$  °C). Mechanical testing revealed the highest Young's modulus at 6% extract ( $3716 \pm 168$  MPa). Release studies indicated that films with 6% and 7% of the extract fit Higuchi and First-order models, respectively. These results demonstrate the feasibility of developing plant-based OFs with tailored release profiles and therapeutic potential.

## Highlights

- Ethanolic extract of *Crescentia cujete* L. fruit pulp may be a source of phenolic compounds.
- Gelatin/CMC active films produced by casting have a uniform structure.
- Extract concentration affects the physical, chemical, and biological properties of the films.
- Extract concentration affects the intermolecular interactions with the polymeric matrix, influencing the kinetics and mechanism of release.

**Keywords** Antimicrobial · Antioxidant · Films · Natural extracts · Release · Totumo

## Introduction

*Crescentia cujete* L., the pumpkin tree, is a tropical plant widely distributed in Mexico, Central American countries, South America, Central Africa, the West Indies, and tropical areas worldwide [1, 2]. Its fruit, characterized by a hard shell and gelatinous white pulp rich in seeds, takes about six months to mature. [2, 3]. Traditionally used in crafts, livestock feed, and folk medicine due to its pulp, which contains phenolic compounds with antioxidant and anti-inflammatory effects, which have been used for the treatment of respiratory conditions such as asthma, respiratory illnesses like flu, and diabetes, and for reducing body cholesterol by

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avoiding its reabsorption [3–5]. Antioxidant [6] antimicrobial, antibacterial, anthelmintic, anticholesterol, antidiabetic, and anti-inflammatory activity are the main properties identified in the extracts obtained in the different parts of the plant [5]. Ethanolic extracts of the leaves and fruit have shown the highest concentration of biologically active compounds [2, 4, 7, 8].

Various analytical techniques, including chromatography, spectroscopy, colorimetric, and bioassays, have been employed to characterize the chemical and biological properties of *C. cujete* L. leaf and fruit extracts for potential applications in food, pharmaceutical, and medical fields [9–11]. Advanced studies using an untargeted metabolomics approach combined with UPLC-MS/MS (Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry)-based molecular networking with NMR methods, in silico molecular docking with ADME (Absorption, Distribution, Metabolism, and Excretion) prediction, and statistical analyses have identified flavonoids and confirmed their biological activity [4, 12, 13]. These results support the valorization of *C. cujete* L. biomass as part of a sustainable, circular bioeconomy in tropical regions [14–16].

The development of therapies based on plant-derived nutraceuticals with cost-effective approaches with easy administration is a growing research focus [17, 18]. Orally disintegrating films (ODFs) offer rapid disintegration in the mouth, forming a suspension in saliva [19, 20]. Initially used to treat halitosis, and later for dysphagia [20] ODFs now deliver various active compounds that improve solubility, absorption, load capacity, and patient compliance [21]. Their advantages include flexible dosing, portability, rapid absorption via the oral mucosa, and bypassing hepatic first-pass metabolism [20–22]. ODFs are composed of single or multilayers of hydrophilic polymers that affect mechanical properties and dissolution, along with excipients like plasticizers, sweeteners, and surfactants that influence drug loading and release. Current research aims to enhance load capacity and preserve bioactive compounds by optimizing materials, administration routes, and methods to obtain films [20–22]. The structural properties of oral films, including surface morphology, porosity, and thickness, are directly related to their performance in the oral cavity. These features influence mechanical resistance, disintegration rate, and the release profile of bioactive compounds, making the link between film structure and oral applicability highly relevant. A homogeneous and flexible structure is essential to ensure patient comfort and uniform release, while irregularities or micro-porous features may accelerate degradation or alter bioavailability [23].

In this study, oral films (OFs) were developed using natural extracts obtained from the fruit pulp of *C. cujete* L. These films were characterized in terms of morphology,

mechanical properties, antioxidant and antibacterial activities, thermal behavior, and release profile and kinetics. Among the extracts, those with higher phenolic content demonstrated notable bioactivity. Their incorporation into gelatin/CMM-based films significantly influenced key functional properties, including antioxidant and antimicrobial performance, tensile strength, and the capacity for controlled release. These findings highlight the potential of *C. cujete* L.-enriched films as promising candidates for oral delivery systems targeting therapeutic applications.

## Materials and Methods

### Materials

Commercial ethanol (96%) and gelatin (bovine, 85%, bloom 280) were used. 2, 2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid radical (ABTS<sup>•+</sup>), carboxymethylcellulose (CMC), ascorbic acid, and quercetin standard were supplied by Alfa Aesar (Stoughton, MA, USA). Acetonitrile and methanol HPLC grade  $\geq 99.9\%$ , Folin-Ciocalteu reagent, Tween 80, sodium phosphate dibasic heptahydrate, potassium persulfate, and sodium phosphate monobasic monohydrate were supplied by Panreac AppliChem (Darmstadt, Germany). Gallic acid standard (ACS reagent grade,  $\geq 98.0\%$ ), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), trichloro aluminum (AlCl<sub>3</sub>), 85% orthophosphoric acid, acrylic Petri dishes (150 mm  $\times$  15 mm) and (60 mm  $\times$  15 mm), Whatman® qualitative filter paper No.1/47 mm, and Whatman® syringe filters PVDF membrane/0.22  $\mu$ m, were purchased from Sigma-Aldrich (Darmstadt, Germany). Milli-Q water was obtained from HYDROLAB (Polsky Rejestr Statków S.A., Straszyn, Poland). Commercial vegetable paper 41 g/m<sup>2</sup>, 1/8 (Ardèche, Francia). Fisher Scientific (Massachusetts, USA) supplied sorbitol (BioReagents, 91–100% purity).

Antimicrobial activity: *Staphylococcus aureus* (ATCC 25923) is a Gram-positive (G<sup>+</sup>) bacterium characteristic of skin and mucosal infections. *Streptococcus mutans* is a Gram-positive bacterium characteristic of the oral cavity. *Escherichia coli* (ATCC 25922) is a Gram-negative (G<sup>-</sup>) bacterium characteristic of the intestine. All of them were supplied by MICROBIOLOGICS (Granite Partners Company, Saint Cloud, Minnesota, USA).

### Obtention and Characterization of Natural Extracts from *C. cujete* L. Fruit Pulp

The physicochemical properties of fruit pulp extracts were evaluated based on ripening state (green-G and ripe-R), pulp pretreatment (fresh-F and freeze-dried-Fd), and extraction

method (magnetic stirring-*Ms*, ultrasound-*U*, and Soxhlet-*Sox*). Fruits were acquired from a local market in Bogotá, Colombia, and pulp was treated with ascorbic acid 1.6% (w/v) to avoid oxidation [24]. Freeze-dried samples were obtained using liquid nitrogen and a freeze-dryer (BNFD-L18MY model supplied by Hangzhou Boyn Instrument Co., Ltd, Hangzhou, China) for 72 h. The extraction was carried out as described following [18]:

**Magnetic stirring method (*Ms*):** Samples GFd and RFd were treated with 96% ethanol (10%, w/v). Fresh (*F*) samples were used at 33% (w/v). using the conditions: 500 rpm for 72 h at room temperature (r.t) in a Hei-PLATE Mix 'n' Heat Core (supplied by Heidolph, Chicago, IL, USA), keeping them in the absence of light.

**Ultrasound method (*U*):** Samples were sonicated for 30 min at 25 °C, frequency 40 kHz, and power 120 W in an ultrasound equipment ULTRASONIC CLEANER model KSL5120-5 (supplied by Huiya International Mansion, Ningbo, China).

**Soxhlet method (*Sox*):** Freeze-dried (*Fd*, 10%) and Fresh (*F*, 15%) samples were prepared in a minor concentration due to the capacity of the extraction thimbles. The samples were treated in a solvent extractor SER 148/6 (supplied by VELP Scientifica, NY, USA), using 70 mL of ethanol 96% (v/v), a cycle of immersion at 110 °C for 60 min, washing for 60 min and cooling for 15 min.

All extracts were filtered (Whatman No.1), stored in an amber bottle at 0 °C, and characterized by total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity (DPPH<sup>•</sup> and ABTS<sup>•+</sup> free radicals), HPLC, UV–Vis, FTIR, and antibacterial assays.

The following nomenclature was established to identify the samples: GFMs: “Green fresh magnetic stirring”, GFU: “Green fresh ultrasound”, GFSox: “Green fresh soxhlet”, GFdMs: “Green freeze-dried magnetic stirring”, GFdU: “Green freeze-dried ultrasound”, GFdSox: “Green freeze-dried soxhlet”, RFMs: “Ripe fresh magnetic stirring”, RFU: “Ripe fresh ultrasound”, RFSox: “Ripe fresh soxhlet”, RFdMs: “Ripe freeze-dried magnetic stirring”, RFdU: “Ripe freeze-dried ultrasound”, RFdSox: “Ripe freeze-dried soxhlet”.

### Total Phenolic Content (TPC)

In an Eppendorf was mixed 100 µL of the raw extract (10–33% w/v), 900 µL of ethanol, 500 µL of the Folin-Ciocalteu reagent (diluted tenfold), and 400 µL of sodium carbonate (2%, w/v). After a 60 min incubation, absorbance was recorded at 760 nm. Results were calculated using a standard curve of gallic acid (0.5–9 µg/mL,  $Y=0.1034X+0.0313$ ,  $R^2=0.9984$ ) and expressed as milligrams of gallic acid per gram of the sample (mg GA/g) [25, 26].

### Total Flavonoid Content (TFC)

In an Eppendorf tube, 50 µL of the raw extract, 50 µL of aluminum chloride (2%, w/v), and 1150 µL of 96% ethanol were mixed. After 40 min of incubation, absorbance was recorded at 415 nm. Results were calculated using a standard curve of quercetin (0.6–9.6 µg/mL;  $Y=0.0537X+0.0593$ ,  $R^2=0.9658$ ) and expressed as milligrams of quercetin per gram of sample (mg Q/g) [25, 26].

### Antioxidant Capacity Assays

#### DPPH<sup>•</sup> free radical scavenging assay

Different volumes of the raw extract (15–200 µL) were mixed with 1800 µL DPPH<sup>•</sup> (72 µM) radical and adjusted to 2000 µL with 96% ethanol. A blank was measured immediately at 515 nm. Samples were incubated in the dark for 60 min, and absorbance was recorded. The DPPH<sup>•</sup> radical scavenging percentage was calculated using Eq. 1 (Eq. 1), where  $ABS_{DPPH^{\bullet}}$ =blank absorbance, and  $ABS_{Sample}$ =sample absorbance.

$$DPPH^{\bullet} (\%) = \frac{ABS_{blank} - ABS_{Sample}}{ABS_{blank}} * 100 \quad (1)$$

#### ABTS<sup>•+</sup> Free Radical Scavenging Assay

A solution of ABTS<sup>•+</sup> radical (7 mM) and potassium persulfate (2.45 mM) (1:0.5) was kept in the dark for 12 h. Then 950 µL of this solution was diluted with 50 µL ethanol to reach an absorbance of 0.70–0.735, at 734 nm. Different aliquots of the raw extract (10, 20, 30, and 40 µL) were added to 950 µL ABTS<sup>•+</sup> solution, and the total volume was adjusted to 1000 µL with 96% ethanol. A blank was measured immediately at 734 nm, and absorbance was recorded again after 6 min of incubation in the dark. Similarly, the percentage of ABTS<sup>•+</sup> radical scavenging capacity was calculated using Eq. 1.

The antioxidant capacity against DPPH<sup>•</sup> and ABTS<sup>•+</sup> radicals was expressed as the EC<sub>50</sub> (half-maximal effective concentration) which is the concentration of the extract that gives the half-maximal response. For that, antiradical curves were plotted mL of the extract vs %DPPH<sup>•</sup> or ABTS<sup>•+</sup>. These essays were performed following some methods with some changes [26–29].

The TPC, TFC, and antioxidant capacity assays were performed at room temperature under light-free conditions. The absorbances were recorded at the specific wavelength in a UviLine 9600 UV–Vis spectrophotometer with SPECTRALAB software (supplied by SECOMAM SA, Alés,

Francia) against the reagent blank. All tests were conducted in triplicate, and the results are presented as the mean  $\pm$  standard deviation (SD).

### Antibacterial Activity

The agar diffusion method was used to evaluate the antimicrobial properties of natural extracts [26, 30]. Briefly, plates with nutrient agar were individually inoculated with fresh cultures of *S. aureus* and *S. mutans* ( $10^8$  colony-forming units CFU/mL). *E. coli* ( $10^8$  CFU/mL) cultures were inoculated on Lactose agar plates. Filter paper disks of 6 mm diameter impregnated with 20  $\mu$ L of natural extracts were placed onto the surface of the plates. Positive and negative controls were defined by chloramphenicol (2.5% w/v) and absolute ethanol, respectively. After incubation at 37° C for 24 h, the diameter of the inhibition zone (mm) was measured considering the diameter of the disks.

### HPLC Analysis

The analysis was carried out in HPLC LC-4000 Series (supplied by company JASCO International Co., Ltd., Tokyo, Japan) with a UV–Vis detector (UV-4070/75) using the gradient method described by Mizzi et al., (2020) without modifications [31]. Standard solutions of gallic acid and quercetin (10 mg/L) were prepared in methanol of HPLC grade for the analysis.

### Chemical and Optical Properties

#### Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were obtained in the range 4000–400  $\text{cm}^{-1}$ . The raw extract was analyzed, and the transmittance was recorded over 56 scans with a resolution of 4  $\text{cm}^{-1}$  in a Jasco FT/IR-4X Spectrometer (instrument distributed by JASCO International Co., Ltd., Tokyo, Japan).

#### UV–Vis Analysis

The samples were analyzed in a UviLine 9600 UV–Vis spectrophotometer with SPECTRALAB software (supplied by SECOMAM SA, Alés, Francia) with a resolution of 1 nm. Natural extracts (100  $\mu$ L of fresh sample and 900  $\mu$ L of ethanol 96%) were analyzed in a quartz cell. UV–Vis spectra were obtained between 200 and 800 nm to determine the absorption peaks.

## Production and Characterization of Oral Films (OFs)

### Production of Oral Films (OFs)

Oral films (OFs) were prepared using FDA-approved food ingredients: carboxymethylcellulose (CMC), gelatin, and sorbitol. CMC (4% w/v, 45 mL) and gelatin (7.8% w/v, 45 mL) solutions were prepared separately with gelatin hydrated and dissolved at 55 °C. After mixing both polymers, 18% sorbitol (20 g/100 g of macromolecule) and varying extract concentrations (F1-0%, F2-4%, F3-5%, F4-6%, and F5-7% (w/w)) were obtained the formulations. The final mixture was adjusted to 120 g with distilled water to be homogenized with a BonLari two-speed immersion blender model BLBI-518 (120 V, ~60 Hz, 200 W, for 5 min at the first speed). Solution was degassed by sonication with an ULTRASONIC CLEANER ultrasonic bath model KSL5120-5 (supplied by Huiya International Mansion, Ningbo, China), keeping a temperature of 55 °C, 40 kHz frequency and power of 120 W. Films were cast into acrylic Petri dishes (25 g per dish, 150 mm  $\times$  15 mm) and dried at room temperature away from light [30]. Once dried, these were stored in desiccators in paper envelopes until further analysis.

### Characterization of Oral Films (OFs)

The films were characterized biologically, physically, and chemically, according to the following techniques:

**Antibacterial Activity and Total Phenolic Content** Antibacterial properties were evaluated using 25 mm disks of the films, which were placed onto plates inoculated with *E. coli*, *S. aureus*, and *S. mutans* cultures. The analysis was performed under the same conditions described previously.

The total phenolic content was evaluated on active film samples; approximately 0.01 g was immersed in 6 mL of distilled water at 50 °C until total solubilization. An aliquot of 1 mL was added to 1 mL of 96% ethanol. Samples were centrifuged at 5000 rpm for 5 min. An aliquot of 0.5 mL was analyzed according to the previous description.

**Color Parameters** The color parameters of films  $a^*$  (red to green),  $b^*$  (yellow to blue),  $L^*$  (lightness), and  $\Delta E^*$  (total difference of color), were determined using a colorimeter spectrophotometer CM-23d (supplied by Konica Minolta, Valencia, España) using the method SCI (Specular Component Included). The Films were placed on a cell with a white background ( $L^*=31.73$ ,  $a^*=-1.11$ , and  $b^*=3.27$ ), and the measurements

were made directly on the surface. These analyses were carried out in triplicate, from 5 random film measurements [30].

**Visual Aspect, Film Thickness, and Moisture Content** Visual analysis was evaluated by selecting homogeneous films with no visible imperfections. Film thickness was measured with a digital micrometer (supplied by Mitutoyo, Kawasaki, Japan) at five distinct positions utilizing an accuracy of  $\pm 0.001$  mm. To determine the moisture content, the films were dried in a digital incubator (Mettler MOD BE 400, Germany) at 105 °C for 24 h, in triplicate [32].

**Water Contact Angle (WCA)** WCA was determined by the sessile drop method in a Contact Angle Goniometer (supplied by Ossila, Leiden, Netherlands). A 10  $\mu$ L droplet of deionized water was added on the surface of the films and the angle was measured from 5 to 60 s. The measurements were replicated 5 times, and the results were expressed as average with their relative standard deviation [33].

**Disintegration Time** According to the previous method [34], the films (20  $\times$  30 mm) were fixed on a support, and a drop of distilled water (200  $\mu$ L) was deposited on the film. The time required to dissolve the film and form a hole was taken as the disintegration time in seconds. The analysis was performed in triplicate.

**Mechanical Properties** Tensile strength, elongation at break, and Young's modulus of the films were determined in a Lloyd LF Plus compact 1kN tabletop tensile test machine (Bognor Regis, UK), according to ASTM D882–12 [33]. Films (75  $\times$  15 mm) were prepared and conditioned for 3 days at 25 °C and 50% RH before testing. Five samples were used for each formulation, and the results were expressed as the mean value with its standard deviation.

**Chemical and Optical Properties** Fourier transform infrared (FTIR) spectroscopy

The films were cut into 12  $\times$  40 mm<sup>2</sup> rectangles. FTIR spectra were obtained in the range 4000–400 cm<sup>-1</sup>. The transmittance was recorded over 56 scans with a resolution of 4 cm<sup>-1</sup> in a Jasco FT/IR-4X Spectrometer (instrument distributed by JASCO International Co., Ltd., Tokyo, Japan).

UV–Vis analysis

The films were cut into 12  $\times$  40 mm<sup>2</sup> rectangles. The conditions previously indicated in the extracts were also used to record the absorbance using the same equipment.

**Scanning Electron Microscopy (SEM)** Film morphology was analyzed in a Dual Beam Field Emission Scanning Electron Microscopy (FESEM) Scios 2 LoVac model (supplied by Thermo Fisher Scientific, Massachusetts, USA) and in a Scanning Electron Microscopy JEOL JSM 6490 LV model (supplied by JEOL, Massachusetts, USA), operating at an acceleration voltage of 0.8–10 kV, and magnification of X1500–X15000. The samples were covered with gold [35].

**Thermal Properties** Differential scanning calorimetry (DSC) was performed using an analyzer (Q-100, TA Instruments, distributed by Lanzetta Rengifo & CIA Ltd., Bogotá, Colombia), with approximately 6–8 mg of polymer. Samples were weighed into aluminum pans. The pan was placed in the calorimeter and heated from 30 to 300 °C at a rate of 10 °C/min under a nitrogen atmosphere, with an empty pan used as a reference. Data analysis was determined automatically with the data processing software TA Universal Analysis [35, 36].

### Profile and Kinetic Release of OFs

The in vitro release of extracts from OFs was evaluated by measuring TPC using UV–Vis spectroscopy. Films (6 cm diameter) were placed in phosphate buffer (pH 6.8, 50 mL, 0.05 M) with 0.2% (v/v) Tween 80, with constant stirring at 500 rpm at 37 °C. Samples were taken at set intervals (2 mL), and 500  $\mu$ L was treated with Folin-Ciocalteu reagent to determine TPC. Tests were performed in triplicate. Release data were fitted to various kinetic models (zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixon-Crowell), according to previous studies [37, 38]; and the model with the highest correlation coefficient ( $R^2$ ) was selected as the best fit [22, 38, 39].

### Statistical Analysis

All data are presented as mean  $\pm$  standard deviation ( $\pm$  SD) from at least three measurements. Means were compared between groups by one-way analysis of variance (ANOVA) using the software Statgraphics Centurion XVI.I.  $p < 0.05$  value was considered statistically significant. To analyze the extracts, the variables considered were total phenolic and flavonoid contents, antioxidant capacity, and antibacterial activity. The factors studied were the fruit's ripening state, pulp pretreatment, and extraction method. One extract was selected to evaluate the physical, chemical, and biological properties of the OFs at different concentrations.

## Results and Discussions

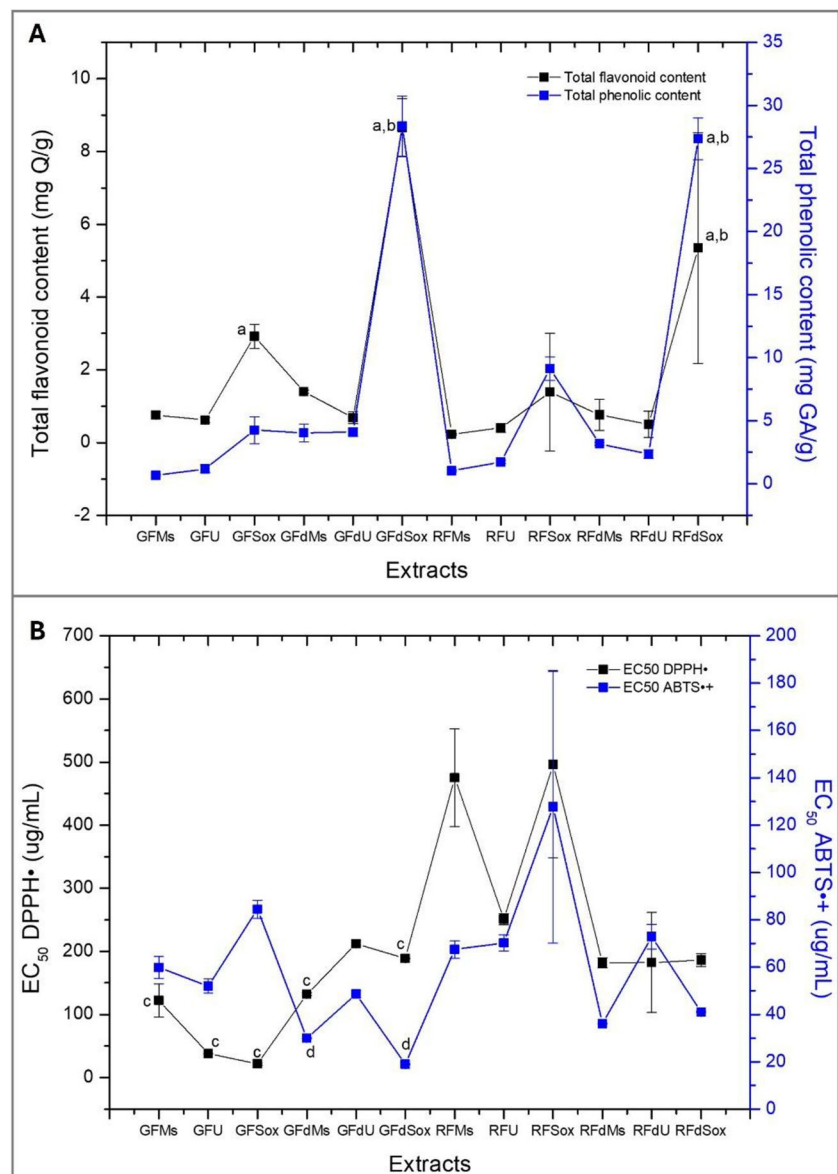
### Characterization of Natural Extracts Obtained from *C. cujete* L. Fruit Pulp

#### Total Flavonoid and Phenolic Content, and Antioxidant Capacity

Statistical analysis revealed that TPC was significantly influenced by pulp pretreatment (F and Fd) and extraction methods (Ms, U, and Sox),  $p < 0.0000$ , Fig. 1A, right y-axis, but not by fruit ripening state (G and R). The highest TPC values were found for the samples: GFdSox:  $28.4 \pm 2.4$  and RFdSox:  $27.4 \pm 1.7$  mg GA/g extract, with no difference between them. TFC, and antioxidant activity (DPPH $^{\bullet}$  and ABTS $^{++}$ ) were significantly affected by all three factors. Figure 1A,

left y-axis presents TFC values: GFdSox ( $8.7 \pm 0.8$ ) and RFdSox, ( $5.3 \pm 3.2$  mg Q/g extract). The most effective antioxidants, indicated by the lowest EC $_{50}$  values ( $\mu\text{g/mL}$ ) were: GFSox ( $21.8 \pm 2.0$ ) > GFU ( $37.8 \pm 0.2$ ) > GFMs ( $122.3 \pm 26.2$ ) > GFdMs ( $132.0 \pm 2.2$ ) > GFdSox ( $188.9 \pm 0.4$ ), Fig. 1B, left y-axis. Regarding ABTS $^{++}$  radical scavenging activity, significant differences were found for all three factors ( $p < 0.0024$ ). The extracts with the lowest EC $_{50}$  values ( $\mu\text{g/mL}$ ) were GFdSox ( $19.0 \pm 0.2$ ) and GFdMs ( $29.9 \pm 0.2$ ), Fig. 1B, right y-axis. It is worth noting that the ABTS $^{++}$  assay is more sensitive and can detect both hydrophilic and hydrophobic antioxidants [29]. Previous studies using water, 50% ethanol, and 100% ethanol as solvents for fruit extraction reported TPC values:  $8.56 \pm 1.47$ ,  $16.34 \pm 0.53$ , and  $166.12 \pm 3.81$  mg GAE/100 g extract, respectively. DPPH $^{\bullet}$

**Fig. 1** A. Total flavonoid content (mg Quercetin/g extract) (—■—) and total phenolic content (mg Gallic acid/g extract) (—■—). Analysis of variance (multivariate ANOVA), <sup>a</sup> $p < 0.000$ . B. EC $_{50}$  DPPH $^{\bullet}$  (—■—) and ABTS $^{++}$  (—■—) of the ethanolic crude extracts. Analysis by Comparing Multiple Samples <sup>c</sup> $p < 0.000$ , and <sup>d</sup> $p < 0.0024$  for the factors: fruit's ripening state (G and R), pulp pretreatment (F and Fd), and extraction method (Ms, U, and Sox). Each value was expressed as mean  $\pm$  SD ( $n = 3$ )



essay showed the strongest radical scavenging in the extract obtained with 100% ethanol [40].

Most studies on *C. cujete* L. plant have evaluated the antioxidant and antibacterial effects of extracts directly on microorganisms [3, 5, 12, 13], without assessing extract characteristics before application. Recent studies based on networking molecular and mass spectrometry analyses have identified key bioactive compounds in the fruit, such as flavonoids, glycosides, and phenolic acids [12]. In silico studies confirm the potential of flavonoids to inhibit proteins involved in inflammatory and oxidative pathways [4]. Extracts from leaves showed higher bioactivity when ethyl acetate was used as the solvent, compared to ethanolic extracts: IC<sub>50</sub> (80.21 µg/mL), TPC (28.07±9.47 mg GAE/g<sub>e</sub>), and TFC (139.57±3.75 mg GAE/g<sub>e</sub>) [6]. In this study, ethanolic extract from the GFMs sample showed significantly lower bioactivity values: EC<sub>50</sub> (122.3±26.2 µg/mL), TPC (0.7±0.1 mg GA/g<sub>e</sub>), and TFC (0.8±0.1 mg Q/g<sub>e</sub>).

### Antibacterial Activity

The antibacterial activity of the extracts was tested against *E. coli*, *S. aureus*, and *S. mutans* bacteria. The GFdSox extract showed the largest inhibition zones for all three strains, with significant effects from the extraction method (U, Sox) and pulp treatment (Fd),  $p < 0.0000$ , Table 1 and Fig. S1. This antibacterial activity was consistent with its high TPC, TFC, and antioxidant capacity.

Previous studies attribute antimicrobial effects against *R. microplus* due to the action of cinnamic, benzoic, and hexadecenoic acid [8], as well as against *P. aeruginosa* by the presence of flavonoids [25]. Stem bark extract obtained with ethyl acetate has shown inhibitory effects on *S. aureus*,

possibly due to compounds such as quercetin [41, 42]. HPLC analysis in our study revealed the presence of quercetin at a retention time of 21.7 min in the GFdU, GFdSox, RFdU, and GFdSox extracts (Fig. S2); however, similar reports specifically using *C. cujete* L. fruit pulp were not found to compare.

Generally, these extracts exhibit stronger inhibitory effects on gram-positive bacteria due to their simpler structure [5]. However, despite being gram-positive, *S. aureus* did not exhibit sensitivity to the extracts tested. A recent study suggests that *S. aureus* displays resistance to chlorhexidine, while *S. mutans* remains susceptible. This may be attributed to the repair mechanism of the cell wall of *S. aureus*, which prevents the accumulation of active compounds in the cytoplasm by an efflux system that reduces intracellular concentrations [43].

### Chemical and Optical Properties

The optical analysis of the extracts showed UV–Vis absorption between 320–380 nm, consistent with the typical absorption of quercetin at ~375 nm [44], though the intensity varied due to differences in the extract concentration (Figs. S3A and S3B). However, the FTIR spectra revealed more consistent trends, especially in freeze-dried (Fd) samples extracted via “Sox” and “U” methods, which exhibited stronger peak intensities (Fig. 2). The peak at 1723 cm<sup>-1</sup> is characteristic of the carbonyl (C=O) stretch, while the signal at 1368 cm<sup>-1</sup> was attributed to methyl groups, both for ketone group. The peaks at 1277 and 1280 cm<sup>-1</sup> were related to C-O stretching vibrations, while the bands at 1110 and 1120 cm<sup>-1</sup> are attributed to the stretching of C-O in -CHOH [44]. Stronger FTIR signals in GFdSox, GFdU, RFdSox, and RFdU correlated with higher TPC, TFC, antioxidant, and antibacterial activities, supporting the presence of quercetin-like compounds. Additionally, FTIR helped identify intermolecular interactions influenced by extract concentration like previous studies with green tea extract [45], reinforcing the potential of GFdSox as a highly bioactive ingredient for oral film applications.

### Characterization of Oral Films (OFs)

#### Antimicrobial Activity and Antioxidant Capacity

Oral films were prepared with fixed amounts of gelatin, CMC, and sorbitol, while varying the extract concentration (0–7%, w/w). TPC increased with extract concentration, F5 (7%) showed the highest value (7.8±0.5 mg GA/g), it confirms the formation of bioactive films, Table 2.

All films showed antibacterial activity against *S. mutans*, Fig. S4. Inhibition zones were observed to range from

**Table 1** Inhibition zone diameters (mm) of natural extracts against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Streptococcus mutans* (*S. mutans*)

| Extract | * <i>E. coli</i><br>(mm)±DS | * <i>S. aureus</i><br>(mm)±DS | * <i>S. mutans</i><br>(mm)±DS |
|---------|-----------------------------|-------------------------------|-------------------------------|
| GFMs    | -                           | -                             | -                             |
| GFU     | 7.7±0.6                     | -                             | *9.7±0.6                      |
| GFSox   | -                           | -                             | *8.0±0.0                      |
| GFdMs   | -                           | -                             | 7.0±0.0                       |
| GFdU    | -                           | -                             | *7.3±0.6                      |
| GFdSox  | 12.7±4.6                    | 7.3±0.6                       | *9.3±2.5                      |
| RFMs    | -                           | -                             | 7.0±0.0                       |
| RFU     | -                           | -                             | *9.0±0.0                      |
| RFSox   | 9.3±1.2                     | -                             | *8.0±0.0                      |
| RFdMs   | -                           | -                             | -                             |
| RFdU    | 9.7±1.5                     | -                             | *7.7±1.2                      |
| RFdSox  | 8.0±0.0                     | 8.3±0.6                       | *8.7±2.1                      |

Data represent the mean±SD ( $n=3$ ). Statistically significant: \* $p=0.0000$

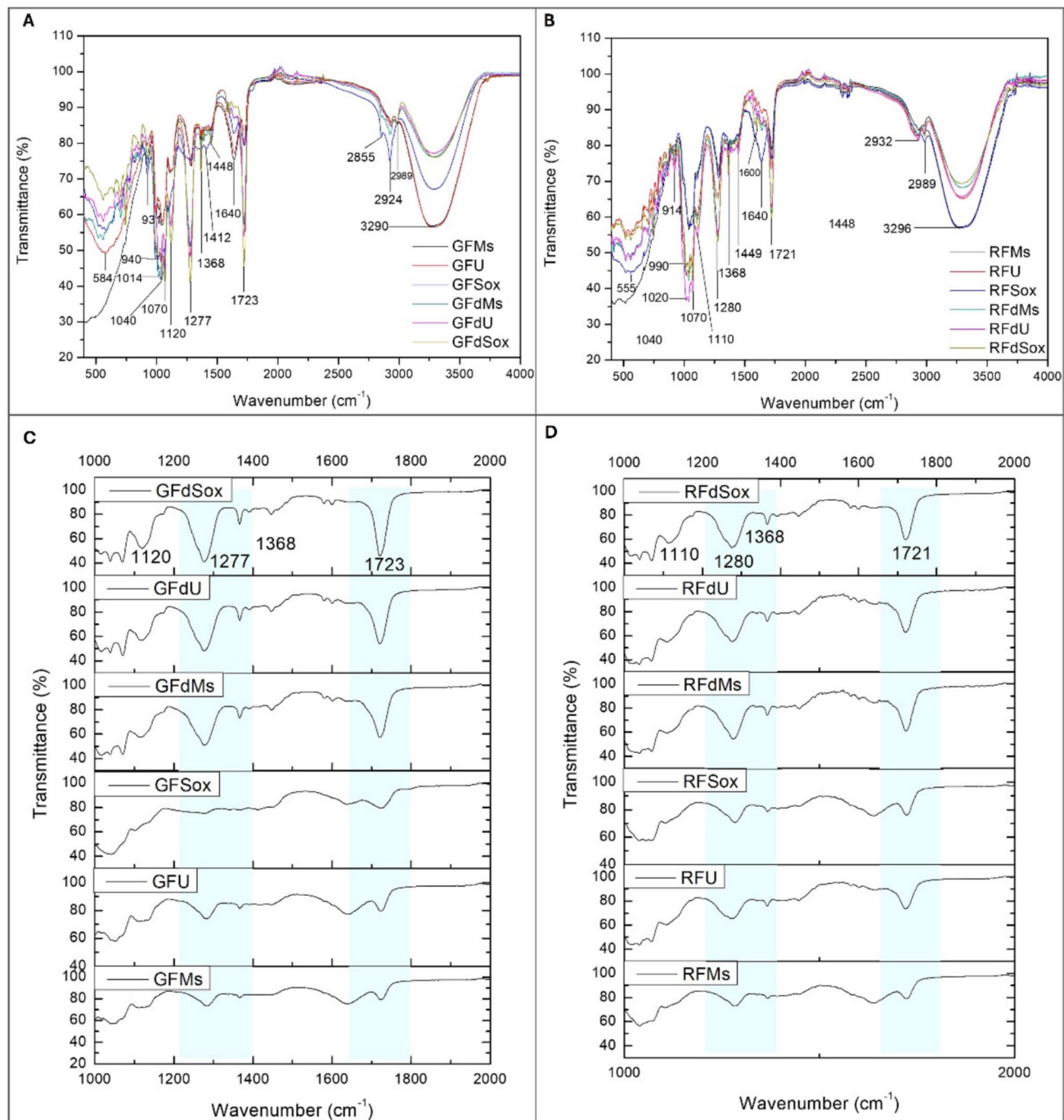


**Fig. 2** FTIR spectra of the extracts obtained using different extraction methods (magnetic stirring “Ms”, ultrasound “U”, and Soxhlet “Sox”), pulp pretreatment (fresh “F” and freeze-dried “Fd”), and fruit’s ripen-

ing state (green “G” and ripe R”). Figures **A** and **B** display the full spectra. Figures **C** and **D** highlight the spectral region between 1000–2000  $\text{cm}^{-1}$  for green and ripe stage samples, respectively

4.4 mm (F3-5%) to 4.8 mm (F2-4%; F4-6%; F5-7%); however, inhibition zones at higher concentrations suggest a saturation point.

Similar TPC values in F2 and F3 may result from strong interactions between the extract and the polymer matrix. Electrostatic interactions and hydrogen bonding between

gelatin (amino acid groups) and CMC (carboxylate groups) create an amphiphilic environment that improves the compatibility and solubility of the extract within the film matrix. However, these strong interactions may also restrict the release of bioactive compounds. Previous studies have reported that inter-polymeric complexes formed between

gelatin/CMC result in homogeneous and compact structures with low free volume, which limits the diffusion and release of the compounds [46, 47].

### WCA, Moisture, and Disintegration Time of the Films

According to the results reported in Tables 2 and 3, a correlation heatmap was generated to analyze the TPC, antibacterial activity, thickness, WCA, disintegration time, and moisture percentage (Fig. S5). Three distinct groups were identified: F1, F2-F3, and F4-F5. Formulations F4 and F5 exhibited the highest values for WCA (66.7°, 69.9°), disintegration time (>49 s), TPC (5.1–8.3 mg GA/g), and antibacterial activity. This indicates an increase in hydrophobic nature by the increase in extract concentration, which was consistent with the elevated TPC values. The presence of these compounds likely reduces water diffusion into the polymeric matrix, thereby delaying the swelling and dissolution processes of the film, increasing the disintegration time [48, 49]. In contrast, F2 and F3 exhibited lower values for TPC (0.9–1.4 mg GA/g), WCA (64.2°, 58.9°), and disintegration time (<23 s), indicating a hydrophilic behavior. Extract concentration did not notably influence the thickness and moisture; however, notable differences were observed, particularly between F1 and F2-F4 samples.

**Table 2** Total phenolic content and antimicrobial activity

| Formulation | *Total phenolic content<br>(mg GA/g)±DS | *S.<br>mutans<br>(mm)±DS |
|-------------|-----------------------------------------|--------------------------|
| F1-0%       | -                                       | -                        |
| F2-4%       | 1.0±0.1                                 | 4.8±0.3                  |
| F3-5%       | 1.1±0.3                                 | 4.4±0.1                  |
| F4-6%       | 5.4±0.5                                 | 4.8±0.2                  |
| F5-7%       | 7.8±0.5                                 | 4.7±0.1                  |

Data represent the mean±SD ( $n=3$ ). Statistically significant: \* $p=0.0000$

**Table 3** Physical properties of the films obtained with the extract GFd-Sox at different concentrations: thickness, WCA, disintegration time, and % moisture

| Formulation | Thickness<br>(mm)±DS | WCA average<br>(degree)±DS <sup>a</sup> | Disinte-<br>gration<br>time<br>(s)±DS <sup>b</sup> | % Mois-<br>ture±DS |
|-------------|----------------------|-----------------------------------------|----------------------------------------------------|--------------------|
| F1-0%       | *0.05±0.01           | <sup>a</sup> 53.7±2.0                   | <sup>b</sup> 20.5±2.1                              | *13.1±0.2          |
| F2-4%       | 0.06±0.01            | <sup>a</sup> 64.2±2.4                   | <sup>b</sup> 18.0±4.3                              | 9.9±0.9            |
| F3-5%       | 0.06±0.01            | <sup>a</sup> 58.9±0.9                   | <sup>b</sup> 19.5±3.5                              | 11.4±1.0           |
| F4-6%       | *0.04±0.01           | 66.7±2.1                                | 49.1±5.5                                           | *11.2±2.1          |
| F5-7%       | 0.05±0.01            | 69.9±1.0                                | 49.0±1.4                                           | 11.9±0.9           |

Data represent the mean±SD ( $n=3$ ). Statistically significant: <sup>a,b</sup> $p=0.0000$ . WCA: water contact angle

**Table 4** Colorimetric properties of the oral disintegration films

| Formulation | *L         | *a         | *b        | *ΔE       |
|-------------|------------|------------|-----------|-----------|
| F1-0%       | 98.99±0.01 | -0.22±0.01 | 0.78±0.08 | 7.11±0.03 |
| F2-4%       | 98.45±0.05 | -0.42±0.02 | 2.19±0.09 | 6.41±0.05 |
| F3-5%       | 97.42±0.27 | -0.59±0.05 | 4.57±0.50 | 5.89±0.06 |
| F4-6%       | 97.14±0.75 | -0.75±0.06 | 6.06±0.15 | 6.13±0.07 |
| F5-7%       | 96.12±0.09 | -1.11±0.01 | 7.97±0.30 | 7.07±0.19 |

Data represent the mean±SD ( $n=3$ ). Statistically significant: \* $p=0.0000$ . Parameters L (brightness), a (positive values for redness and negative values for greenness), b (negative values for blueness and positive values for yellowness)

### Visual Aspect and Color Parameters

The incorporation of *C. cujete* L. extract at concentrations from 4 to 7% significantly influenced the colorimetric properties of the oral disintegration films, as shown in Table 4 and Fig. 4A. A progressive decrease in L\* values indicated a darkening of the films with increasing extract concentration. Parameter a\* shifted into the negative range, revealing a tendency toward green hues, while the b\* parameter increased notably, suggesting a stronger yellow component. The total color difference (ΔE) values confirmed perceptible color changes compared to the control formulation (F1-0%). The results observed for the color parameters are consistent with the visual aspect, as can be seen from a macroscopic perspective in Fig. 4A, a darkening of the film is observed when the extract concentration increases. In a quality way, the films F4-6% and F5-7% showed a dark green color. These results demonstrate that the addition of extract has a measurable and visible impact on the visual appearance of the films [50].

### Chemical and Optical Properties

Figure 3A shows visible changes in the appearance of the films. The control film (F1-0%) had over 80% transmittance, while F4 and F5 showed values below 20%, indicating the extract effectively blocked UV light, as it was found in gelatin/CMC films functionalized with bamboo leaf extracts [51].

Figure 3B presents FTIR spectra showing characteristic bands for gelatin (amino acids), CMC (carboxylate groups), and the extract (ketone groups). Signals observed between 3100–3600  $\text{cm}^{-1}$  corresponding to O–H groups and N–H stretching vibrations, peaks at 2937 and 2879  $\text{cm}^{-1}$  assigned to asymmetric and symmetric stretching vibrations of the  $-\text{CH}_2$  bond, respectively, and signals at 1552 and 1632  $\text{cm}^{-1}$  characteristic of carboxylate and amide groups, respectively, confirmed polymer interactions. As extract concentration increased, peak intensities decreased, suggesting stronger intermolecular interactions due to electrostatic interactions

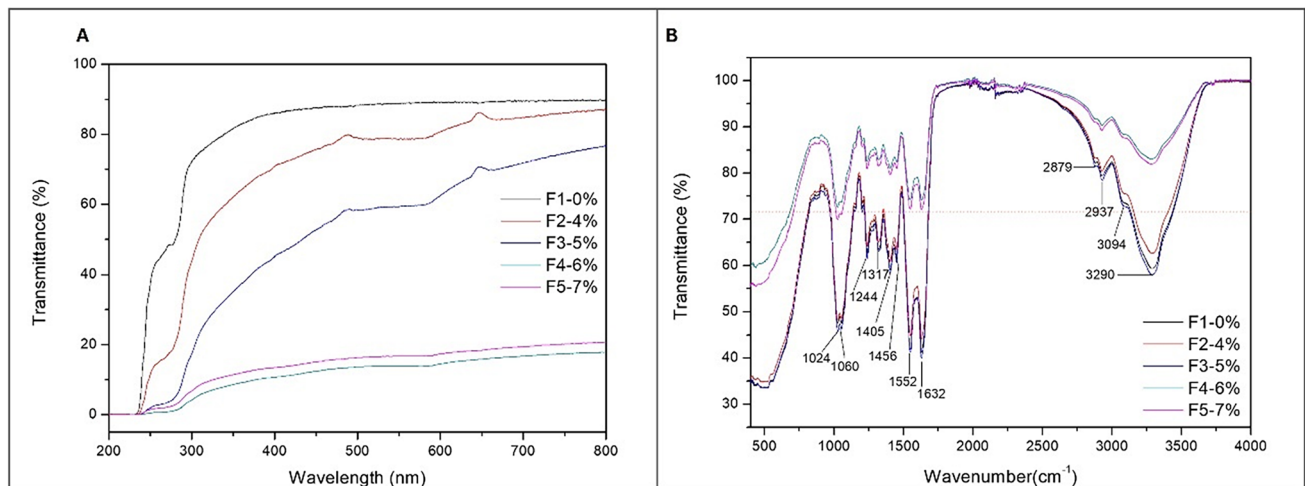


**Fig. 3** A. UV-Vis spectra and B. FTIR spectra of the film obtained with the extract GFdSox at different concentrations: 0, 4, 5, 6, and 7%

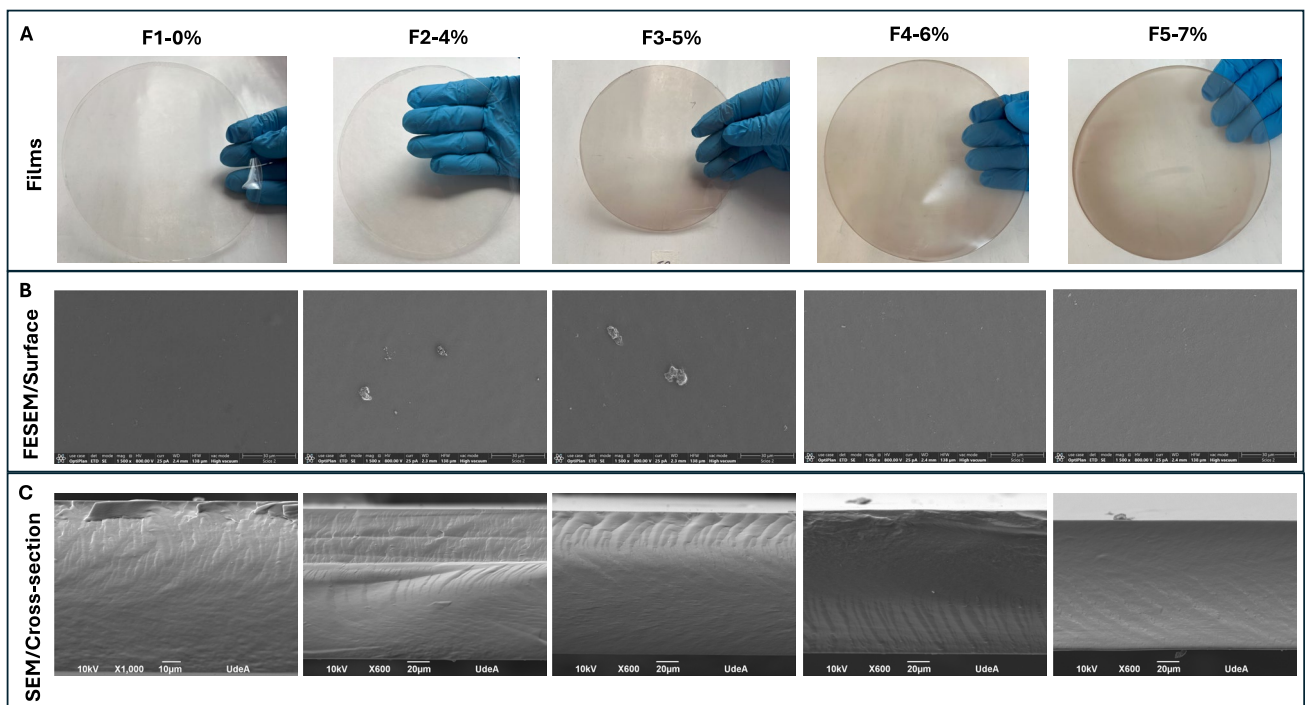


**Fig. 4** A. Digital photographs. B. FESEM images of the film surface (X 1500, scale 30 μm, 800 V) and C. SEM images of the cross-section of the films (× 600, scale 20 μm, 10 kV) with 0, 4, 5, 6, and 7% of the extract GFdSox obtained from the pulp of the fruit *C. cujete* L

and hydrogen bonding, which reduced the vibrational freedom of functional groups [46, 51, 52].

### Thermal, Mechanical, and Morphological Properties

Figures 4B and C show the micrographs of the film surfaces and cross-sections, respectively. The films exhibited homogeneous and smooth structures without phase segregation, indicating that the extract concentration did not alter the structural integrity of the polymeric matrix. This

suggests good compatibility between the extract and the CMC-gelatin film components. A study on active films of gelatin/CMC *Hass* avocado peel extracts reported structural changes; however, bamboo leaf extracts did not affect the morphology of this type of polymeric matrix. These differences are influenced by the ratio gelatin: CMC, the composition, and the extract concentration [50, 51]. SEM conditions revealed a characteristic of a thin film without fractures. Low-voltage FESEM (e.g., around 800 V) was ideal for surface analysis of polymeric films because it minimizes

electron penetration and specimen charging, enhancing surface topography contrast and preserving fragile structures. Conversely, high-voltage SEM (e.g., 10 kV) enables deeper penetration of the electron beam, improving contrast and imaging of cross-sectional and internal features that require more interaction volume [53, 54]. The film thickness was calculated using the ImageJ 1.46r program, obtaining an average value of  $0.05 \pm 0.00$  mm, consistent with the values reported in Table 3.

The mechanical properties of the gelatin/CMC films were notably influenced by the addition of *C. cujete* L. extract (0–7%). As shown in Fig. 5A, tensile strength (TS) decreased from F1 to F4, with the lowest value at F4 ( $37.88 \pm 0.96$  MPa,  $p < 0.05$ ), suggesting disruption of the polymer matrix by the extract. However, TS increased in F5, suggesting structural reorganization at higher extract levels. Young's modulus (YM) (Fig. 5A, blue line) showed

a continuous increase from F1 to F4, reaching its highest value at F4 ( $3716 \pm 168$  MPa). However, in F5, a sharp decline in the modulus was observed, reflecting a more flexible structure at higher extract concentrations.

Figure 5B presents the results for load at break (LB) and stress at break (ST). LB increased from F1 ( $16.2 \pm 1.0$  N) to F3 ( $20.3 \pm 1.0$  N), declined in F4 ( $16.4 \pm 1.4$  N), and peaked significantly in F5 ( $31.1 \pm 1.3$  N). Similarly, ST was stable from F1 ( $57.2 \pm 4.4$  MPa) to F3 ( $56.0 \pm 1.1$  MPa), decreased in F4 ( $37.9 \pm 1.0$  MPa), and recovered in F5 ( $52.2 \pm 1.3$  MPa). These trends suggest that F4 represents a critical point where the extract weakens cohesion, while F5 demonstrates enhanced mechanical performance due to new interactions between extract constituents and the polymer.

Overall, the extract modulates the mechanical properties, initially weakens but later strengthens the films, depending on concentration. Similar trends have been reported in other

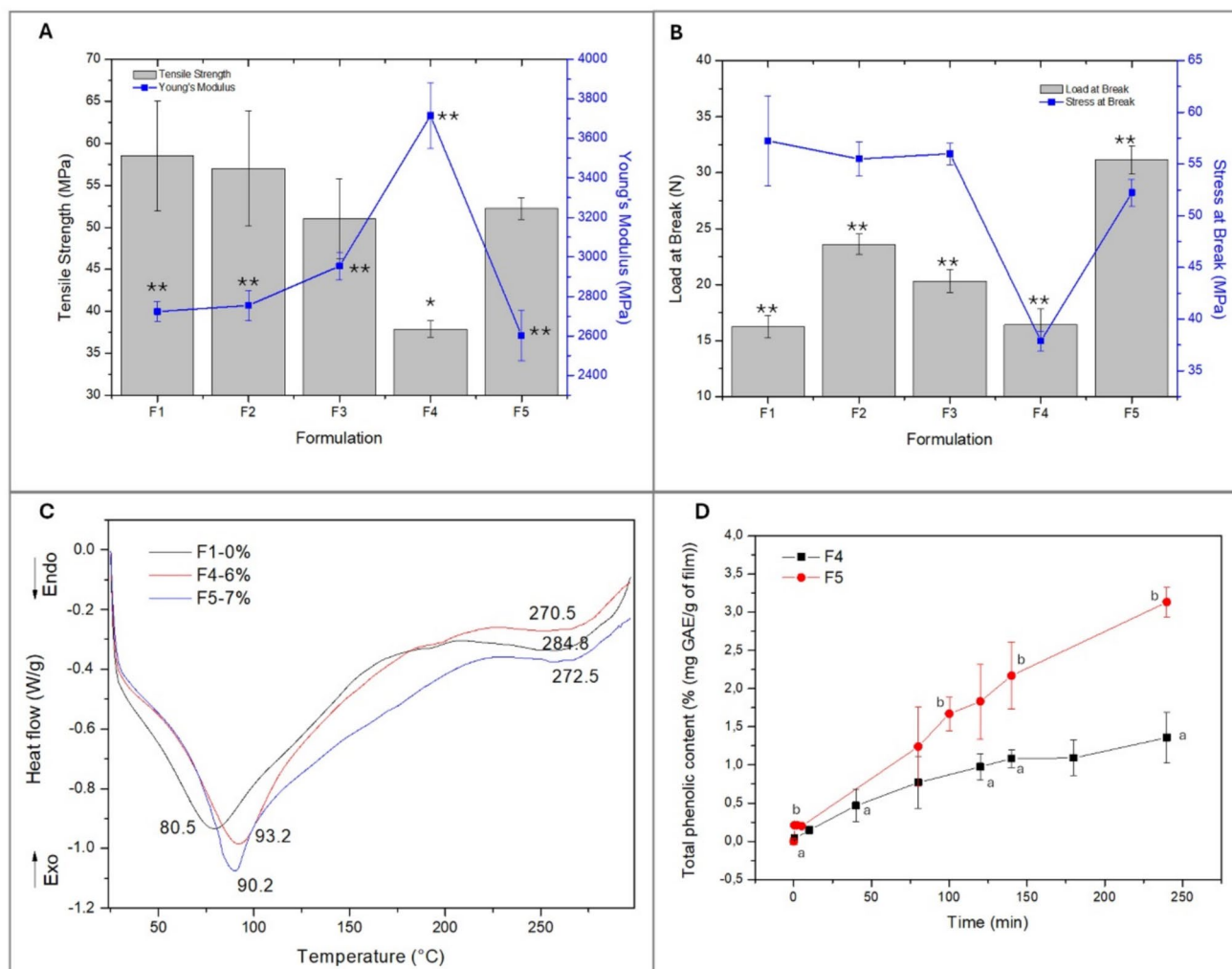


**Fig. 5** A and B. Mechanical properties of the film obtained with the extract GFdSox: Tensile strength (■ bars), and Young's modulus (— line); Load at break (■ bars), and Stress at break (— line), respectively. C. DSC curves. D. Release profile of the extracts from the

formulation F4-6% (—■— line), and F5-7% (—■— line), in a simulated medium ( $p = 0.0365$ ). Data represent the mean  $\pm$  SD ( $n = 3$ ). Statistically significant:  $*p = 0.0028$ ,  $***p = 0.0000$ ,  $^b p = 0.0006$

systems with ratio: gelatin:CMC 9:1 and 8:2, supporting the trends observed here with a ratio 7:1. The interactions between amino acids, hydroxyl, and carboxylate groups form a complex, which removes water during the process, decreasing the plasticizer effect of the water, and the ST values decrease. As was studied with extracts of bamboo leaves between 0–0.4% (v/v) in gelatin: CMC (5:5) matrix [46, 51].

Thermal analysis of F4 and F5 revealed two endothermic peaks (Fig. 5C). The first peak (80.5–93.2 °C) corresponds to water evaporation and appeared at a lower temperature than in other studies due to the specific gelatin/CMC ratio. The second peak, related to the film melting, showed that the control film (F0) had a higher melting temperature ( $T_m=284.8$  °C) than F4 (270.5 °C) and F5 (272.5 °C). This reduction in  $T_m$  indicates that the addition of *C. cujete* L. extract weakens intermolecular interactions in the polymer matrix, lowering thermal stability [51].

### Profile, Kinetic, and Mechanism of Release of the OFs

The release of phenolic compounds from *C. cujete* L. extracts in gelatin/CMC-based oral films was modeled using kinetic approaches. Table 5 and Fig. 5D present the corresponding fitting results. The Higuchi model described the slower release from formulation F4 ( $R^2=0.9884$ ), indicating diffusion controlled by partial polymer matrix dissolution due to specific interactions between the extract and the polymer [55]. In contrast, F5, with a higher extract concentration showed a faster release, concentration-dependent release within 4 h, fitting a First-order model ( $R^2=0.9869$ ). This behavior is linked to greater compound distribution and enhanced matrix solubility from hydrogen bonding [56, 57]. Both formulations followed the Korsmeyer-Peppas

**Table 5** Phenols release data fitted to various kinetic models: zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixon-Crowell

| Formulation | Kinetic model    | $R^2$  | k                    | n      |
|-------------|------------------|--------|----------------------|--------|
| F4-6%       | Zero-order       | 0.9307 | $6.0 \times 10^{-5}$ | -      |
|             | First-order      | 0.9314 | $6.0 \times 10^{-5}$ | -      |
|             | Higuchi          | 0.9884 | 0.0009               | -      |
|             | Korsmeyer-Peppas | 0.9861 | 0.0005               | 0.5992 |
|             | Hixon-Crowell    | 0.9312 | $2.0 \times 10^{-5}$ | -      |
| F5-7%       | Zero-order       | 0.9860 | 0.0001               | -      |
|             | First-order      | 0.9869 | 0.0001               | -      |
|             | Higuchi          | 0.9684 | 0.0019               | -      |
|             | Korsmeyer-Peppas | 0.9175 | 0.0017               | 0.4849 |
|             | Hixon-Crowell    | 0.9866 | $4.0 \times 10^{-5}$ | -      |

$k$  release rate constant,  $n$  diffusion or release exponent, where  $0 < n < 0.45$ : quasi-Fickian diffusion.  $n=0.45$ : Fickian diffusion (Case I),  $n: 0.45 < n < 1$ : Anomalous transport,  $n=1$ : Non-Fickian transport (Case II), and  $n > 1$ : Super case II

model with anomalous transport ( $n=0.6$  for F4 and  $n=0.5$  for F5), suggesting a combined mechanism of diffusion and polymer relaxation [37, 58, 59].

## Conclusions

This study highlighted the potential of *C. cujete* L. extracts as bioactive agents for developing functional oral films. It analyzed how extract concentration influences the mechanical, thermal, functional, and release properties of the films. Extracts obtained via ultrasound-assisted and Soxhlet methods from freeze-dried pulp exhibited significant bioactivity, particularly antioxidant capacity and antimicrobial effects against *S. mutans* and *E. coli*. The GFdSox extract showed the highest TPC and TFC, antioxidant capacity (ABTS<sup>•+</sup>), and bacterial inhibition zones. Spectroscopic (UV–Vis, FTIR) and chromatographic (HPLC) analyses confirmed the presence of quercetin-like compounds.

A direct correlation was observed between extract concentration and films properties, affecting disintegration time, WCA, release behavior, visual aspect and color. Tensile strength decreased while Young's modulus increased up to formulation F4, with a structural reorganization at F5 improving mechanical strength. Thermal analyses showed reduced melting temperature ( $T_m$ ), indicating lower intermolecular cohesion due to phenolic compounds.

Phenol release kinetics revealed that F4 followed the Higuchi model, while F5 fit a First-order model. Both exhibited anomalous transport mechanisms according to the Korsmeyer–Peppas model, indicating combined diffusion and polymer relaxation mechanisms.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11483-025-10042-3>.

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**Author Contribution** P.M: She wrote the manuscript. She characterized the antioxidant capacity, thermal, and morphological properties of the films. She developed the statistical analysis and HPLC analysis. She developed the profile and kinetic release of OFs. She analyzed and graphed the data. She obtained extracts from the totumo and the films. G.C: He performed the antimicrobial activity and obtained the FTIR data of the extracts and films. He wrote the extraction procedure. He developed the experiment for disintegration time, moisture content, and visual appearance. He obtained extracts from the totumo and the films. L.R: She performed the antimicrobial activity assay of the extracts and films. She obtained the mechanical properties data, the color parameter, and the contact angle data. She wrote the procedures for the antioxidant capacity and antibacterial activity assays. She reviewed the final version of the manuscript.

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**Data Availability** No datasets were generated or analysed during the current study.

## Declarations

**Consent to Participate** The authors express their consent to cooperate in this article.

**Competing interests** The authors declare no competing interests.

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