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Development and Characterization of Functional Cassava Starch–Chitosan Polysaccharide Blends Incorporating Grape Seed Extract for Chilled Shrimp Preservation

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Abstract

Biodegradable films based on polysaccharides have attracted attention as sustainable alternatives for food preservation. In this study, films and films were developed using cassava starch, chitosan, and grape seed extract, either individually or in polymeric blends, and their physicochemical, mechanical, microstructural, and preservative properties were evaluated. The films were applied to peeled shrimp stored under refrigeration for six days. Microbiological analysis showed a reduction in aerobic mesophilic bacterial counts in coated samples, indicating improved preservation. Films containing cassava starch and chitosan provided greater pH stability during storage. Film characterization revealed that grape seed extract influenced thickness and solubility, particularly in chitosan-based formulations. Cassava starch films exhibited the best water vapor permeability, while blended systems demonstrated improved mechanical performance. The highest tensile strength was observed for the chitosan-based film with extract, whereas starch-containing blends showed balanced strength and flexibility. Scanning electron microscopy revealed more cohesive and continuous structures in polymer blends, while extract-only films presented internal voids, explaining their lower mechanical resistance. Thus, the synergistic combination of cassava starch and chitosan, modulated by grape seed extract, produced

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films with suitable barrier, mechanical, and structural properties. These biodegradable polymeric films show promising potential for extending the shelf life of refrigerated shrimp and for application in sustainable food packaging.

Keywords: biodegradable films; cassava starch; chitosan; grape seed extract; shrimp preservation; polymer blends

1. Introduction

The growing demand for sustainable food preservation technologies has intensified research into biodegradable polysaccharide-based materials with tailored structural and functional properties [1–3]. These modern packaging systems are expected not only to protect food from external contamination but also to actively contribute to shelf-life extension through physicochemical and biological mechanisms [4,5]. In this context, polysaccharides have attracted considerable attention due to their renewability, biodegradability, film-forming ability, and structural versatility [6–8].

Among the natural polysaccharides, cassava starch (*Manihot esculenta*) and chitosan stand out because of their complementary physicochemical characteristics [9]. Cassava starch is a low-cost and abundantly available biopolymer composed primarily of amylose and amylopectin, whose hydroxyl-rich structure enables hydrogen bonding and film formation [10,11]. These films generally exhibit high water solubility and limited mechanical resistance due to their hydrophilic nature [12]. On the other hand, chitosan, a partially deacetylated derivative of chitin obtained from crustacean shells, presents a cationic character, good film-forming ability, intrinsic antimicrobial activity, and the capacity to interact electrostatically and through hydrogen bonding with other polymers [13]. Blends containing cassava starch and chitosan represent a promising strategic approach capable of modulating intermolecular interactions and improving functional performance [14].

Among the modes of interaction in this polymer blending, supramolecular arrangements formed by hydrogen bonding, electrostatic interactions, and chain entanglement between starch and chitosan macromolecules stand out [9]. These interactions may alter crystallinity, barrier properties, and mechanical resistance, generating materials with characteristics distinct from those of the individual polymers [15]. When compared to complex polymerization routes, this blending offers a versatile and economically viable strategy for tailoring biodegradable materials [16].

The incorporation of natural bioactive compounds further expands the functionality of polysaccharide matrices [17]. Among the natural bioactive compounds, grape seed (*Vitis vinifera*) extract stands out as a mixture rich in polyphenolic compounds, including proanthocyanidins, which exhibit both antioxidant and antimicrobial activities [18,19]. When incorporated into starch–chitosan systems, these compounds may establish additional interactions with hydroxyl and amino groups of polymer chains, influencing network organization and molecular mobility [20]. Such effects can contribute to the development of active films capable of retarding oxidative degradation and microbial proliferation in perishable foods [21], particularly when using grape seed extract, a rich source of polyphenolic compounds with well-established antioxidant and antimicrobial properties, obtained from an abundant agro-industrial by-product of the wine and juice industries.

In this context, the whiteleg shrimp (*Litopenaeus vannamei*) represents a suitable model for evaluating active biodegradable films. Due to its near-neutral pH, high water activity, and high nutrient content, this species is highly susceptible to microbial spoilage [22]. Additionally, the processing of this food delicacy generates large amounts of shell residues

rich in chitin, reinforcing the circular economy potential of converting these by-products into chitosan for advanced packaging applications [23,24].

From a structural standpoint, cassava starch–chitosan blends containing grape seed extract represent a multifactorial system in which polymer compatibility promotes molecular interactions that confer morphology and physicochemical stability suitable for food packaging. Understanding how these interactions influence barrier properties, mechanical behavior, and antimicrobial efficiency is essential for designing optimized polysaccharide-based films. Thus, this study aims to systematically characterize the structural, physicochemical, mechanical, and functional properties of cassava starch–chitosan blends incorporated with grape seed extract, and to evaluate their effectiveness as active biodegradable films in delaying quality deterioration and extending the refrigerated shelf life of shrimp.

2. Materials and Methods

2.1. Acquisition of Shrimp

Shrimp (*L. vannamei*) samples were commercially obtained in the city of Mossoró, Rio Grande do Norte, Brazil. Immediately after purchase, the samples were placed in insulated containers containing ice and maintained at approximately 0 °C during transportation to the Food Technology Laboratory (LABA) at the Federal Rural University of the Semi-Arid Region (UFERSA). Upon arrival at the laboratory, the shrimp were minimally processed, including decapitation, peeling, and washing with running distilled water.

2.2. Preparation of Cassava Starch and Chitosan Film-Forming Solutions

The chitosan film-forming solution was prepared according to the method described by Xiao and collaborators [25], with modifications. Commercially obtained chitosan (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in a 1% (*v*/*v*) acetic acid (Merck, Darmstadt, Germany) solution containing glycerol (Vetec Química Fina Ltd., Rio de Janeiro, Brazil) (20% *w*/*w* relative to the dry polymer mass), resulting in a final chitosan concentration of 2% (*w*/*v*). The solution was stirred continuously for 24 h at room temperature using a magnetic stirrer (SL-95: Solab, Piracicaba, Brazil). Subsequently, the dispersion was heated in a water bath (SL-150: Solab, Piracicaba, Brazil) at 60 °C for 2 min and stirred again until complete dissolution of the chitosan was achieved.

The cassava starch film-forming solution was prepared following the methodology: cassava starch (local commercial supplier, Mossoró, Brazil) (2% *w*/*v*) and glycerol (20% *w*/*w* relative to the dry polymer mass) were solubilized in deionized water. The mixture was heated under continuous stirring using a magnetic stirrer with heating (SP-160: SPLABOR, Presidente Prudente, Brazil). The temperature was monitored with an infrared thermometer (Instrutherm, São Paulo, Brazil) and gradually increased to 70 °C. The solution was maintained at this temperature for 15 min under constant stirring, ensuring that the temperature did not exceed 70 °C to promote complete starch gelatinization.

2.3. Preparation of Hydroalcoholic Grape Seed Extract

Purple grapes (*V. vinifera*) were commercially obtained from a local producer in Apodi, Rio Grande do Norte, Brazil, and transported to LABA–UFERSA. Upon arrival, the fruits were sanitized in accordance with RDC Nº. 352/2002 (https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2002/rdc0352_23_12_2002.html, accessed on 25 March 2026). The grape seed extract was prepared based on methodologies described and published elsewhere [26,27], with modifications. After sanitization, the grapes were manually separated to obtain the seeds, which were washed with deionized water and dried in a forced-air circulation (SL-102: Solab, Piracicaba, Brazil) oven at 40 °C for 48 h. The dried seeds were then ground using a mortar and pestle and sieved to obtain a fine and homogeneous powder.

For hydroalcoholic extraction, the seed powder was mixed with 70% (*v*/*v*) ethanol (Merck, Darmstadt, Germany) at a 1:1 (*w*/*v*) ratio. The mixture was maintained under continuous stirring using a magnetic stirrer (SP-160: SPLABOR, Presidente Prudente, Brazil) at room temperature for 24 h in a closed container to minimize solvent evaporation. After extraction, the suspension was allowed to stand for 24 h to promote decantation and phase separation. The supernatant was carefully collected using a sterile pipette and transferred to a volumetric flask without a stopper to allow solvent evaporation. The solvent was removed by heating the extract at 50 °C under magnetic stirring for 48 h until ethanol evaporation was complete, yielding the concentrated grape seed extract used in subsequent formulations.

2.4. Application of Film-Forming Treatments and Experimental Design

The experiment was conducted using a completely randomized design consisting of seven treatments and one control group. All analyses were performed in quintuplicate for each treatment (Table 1).

Table 1. Composition of polysaccharide film-forming formulations incorporating grape seed extract applied as active films to shrimp samples.

Treatment Code	Description of Film Composition
C (Control)	Uncoated shrimp (no film application)
Q	Chitosan film
QE	Chitosan + grape seed extract
F	Cassava starch film
FE	Cassava starch + grape seed extract
QFE (Polymeric Blend 1)	Chitosan + cassava starch + grape seed extract
QF (Polymeric Blend 2)	Chitosan + cassava starch
E	Grape seed extract solution

The grape seed extract was incorporated into the film-forming solutions at a concentration of 5% (*v*/*v*), based on previous studies [28–30], with modifications. This concentration was also supported by preliminary assays conducted to evaluate the influence of the extract on film formation, particularly regarding film adhesion to the casting surface and the maintenance of biopolymer matrix integrity.

The films were applied using the dipping method as described by Costa and collaborators [31], with adaptations. Briefly, shrimp were immersed in the respective film-forming solutions three consecutive times, with 5 s between immersions to allow uniform layer formation. After film application, the samples were packaged in polyethylene bags (local commercial supplier, Mossoró, Brazil) and hermetically sealed using an industrial heat sealer (Selovac, Barueri, Brazil).

The treated shrimp were stored under refrigeration at 7 ± 1 °C (Consul, Joinville, Brazil) and evaluated on days 0, 3, and 6 for microbiological and physicochemical quality parameters. All analyses were carried out with five independent replicates per treatment.

2.5. Microbiological Analyses

The microbiological analyses included the identification of aerobic mesophilic bacteria and coagulase-positive *Staphylococcus* spp. All procedures were conducted in a laminar flow hood (Pachane, Piracicaba, Brazil) using sterilized materials to prevent external contamination. Decimal serial dilutions were prepared by aseptically transferring 25 g of sample into 225 mL of sterile peptone water (0.1% Kasvi, São José dos Pinhais, Brazil) and homogenizing to obtain the 10^{-1} dilution. Subsequent dilutions were prepared by

transferring 1 mL of the previous dilution into 9 mL of sterile diluent, followed by thorough mixing, to obtain the desired dilution levels.

Culture media were prepared according to the manufacturer's instructions. The surface plating (spread plate) technique was employed, in which 0.1 mL of each selected dilution was inoculated onto the surface of previously prepared agar plates specific for each microbial group. The inoculum was evenly distributed using a sterile Drigalski spatula.

For the identification of aerobic mesophilic bacteria, Plate Count Agar (Himedia, Mumbai, India) was used. After inoculation and spreading, plates were labeled according to treatment and dilution and incubated (Quimis, Diadema, Brazil) at 36 ± 1 °C for 48 h. Colonies were subsequently counted, and results were expressed as colony-forming units per gram (CFU/g).

For the detection of coagulase-positive *Staphylococcus*, the procedure described in IN 62/2003 (https://www.legisweb.com.br/legislacao/?id=75773, accessed on 10 November 2025) was followed. Diluted samples were inoculated onto Baird–Parker agar (Himedia, Mumbai, India) supplemented with egg yolk emulsion (Himedia, Mumbai, India) and 1% potassium tellurite (Sigma-Aldrich, St. Louis, MO, USA). Supplementation was performed at 45–50 °C prior to solidification. Plates were incubated at 36 ± 1 °C for 48 h.

Typical colonies were subjected to confirmatory tests, including Gram staining, catalase, thermonuclease, and coagulase tests. Presumptive colonies were first transferred to Brain Heart Infusion (Himedia, Mumbai, India) broth and incubated at 36 ± 1 °C for 24 h. Catalase activity was evaluated, followed by confirmation of coagulase production using rabbit plasma (Newprov, Pinhais, Brazil). Coagulase reactions were monitored after 1, 2, 4, 6, 12, and 24 h of incubation at 36 ± 1 °C.

2.6. Physicochemical Analyses

2.6.1. Instrumental Color Analysis

Instrumental color measurements were performed using a Delta Vista 450G colorimeter (Delta Color, São Paulo, Brazil) operating in the CIELAB system. The parameters recorded were L* (lightness), a* (red–green coordinate), and b* (yellow–blue coordinate).

For opacity determination, measurements were conducted against both black and white standard backgrounds, following the methodology described by Fakhouri and collaborators [32], with modifications. Three replicates were performed for each sample. Film opacity (%) was calculated using Equation (1):

$$Opacity\ (\%) = (Op_B/Op_W) \times 100 \quad (1)$$

where:

Op_B = film opacity measured against a black background;

Op_W = film opacity measured against a white background.

2.6.2. pH Determination

For pH analysis, 1 g of each coated shrimp sample was weighed using an analytical balance (Shimadzu, Kyoto, Japan), macerated in a mortar and pestle, and homogenized with 5 mL of distilled water. The homogenate was analyzed using a previously calibrated digital bench-top pH meter (Quimis Q400AS, Diadema, Brazil). After stabilization (approximately 30 s), the pH value was recorded.

2.6.3. Determination of Total Phenolic Compounds in Grape Seed Extract

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method, based on the oxidation–reduction reaction between phenolic compounds and the Folin reagent under alkaline conditions [33]. Briefly, the grape seed extract was appropri-

ately diluted in ethanol. An aliquot of the diluted extract was reacted with Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), followed by the addition of sodium carbonate solution (Vetec Química Fina Ltd., Rio de Janeiro, Brazil). After incubation under controlled conditions, absorbance was measured using a UV–Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at the appropriate wavelength.

A calibration curve was constructed using gallic acid as the standard (Sigma-Aldrich, St. Louis, MO, USA). The relationship between absorbance (Y) and gallic acid concentration (X) was described by the linear Equation (2):

$$Y = ax + b \quad (2)$$

where:

Y = absorbance;

x = gallic acid concentration;

a = slope of the calibration curve;

b = intercept.

The coefficient of determination (R^2) was required to be $\geq$0.98. Results were expressed as milligrams of gallic acid equivalents per 100 g of sample (mg GAE·100 g^{-1}). When using spectrophotometers without integrated calibration software, absorbance values were plotted against known gallic acid concentrations to obtain the linear regression equation. This equation was subsequently applied to calculate the total phenolic content of the samples.

2.7. Film Characterization

Film preparation was carried out according to Espitia and collaborators [34] using the casting method. The film-forming solutions were poured onto leveled acrylic plates (local manufacturer, Mossoró, Brazil) and dried in a forced-air circulation oven at 50 °C for approximately 6–8 h, depending on the formulation, until complete solvent evaporation. After drying, the films were carefully detached from the plates and conditioned prior to analysis. The films were then evaluated for thickness, water vapor permeability, mechanical properties, and surface morphology by scanning electron microscopy (SEM).

2.7.1. Thickness

Film thickness was measured using a digital micrometer (digital micrometer 0–25 mm: Mitutoyo, Kawasaki, Japan; accuracy ±0.001 mm). Measurements were taken at five random points on each film sample (center and four peripheral positions), following Luchese and collaborators [35]. Results were expressed as the mean thickness (mm).

2.7.2. Water Vapor Permeability (WVP)

Water vapor permeability was determined using a gravimetric method based on the mass loss of water through the film. Circular film samples (2 cm × 2 cm) were sealed over the opening of PVC permeability cells (custom-built, local workshop, Mossoró, Brazil) containing 6 mL of distilled water. Each cell was fitted with a rubber gasket and tightly sealed with a lid to ensure that water vapor transmission occurred exclusively through the film surface [36]. The initial mass (Shimadzu, Kyoto, Japan) of the assembled system (cell + water + film) was recorded. The assemblies were placed in a desiccator (Permution, Curitiba, Brazil) maintained at 50% relative humidity and 29 °C. The cells were weighed at 1 h intervals for a total period of 8 h. The mass loss over time corresponded to water vapor transmission through the film. Water vapor transmission rate (WVTR) was calculated using Equation (3):

$$WVTR = W/A \cdot t \quad (3)$$

where:

W = mass of water lost (g);

A = exposed permeation area (m^2);

t = permeation time (s).

A linear regression of mass loss versus time was performed, and the slope of the curve was used to determine the water vapor transmission rate ($g \cdot s^{-1}$).

2.7.3. Water Solubility

Filter papers (Whatman No. 1, GE Healthcare, Chicago, IL, USA) and film samples (7 cm × 4 cm) were dried in a forced-air circulation oven at 105 °C for 4 h. After drying, samples were cooled to room temperature in a desiccator and their initial mass (m_1) was recorded.

Following adaptations of the methodologies described by Rhim and collaborators [37], the dried films were placed in Erlenmeyer flasks containing 50 mL of distilled water and agitated for 24 h under gentle stirring (100–180 rpm) using a TE-140 (Tecnal, Piracicaba, Brazil) orbital shaker. After agitation, the insoluble film residues were recovered by filtration. The filter paper containing the residual film material was dried again in a forced-air oven at 105 °C until constant mass was achieved. The samples were then cooled in a desiccator and weighed to obtain the final mass (m_2). Drying time varied depending on the formulation. Five replicates were performed for each film formulation. Water solubility (%) was calculated using Equation (4):

$$S\ (\%) = ((m_1 - m_2)/m_1) \times 100 \quad (4)$$

where:

m_1 = initial dry mass of the film (g);

m_2 = final dry mass after immersion (g).

2.7.4. Color and Opacity of the Films

The color parameters of the films were measured using a Delta Vista 450G colorimeter operating (Delta Color, São Paulo, Brazil) in the CIELAB system. The parameters recorded were L* (lightness), a* (red–green coordinate), and b* (yellow–blue coordinate). Film opacity was determined by measuring the film against both black and white standard backgrounds, following Fakhouri and collaborators [32], with three replicates per sample. Opacity (%) was calculated using Equation (5):

$$Op\ (\%) = (Op_B/Op_W) \times 100 \quad (5)$$

where:

Op = film opacity (%);

Op_B = opacity measured against a black background;

Op_W = opacity measured against a white background.

2.7.5. Mechanical Properties

Mechanical properties were determined according to ASTM D882-91 (1996). Tests were performed in quintuplicate for each formulation. Film samples were cut into strips measuring 6 cm in length and 0.5 cm in width. The strips were positioned between the grips of a universal testing machine (DI 5000/1000: EMIC, São José dos Pinhais, Brazil). The mechanical tests were performed under the following conditions: an initial grip separation (gauge length) of 50 mm and a crosshead (traverse) speed of 1.0 $mm \cdot s^{-1}$, in accordance with ASTM D882-91 (1996) recommendations for thin films. Tensile force was applied at a

constant rate until film rupture occurred. From the obtained data, stress–strain curves were generated to determine tensile strength and elongation at break. Tensile strength (σ, MPa) was calculated using Equation (6):

$$\sigma = F_{max}/A \quad (6)$$

where:

σ = tensile strength (MPa);

F_{max} = maximum force at rupture (N);

A = cross-sectional area of the film (m^2).

Elongation at break (ε, %) was calculated using Equation (7):

$$\varepsilon\ (\%) = (L - L_0/L_0) \times 100 \quad (7)$$

where:

L_0 = initial gauge length (m);

L = length at rupture (m).

2.7.6. Scanning Electron Microscopy (SEM)

The microstructure of the films was analyzed using a VEGA 3 LMU scanning electron microscope (TESCAN, Brno, Czech Republic) operated at an accelerating voltage of 15 kV. Prior to analysis, film samples were cut into small sections and mounted onto aluminum stubs (Ted Pella Inc., Redding, CA, USA). The samples were sputter-coated with a thin layer of gold for 360 s at 20 mA using a Q150R vacuum sputter coater (Quorum Technologies, Lewes, UK) to improve electrical conductivity. Both surface and cross-sectional morphologies were examined. Cross-sections were obtained by fracturing the films after immersion in liquid nitrogen (White Martins, Rio de Janeiro, Brazil) to ensure a clean and brittle break. Micrographs were acquired at magnifications of 1000×, 4000×, and 6000×. Five replicates were analyzed for each film formulation.

2.7.7. Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) in a split-plot experimental design, in which storage time was considered the subplot factor and film treatment the main plot factor. When significant effects were detected, mean comparisons were performed using Tukey's test at a 5% significance level ($p < 0.05$). Statistical analyses were conducted using SISVAR software v.5.6 (Universidade Federal de Lavras, Lavras, Brazil), and graphs were generated using SigmaPlot v.12.5 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Microbiological Analyses

The counts of mesophilic aerobic bacteria in shrimp samples coated with different formulations during refrigerated storage at 7 °C are shown in Table 2. At day 0, significant differences were observed among treatments. The lowest initial counts were recorded for QFE (5.5906 $\log_{10}$ CFU g^{-1}) and QE (5.8720 $\log_{10}$ CFU g^{-1}), followed by FE (6.0396 $\log_{10}$ CFU g^{-1}) and F (6.2749 $\log_{10}$ CFU g^{-1}). Higher values were observed for the control (6.5367 $\log_{10}$ CFU g^{-1}), Q (6.6988 $\log_{10}$ CFU g^{-1}), and E (6.4040 $\log_{10}$ CFU g^{-1}), indicating variability in the initial microbial load among treatments.

After 3 days of storage at 7 °C, microbial growth increased in all samples; however, statistically significant differences remained. The lowest bacterial counts were observed for FE (6.1729 $\log_{10}$ CFU g^{-1}) and QFE (6.1972 $\log_{10}$ CFU g^{-1}), followed by QE (6.5047 $\log_{10}$ CFU g^{-1}) and F (6.4568 $\log_{10}$ CFU g^{-1}). The control presented a

higher value (6.8360 $\log_{10}$ CFU g^{-1}), while intermediate counts were observed for Q (6.7144 $\log_{10}$ CFU g^{-1}), QF (6.3902 $\log_{10}$ CFU g^{-1}), and E (6.4239 $\log_{10}$ CFU g^{-1}).

Table 2. Counts of mesophilic aerobic bacteria in coated shrimp samples during refrigerated storage at 7 °C (0, 3, and 6 days). Values are expressed as $\log_{10}$ CFU g^{-1} (mean ± standard deviation). Data were analyzed by ANOVA in a split-plot design followed by Tukey's test ($p < 0.05$). Different lowercase letters in the same column indicate statistically significant differences; means sharing at least one letter do not differ significantly.

Treatment	Day 0	Day 3	Day 6
C	6.5367 ± 0.16 ^{e}	6.836 ± 0.08 ^{d}	7.9010 ± 0.05 ^{d}
Q	6.6988 ± 0.09 ^{ab}	6.7144 ± 0.20 ^{d}	7.7666 ± 0.13 ^{cd}
QE	5.872 ± 0.07 ^{bc}	6.5047 ± 0.09 ^{cd}	7.5759 ± 0.08 ^{abc}
F	6.2749 ± 0.17 ^{de}	6.4568 ± 0.11 ^{bcd}	7.5962 ± 0.10 ^{ab}
FE	6.0396 ± 0.04 ^{cd}	6.1729 ± 0.12 ^{a}	7.3238 ± 0.17 ^{a}
QFE	5.5906 ± 0.09 ^{a}	6.1972 ± 0.12 ^{ab}	7.3166 ± 0.10 ^{a}
QF	6.3980 ± 0.15 ^{e}	6.3902 ± 0.21 ^{abc}	7.4913 ± 0.14 ^{ab}
E	6.4040 ± 0.12 ^{e}	6.4239 ± 0.24 ^{abc}	7.6136 ± 0.09 ^{bc}

Abbreviations: C = control; Q = chitosan; QE = chitosan + extract; F = cassava starch; FE = cassava starch + extract; QFE = chitosan + starch + extract; QF = chitosan + starch; E = extract.

On day 6 of refrigerated storage at 7 °C, bacterial counts continued to increase in all treatments. The lowest values were recorded for QFE (7.3166 $\log_{10}$ CFU g^{-1}) and FE (7.3238 $\log_{10}$ CFU g^{-1}), followed by QF (7.4913 $\log_{10}$ CFU g^{-1}) and F (7.5962 $\log_{10}$ CFU g^{-1}). Higher counts were observed for QE (7.5759 $\log_{10}$ CFU g^{-1}), Q (7.7666 $\log_{10}$ CFU g^{-1}), and the control (7.9010 $\log_{10}$ CFU g^{-1}). These results indicate that films containing chitosan, cassava starch, and grape seed extract were more effective in controlling microbial growth during storage.

Regarding chitosan-based films, the addition of grape seed extract reduced bacterial counts, as QE (7.5759 $\log_{10}$ CFU g^{-1}) showed lower values than Q (7.7666 $\log_{10}$ CFU g^{-1}) on day 6. Overall, microbial growth increased during storage but remained higher in the uncoated control samples throughout the evaluation period (Table 2).

Among the 40 shrimp samples analyzed, all showed growth of *Staphylococcus* spp., and seven samples exhibited colonies morphologically compatible with coagulase-positive *Staphylococcus*. However, after performing the coagulase test, all results were negative, indicating the absence of coagulase-positive *Staphylococcus* in the analyzed samples.

3.2. Physicochemical Analysis of Coated Shrimp

3.2.1. Color

The visual appearance of shrimp subjected to different film treatments and stored under refrigeration (7 °C) for six days is presented in Figure 1. No pronounced discoloration or surface darkening was observed among treatments during storage. All samples maintained a characteristic translucent-whitish appearance, although slight differences in brightness and opacity were noticeable at the end of the storage period, particularly for the control samples.

For the luminosity parameter (L*), statistically significant differences were observed among treatments throughout storage (Figure 2). At day 0, L* values ranged approximately from 42.7 to 46.8, with higher luminosity observed for treatments containing cassava starch and extract-based films. After three days of storage at 7 °C, a general reduction in L* values was observed, reaching values close to 38.0–44.0 depending on the formulation. The lowest luminosity at this time was observed for treatments containing chitosan and cassava starch blends, while shrimp coated with grape seed extract combined with starch or chitosan

maintained higher L* values (approximately 43–44). On day 6, a slight recovery in brightness was observed for some treatments, particularly QE and QFE, with L* values around 44.4 and 41.4, respectively. Despite these fluctuations, all treatments remained within a narrow range, indicating limited visual changes in brightness during refrigerated storage.

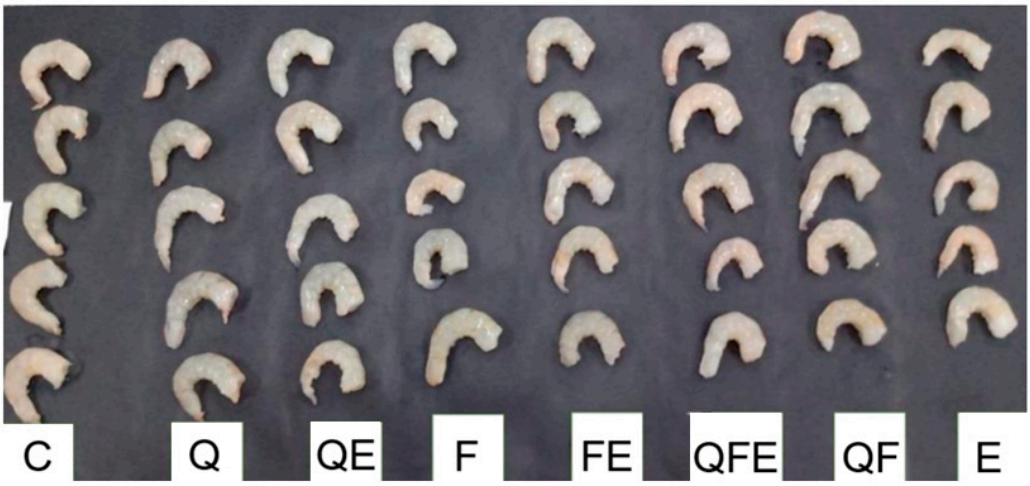


Figure 1. Visual appearance of shrimp samples coated with different formulations (C, Q, QE, F, FE, QFE, QF, and E) during refrigerated storage at 7 °C for six days. Abbreviations: C = control; Q = chitosan; QE = chitosan + extract; F = cassava starch; FE = cassava starch + extract; QFE = chitosan + starch + extract; QF = chitosan + starch; E = extract.

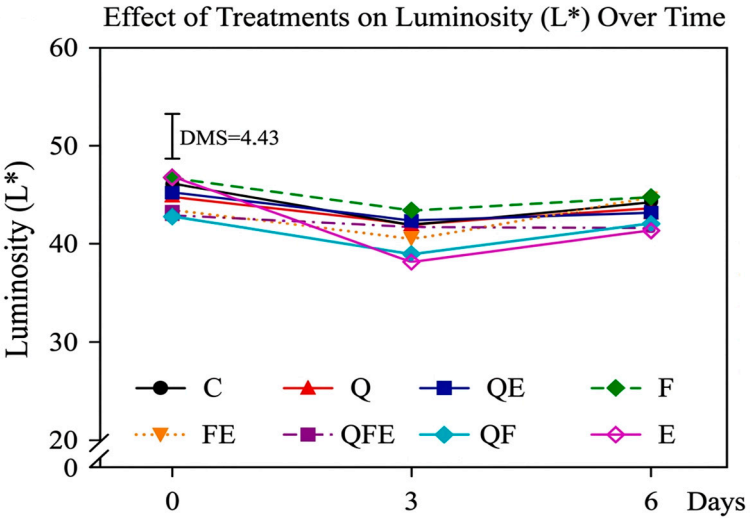


Figure 2. Changes in luminosity (L*) of shrimp coated with different formulations during refrigerated storage at 7 °C for 0, 3, and 6 days. Values represent mean measurements. Different treatments are indicated in the legend. Abbreviations: C = control; Q = chitosan; QE = chitosan + extract; F = cassava starch; FE = cassava starch + extract; QFE = chitosan + starch + extract; QF = chitosan + starch; E = extract.

The evolution of the a* coordinate, representing the red–green axis, is shown in Figure 3. At day 0, coated shrimp exhibited positive a* values ranging approximately from 0.5 to 2.0, while the control showed negative values near −2.5, indicating a greener tonal tendency in uncoated samples. After three days of storage, most treatments showed an increase in a* values, reaching approximately 3.0–4.5, with the highest values observed for films containing chitosan and starch blends. This increase suggests a slight intensification of reddish coloration during storage. On day 6, a reduction in a* values was observed for most treatments, remaining between approximately 2.0 and 3.2. The QE treatment showed

the highest value on the last storage day, whereas the control remained relatively stable and negative throughout the storage period.

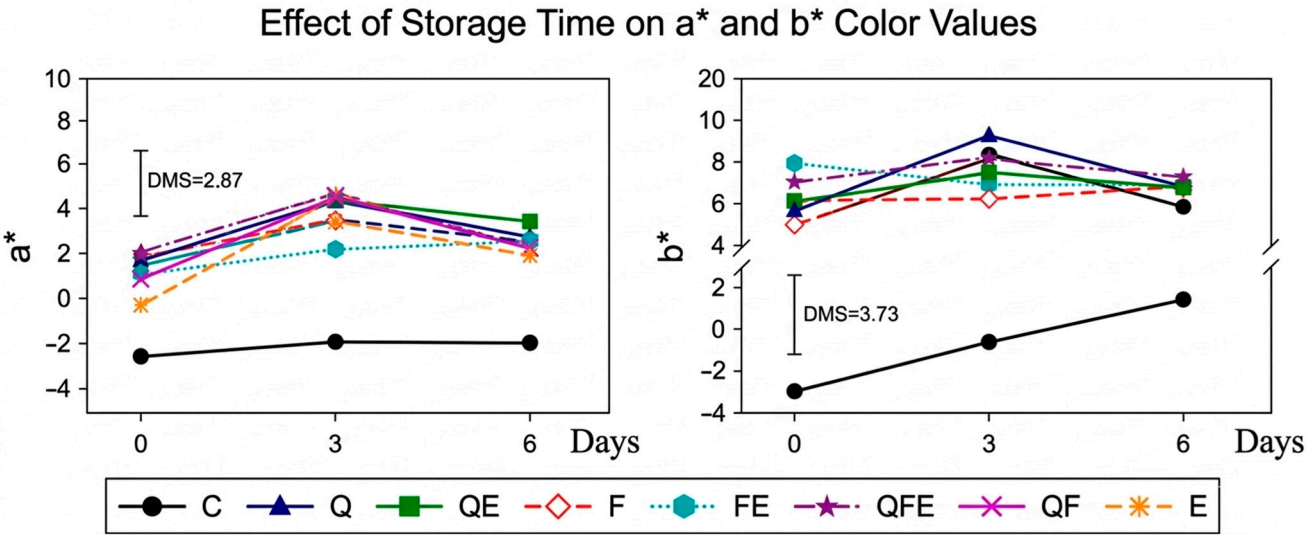


Figure 3. Changes in color coordinates (a* and b*) of shrimp coated with different formulations during refrigerated storage at 7 °C for 0, 3, and 6 days. Values represent mean measurements. Data points correspond to discrete sampling times, and the connecting lines are included only as visual guides to facilitate comparison among treatments over time; they do not represent continuous measurements. Abbreviations: C = control; Q = chitosan; QE = chitosan + extract; F = cassava starch; FE = cassava starch + extract; QFE = chitosan + starch + extract; QF = chitosan + starch; E = extract.

Regarding the b* coordinate (yellow–blue axis), differences among treatments were also observed (Figure 3). At day 0, films containing grape seed extract exhibited higher b* values, ranging approximately from 7.0 to 8.0, while the control presented the lowest value, near −3.0. After three days of refrigerated storage, most coated samples showed an increase in b* values, with QE reaching approximately 9.0, followed by FE and QF with values close to 8.0–8.5. On day 6, a slight reduction in b* values was observed for most treatments, remaining between 6.0 and 7.5, whereas the control increased slightly to values near 1.5 but remained lower than coated samples.

Although instrumental color parameters (L*, a*, and b*) exhibited statistical variations during refrigerated storage at 7 °C, these changes were relatively small and consistent with the visual evaluation (Figure 1). Films containing chitosan, cassava starch, and grape seed extract contributed to maintaining brightness and color stability, particularly by preserving higher L* values and positive a* and b* coordinates compared with the uncoated control during the six-day storage period.

3.2.2. pH

The pH values of shrimp coated with different polymeric formulations during refrigerated storage at 7 °C are presented in Table 3. On day 0, the pH values of all treatments were close to neutrality, ranging from 6.634 to 6.880, with no statistically significant differences among samples ($p > 0.05$). The lowest initial pH was observed for the chitosan film (Q, 6.634 ± 0.05), followed by QF (6.740 ± 0.04), whereas the highest values were recorded for treatments containing cassava starch, namely F (6.880 ± 0.13) and FE (6.880 ± 0.08). The control sample presented an intermediate value (6.866 ± 0.13).

After three days of storage at 7 °C, a general decrease in pH was observed for most treatments (Table 3). The most pronounced reduction occurred in the QFE treatment, which decreased from 6.870 ± 0.07 on day 0 to 5.414 ± 0.06 on day 3. Reductions were also

observed for QF (6.740 ± 0.04 to 6.320 ± 0.06) and Q (6.634 ± 0.05 to 6.540 ± 0.05). In contrast, treatments containing cassava starch maintained comparatively higher pH values, particularly F (6.810 ± 0.06) and FE (6.736 ± 0.17). The control sample also showed a slight decrease, reaching 6.836 ± 0.18 on day 3.

Table 3. pH values of shrimp samples coated with different polymeric formulations during refrigerated storage at 7 °C for 0, 3, and 6 days. Values are expressed as mean ± standard deviation. Data were analyzed by ANOVA in a split-plot design followed by Tukey's test ($p < 0.05$). Different lowercase superscript letters in the same column indicate statistically significant differences; means sharing at least one letter do not differ significantly.

Treatment	Day 0 (pH)	Day 3 (pH)	Day 6 (pH)
C	6.866 ± 0.13 ^{a}	6.836 ± 0.18 ^{b}	6.630 ± 0.08 ^{a}
Q	6.634 ± 0.05 ^{a}	6.540 ± 0.05 ^{ab}	6.508 ± 0.05 ^{a}
QE	6.814 ± 0.09 ^{a}	6.624 ± 0.01 ^{ab}	6.608 ± 0.01 ^{a}
F	6.880 ± 0.13 ^{a}	6.810 ± 0.06 ^{b}	6.718 ± 0.05 ^{a}
FE	6.880 ± 0.08 ^{a}	6.736 ± 0.17 ^{b}	6.884 ± 0.51 ^{a}
QFE	6.870 ± 0.07 ^{a}	5.414 ± 0.06 ^{a}	6.678 ± 0.07 ^{a}
QF	6.740 ± 0.04 ^{a}	6.320 ± 0.06 ^{ab}	6.732 ± 0.02 ^{a}
E	6.808 a ± 0.05 ^{a}	6.688 ± 0.07 ^{b}	6.682 ± 0.08 ^{a}

Abbreviations: C = control; Q = chitosan; QE = chitosan + extract; F = cassava starch; FE = cassava starch + extract; QFE = chitosan + starch + extract; QF = chitosan + starch; E = extract.

On day 6, the pH values increased slightly for some treatments, particularly those that had shown a marked reduction on day 3. The QFE treatment increased to 6.678 ± 0.07, while QF rose to 6.732 ± 0.02. The highest pH value on the final storage day was observed for FE (6.884 ± 0.51), followed by QF (6.732 ± 0.02) and F (6.718 ± 0.05). The lowest value at day 6 corresponded to the chitosan film (Q, 6.508 ± 0.05), indicating that this treatment maintained lower pH values throughout storage. The control sample showed a gradual decrease, reaching 6.630 ± 0.08 at the end of storage.

Overall, the pH of shrimp samples tended to decrease during refrigerated storage, with slight increases observed in some treatments on day 6. Films containing chitosan, particularly when combined with grape seed extract, contributed to lower pH values, while formulations containing cassava starch, especially FE, maintained higher pH levels at the end of storage. These variations remained within a narrow range (5.41–6.88), indicating moderate physicochemical changes during the six-day refrigerated storage period.

3.3. Determination of Total Flavonoid and Phenolic Compounds in the Extract

The determination of total phenolic compounds in the grape seed extract showed a color change from green/yellow to blue after reaction with the Folin–Ciocalteu reagent, indicating the presence of phenolic compounds. Spectrophotometric analysis, based on the gallic acid standard curve, yielded a value of 17.09 g GAE (gallic acid equivalents).

Additionally, the quercetin content was quantified, presenting a value of 0.73 g/L^{-1} in the analyzed extract. These results indicate a high concentration of phenolic compounds and flavonoids in the grape seed extract, with predominance of compounds expressed as gallic acid equivalents.

3.4. Characterization of Polymer Blends and Films

3.4.1. Thickness, Solubility, and Water Vapor Permeability (WVP)

The thickness, solubility, and water vapor permeability (WVP) of the developed films are presented in Table 4. Film thickness varied depending on the biopolymer composition. The cassava starch film (F) showed the lowest thickness value, indicating the formation of thinner structures compared with the other formulations. In contrast, chitosan-based

films exhibited greater thickness, particularly QE, which presented one of the highest values, followed by Q. No statistically significant differences were observed between QE and QF, suggesting that the addition of cassava starch to chitosan–extract systems did not substantially alter film thickness. These results indicate that chitosan contributes to thicker film formation, whereas starch favors thinner structures. Overall, these findings are consistent with expected behavior for biopolymer films, in which starch matrices tend to form more compact and thinner structures, while chitosan contributes to increased film thickness due to its molecular organization and film-forming capacity.

Table 4. Thickness, solubility, and water vapor permeability (WVP) of the produced films. Values are expressed as mean ± standard deviation. Data were analyzed by ANOVA in a split-plot design followed by Tukey's test ($p < 0.05$). Different lowercase superscript letters in the same column indicate statistically significant differences; means sharing at least one letter do not differ significantly.

Treatment	Thickness (nm)	Solubility (%)	WVP ($g/m^2 \cdot h$)
Q	0.074760 ^{b}	16.852922 ^{a}	62.180747 ^{c}
QE	0.077880 ^{b}	61.650606 ^{c}	48.821218 ^{b}
F	0.047200 ^{a}	34.438192 ^{b}	36.640472 ^{a}
FE	0.064480 ^{ab}	31.798317 ^{b}	51.178782 ^{b}
QFE	0.072120 ^{ab}	32.953048 ^{b}	57.662083 ^{ab}
QF	0.077880 ^{b}	20.2628886 ^{a}	49.901768 ^{b}

Abbreviations: Q = chitosan; QE = chitosan + extract; F = cassava starch; FE = cassava starch + extract; QFE = chitosan + starch + extract; QF = chitosan + starch.

Regarding water vapor permeability, significant differences were observed among treatments (Table 4). Overall, the obtained values were consistent with expectations for hydrophilic and biodegradable polymeric films. The starch film (F) exhibited the lowest WVP value, indicating superior barrier properties against moisture transfer. Conversely, the chitosan film (Q) showed higher WVP values, suggesting greater permeability to water vapor. The incorporation of grape seed extract influenced WVP depending on the polymer matrix. In chitosan-based films, the addition of extract reduced WVP from approximately 62.18 to 48.82, indicating improved barrier properties. However, in starch-containing films, the addition of extract resulted in increased WVP values, suggesting that extract incorporation disrupted the starch matrix and facilitated water vapor diffusion. Blended films containing both polymers showed intermediate behavior, reflecting the combined structural characteristics of chitosan and starch. When compared to conventional commercial plastic packaging materials, which typically exhibit significantly lower WVP, the values obtained in this study are higher, as expected. Nevertheless, within the context of edible and biodegradable films, formulations such as F and QE demonstrated improved barrier performance, indicating a positive contribution to moisture control. Importantly, no deterioration in barrier properties was observed; instead, the results indicate that polymer blending and extract incorporation can modulate and, in some cases, enhance film functionality.

Film solubility in water also differed significantly among formulations, as revealed by ANOVA in a split-plot design followed by Tukey's test ($p < 0.05$). Chitosan films exhibited lower solubility compared with starch-based films, indicating greater structural resistance to water. However, the addition of grape seed extract significantly increased the solubility of chitosan-based films, reaching approximately 61.65% for QE. This increase suggests that phenolic compounds present in the extract may interfere with polymer–polymer interactions, promoting greater water uptake and dissolution. In contrast, starch-based formulations (F and FE) showed no statistically significant differences in solubility, indicating that extract incorporation did not markedly alter the solubility of starch films. The

blended systems presented intermediate solubility values, reflecting the balance between the hydrophilic nature of starch and the comparatively lower solubility of chitosan. From an application perspective, this increase in solubility, particularly in extract-containing chitosan films, may represent a limitation for certain uses requiring higher water resistance. However, it may also be advantageous for edible films, where partial solubility is desirable. Overall, no unexpected deterioration was observed, but rather a functional trade-off between solubility and barrier properties.

The results indicate that starch contributed to thinner films with lower WVP, while chitosan increased film thickness and reduced solubility. The incorporation of grape seed extract modified the structural properties of the films, reducing WVP in chitosan matrices but increasing solubility, particularly in chitosan-based formulations. These effects demonstrate that both polymer composition and extract incorporation strongly influence the barrier and physicochemical properties of the developed films (Table 4). Taken together, the results are in agreement with the expected behavior of such systems and demonstrate an overall improvement in functional properties when compared to untreated or single-component films, supporting their potential application as active biodegradable films.

3.4.2. Optical Properties

The optical properties of the polymeric films varied according to formulation, as shown in Figures 4 and 5. Differences in color coordinates (a* and b*) indicate that the incorporation of chitosan, cassava starch, and grape seed extract modified the visual appearance of the films.

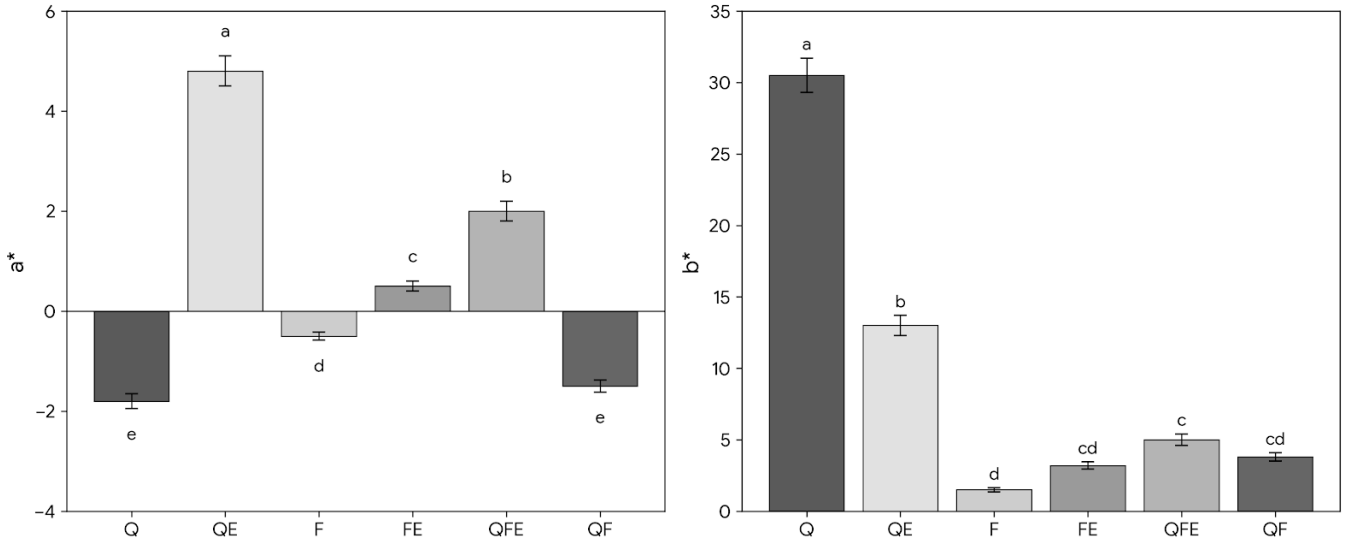


Figure 4. Color coordinates (a* and b*) of polymeric films based on chitosan, cassava starch, and grape seed extract. Values represent mean measurements. Different letters above the bars indicate statistically significant differences among treatments ($p < 0.05$). Abbreviations: Q, chitosan; QE, chitosan + extract; F, cassava starch; FE, cassava starch + extract; QFE, chitosan + starch + extract; QF, chitosan + starch.

Regarding the a* coordinate (red–green axis), significant differences were observed among treatments (Figure 4). The QE film exhibited the highest a* value (approximately +4.8), indicating a more pronounced reddish coloration associated with the presence of grape seed extract in the chitosan matrix. The QFE film showed an intermediate positive value (around +2.0), whereas FE presented a slightly positive value close to +0.5. In contrast, films containing only cassava starch (F) or the chitosan–starch blend (QF) showed negative a* values (approximately −0.5 and −1.5, respectively), indicating a tendency

toward greenish tones. The chitosan film (Q) also showed a negative value (around −1.8), suggesting that the reddish coloration was mainly promoted by extract incorporation.

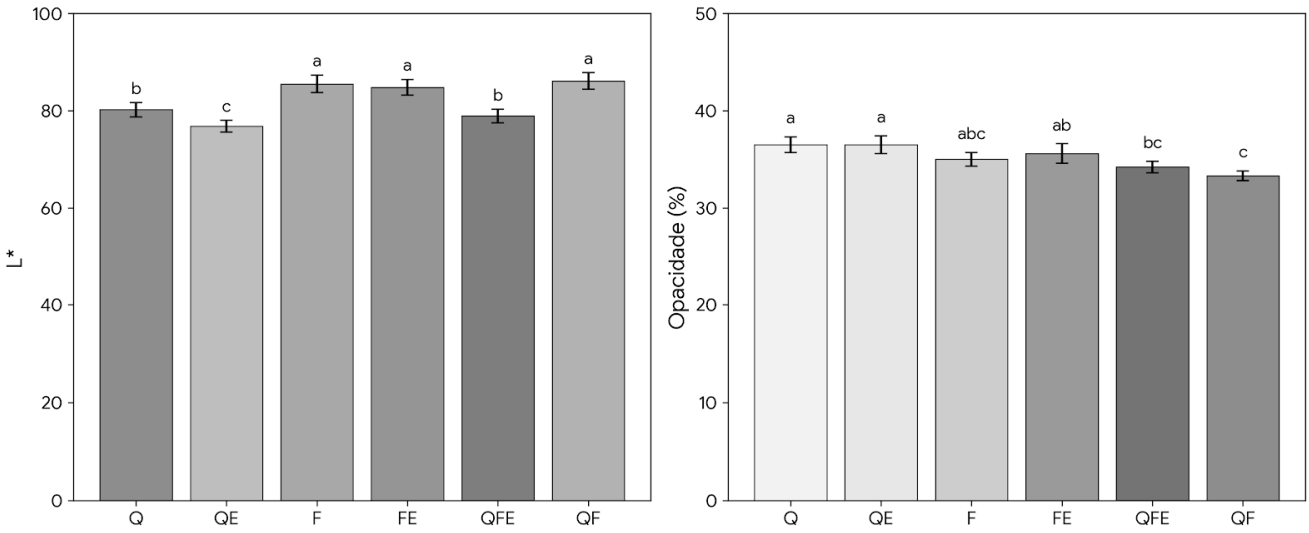


Figure 5. Luminosity (L*) and opacity (%) of polymeric films based on chitosan, cassava starch, and grape seed extract. Values represent mean measurements. Different letters above the bars indicate statistically significant differences among treatments ($p < 0.05$). Abbreviations: Q, chitosan; QE, chitosan + extract; F, cassava starch; FE, cassava starch + extract; QFE, chitosan + starch + extract; QF, chitosan + starch.

For the b* coordinate (yellow–blue axis), the chitosan film (Q) exhibited the highest value, approximately 30, indicating a strong yellowish coloration (Figure 4). The QE formulation presented the second highest value (around 13), while intermediate values were observed for QFE (~5) and QF (~4). The lowest b* values were observed for starch-based films, particularly F (~1.5) and FE (~3), indicating less intense yellow coloration. These results demonstrate that chitosan contributes to yellowish tones, whereas starch produces lighter and less saturated colors.

Luminosity (L*) values ranged from approximately 78 to 88, depending on formulation (Figure 5). The QE film exhibited the lowest luminosity value (~78), indicating a darker appearance. In contrast, starch-containing films such as F, FE, and QF showed the highest L* values (approximately 86–88) and were statistically similar, reflecting lighter and more transparent films. The Q and QFE formulations showed intermediate luminosity values (~80–82). These results indicate that starch promotes brighter films, while the incorporation of grape seed extract, particularly in chitosan-based matrices, reduces luminosity.

Film opacity ranged approximately from 33% to 37% (Figure 5). The highest opacity values were observed for Q and QE (around 36–37%), which were statistically similar, indicating lower transparency. Intermediate opacity values were observed for F and FE (~35–36%), while QFE presented slightly lower values (~34%). The QF formulation exhibited the lowest opacity (~33%), indicating the most transparent film among the tested compositions. These results suggest that the combination of chitosan and starch improves film transparency, whereas the presence of extract slightly increases opacity.

Thus, the incorporation of grape seed extract intensified color (higher a* values), reduced luminosity, and slightly increased opacity, particularly in chitosan-based films. Starch-based matrices produced lighter films with higher L* values and lower color saturation. The optical properties observed demonstrate that polymer composition strongly influences visual appearance, which is relevant for food packaging applications, as films with higher opacity may provide improved protection against light-induced degradation, while higher luminosity and lower opacity favor product visibility (Figures 4 and 5).

3.4.3. Mechanical Properties

The mechanical properties of the polymeric films, including tensile strength, Young's modulus, and elongation at break, are presented in Figure 6A–C. These parameters were evaluated to determine the structural performance and mechanical resistance of the developed matrices.

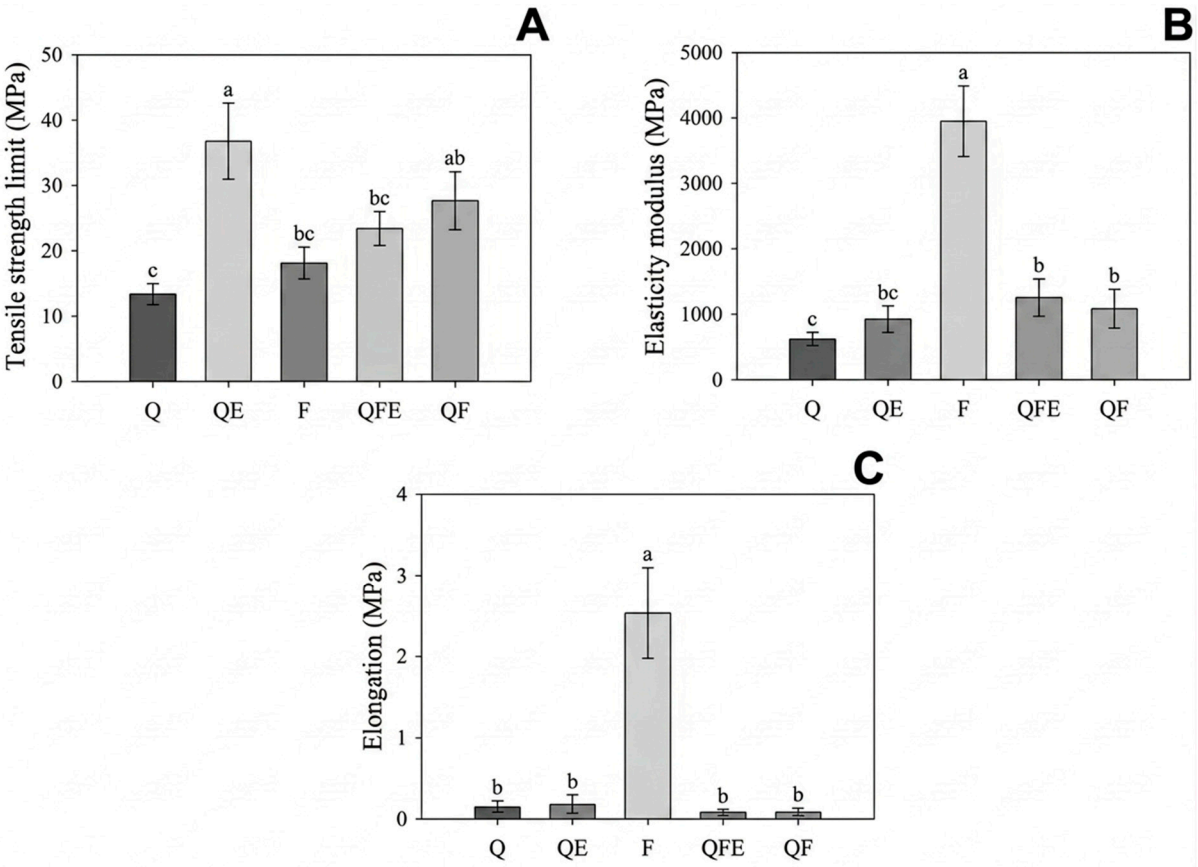


Figure 6. Mechanical properties of polymeric films based on chitosan, cassava starch, and grape seed extract: (**A**) tensile strength (MPa), (**B**) Young's modulus (MPa), and (**C**) elongation at break (%). Values represent mean measurements. Different letters above the bars indicate statistically significant differences among treatments ($p < 0.05$). Abbreviations: Q, chitosan; QE, chitosan + extract; F, cassava starch; FE, cassava starch + extract; QFE, chitosan + starch + extract; QF, chitosan + starch.

Tensile strength values ranged approximately from 13 to 37 MPa (Figure 6A), showing significant differences among formulations. The QE film exhibited the highest tensile strength (≈36–37 MPa), indicating improved resistance after incorporation of grape seed extract into the chitosan matrix. The QF blend showed intermediate values (≈28 MPa), followed by QFE (≈23–24 MPa) and the starch film (F, ≈18–19 MPa). The lowest tensile strength was observed for the chitosan film (Q), with values close to 13 MPa. These results suggest that the addition of extract improved mechanical resistance in chitosan-based films, whereas starch-based and blended films presented intermediate strength values.

Young's modulus values showed greater variability among treatments (Figure 6B), ranging from approximately 600 to 4000 MPa. The cassava starch film (F) exhibited the highest modulus (≈3900–4000 MPa), indicating a more rigid and less flexible structure. Intermediate values were observed for QFE (≈1200 MPa) and QF (≈1100 MPa), while QE presented slightly lower rigidity (≈900 MPa). The lowest modulus was observed for the chitosan film (Q), with values around 600 MPa. These results indicate that starch

contributes to increased film stiffness, whereas chitosan produces more flexible matrices. The blends exhibited intermediate rigidity, reflecting the combined effects of both polymers.

Elongation at break values were generally low for most formulations, indicating limited flexibility (Figure 6C). The starch film (F) showed the highest elongation (≈2.5%), significantly higher than the other treatments. In contrast, Q, QE, QFE, and QF presented very low elongation values, all below 0.3%, suggesting brittle behavior. The incorporation of extract did not substantially increase flexibility in chitosan-based films. These findings indicate that starch improved the deformation capacity of the films, while chitosan-based matrices remained more rigid and prone to rupture.

Overall, the mechanical properties demonstrate that polymer composition strongly influenced tensile strength, rigidity, and flexibility of the films. The incorporation of grape seed extract increased tensile strength in chitosan-based matrices, while starch contributed to higher stiffness and greater elongation at break. Blended formulations presented intermediate mechanical behavior, reflecting modifications in polymer chain interactions and structural organization (Figure 6).

3.4.4. Scanning Electron Microscopy (SEM)

The surface and cross-sectional morphologies of the produced films and polymeric blends were evaluated by scanning electron microscopy, as shown in Figure 7A–C. The micrographs revealed structural differences depending on polymer composition and the incorporation of grape seed extract.

The cross-sectional morphology of cassava starch-based films is presented in Figure 7A. The starch film without extract exhibited a relatively compact structure with visible stratification and some discontinuities along the thickness. In contrast, the film containing grape seed extract showed a more heterogeneous internal organization, with irregular layers and the presence of small voids. These empty spaces suggest incomplete matrix cohesion and possible phase separation induced by the extract. The addition of extract therefore modified the internal structure of the starch matrix, increasing roughness and structural irregularity.

Surface morphology of chitosan-based films is shown in Figure 7B. The chitosan film without extract presented a smoother and more homogeneous surface, with fewer cracks and defects. When grape seed extract was incorporated, the surface became more irregular, showing increased roughness and localized porous regions. These morphological changes indicate that phenolic compounds from the extract interfered with chitosan chain interactions, promoting heterogeneous phase formation. Despite these irregularities, the films maintained structural continuity, suggesting acceptable matrix cohesion.

The cross-sectional morphology of chitosan–cassava starch blends is presented in Figure 7C. The blend containing extract exhibited a heterogeneous structure with visible granules and layered regions, suggesting incomplete dissolution and partial incompatibility among components. Multiple phases could be distinguished, indicating the coexistence of chitosan, starch, and extract domains. In contrast, the blend without extract showed a more compact and stratified morphology, with fewer voids and better structural uniformity. These results indicate that the incorporation of extract increased phase separation and reduced homogeneity in the blended films.

Finally, films produced with a single biopolymer tended to exhibit more homogeneous surfaces, whereas blended formulations showed multiphase structures. The addition of grape seed extract increased roughness and porosity in both starch and chitosan matrices and promoted phase separation in polymer blends. These morphological differences are consistent with the mechanical and barrier properties observed for the films, as structural heterogeneity and void formation may influence permeability and mechanical performance (Figure 7).

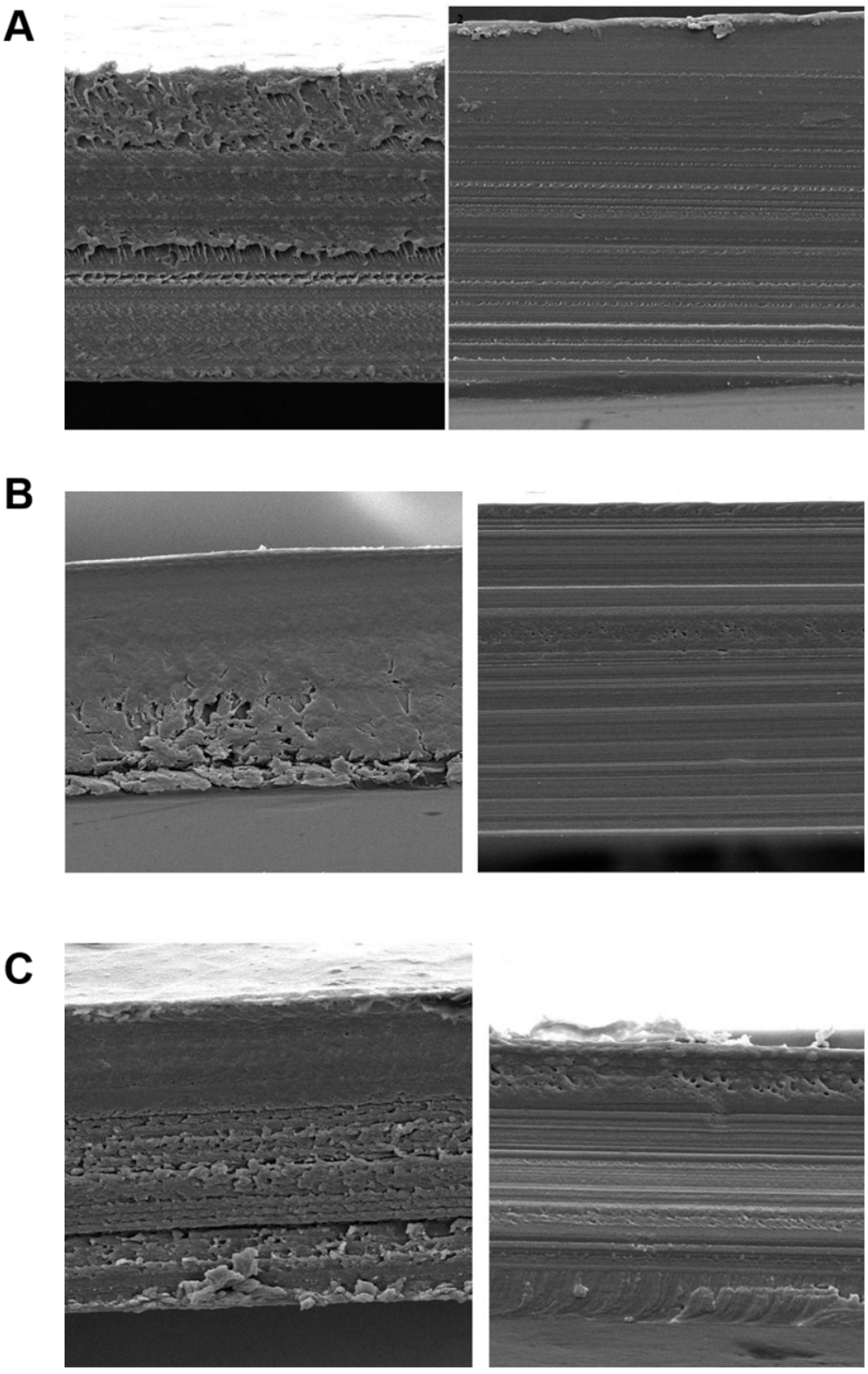


Figure 7. Scanning electron microscopy (SEM) images of the produced films and blends. (**A**) Cross-sectional morphology of cassava starch films with grape seed extract and cassava starch films (thickness of 20 µm) at 3.03 k× magnification. (**B**) Surface morphology of chitosan films with grape seed extract and pure chitosan films (20 µm) at 1.86 k× and 2.51 k× magnifications. (**C**) Cross-sectional morphology of chitosan–cassava starch–extract blends (20 µm) at 3.11 k× and chitosan–cassava starch blends at 3.95 k× magnifications. Images on the left correspond to formulations containing chitosan, cassava starch, and extract, whereas images on the right correspond to chitosan–cassava starch formulations without extract.

4. Discussion

4.1. Microbiological Analyses

The counts of mesophilic aerobic bacteria during refrigerated storage at 7 °C (Table 2) demonstrated a progressive increase in microbial load for all treatments; however, films containing grape seed extract significantly reduced bacterial growth compared to the control. On day 3, the antimicrobial effect of the extract was particularly evident. Treatments containing extract, especially FE (6.1729 ± 0.12 log CFU g^{-1}) and QFE (6.1972 ± 0.12 log CFU g^{-1}), exhibited significantly lower counts than the control (6.836 ± 0.08 log CFU g^{-1}) and chitosan-only film (Q; 6.7144 ± 0.20 log CFU g^{-1}), as determined by ANOVA in a split-plot design followed by Tukey's test ($p < 0.05$). Similarly, QE (6.5047 ± 0.09 log CFU g^{-1}) also showed reduced microbial proliferation relative to the control, indicating that the presence of grape seed extract contributed to antimicrobial activity regardless of the polymer matrix.

The antimicrobial effectiveness observed in extract-containing films can be attributed to the high concentration of phenolic compounds in grape seeds, including flavonoids, tannins, and phenolic acids. These compounds are known to disrupt microbial cell membranes, increase permeability, and interfere with essential cellular processes, leading to leakage of intracellular components and enzyme inactivation [38–40]. Phenolics may also interact with membrane proteins and phospholipids, causing structural disorganization and impairing nutrient transport mechanisms, thereby limiting bacterial proliferation [41,42].

Although FE and QFE treatments presented lower counts at day 3, microbial growth continued gradually until day 6, suggesting that the films exerted primarily a bacteriostatic rather than bactericidal effect. By day 6, the lowest counts remained associated with QFE (7.3166 ± 0.10 log CFU g^{-1}) and FE (7.3238 ± 0.17 log CFU g^{-1}), both significantly lower than the control (7.9010 ± 0.05 log CFU g^{-1}). Treatments without extract, such as Q (7.7666 ± 0.13 log CFU g^{-1}) and F (7.5962 ± 0.10 log CFU g^{-1}), showed intermediate values, reinforcing the contribution of grape seed extract to microbial inhibition. The gradual increase in microbial counts is expected during refrigerated storage; however, the statistically significant differences observed between coated samples and the control highlight the protective role of the applied films.

The barrier properties of the polysaccharide-based films likely contributed to reduced microbial growth by limiting oxygen diffusion and moisture transfer. This semi-permeable structure creates less favorable conditions for aerobic microbial metabolism and slows spoilage. Additionally, the controlled release of phenolic compounds from the polymeric matrix may have prolonged antimicrobial activity throughout storage, explaining the sustained differences observed between treatments [43].

The enhanced antimicrobial performance observed in QFE treatments suggests a synergistic interaction between chitosan and grape seed extract. Chitosan possesses intrinsic antimicrobial properties due to its polycationic nature, which promotes electrostatic interaction with negatively charged bacterial cell walls, leading to membrane disruption and inhibition of DNA transcription. When combined with plant-derived polyphenols, this effect may be potentiated through multiple simultaneous mechanisms, including oxidative stress induction and chelation of essential metal ions required for microbial metabolism [44]. The lower microbial counts observed for QFE compared to films containing only chitosan (Q) or only extract (E) support this synergistic interaction.

Similar results have been reported in previous studies using polysaccharide-based films enriched with plant extracts. Riaz and collaborators [20] reported enhanced antibacterial efficiency in films combining chitosan with apple peel extract, attributing the improved activity to synergistic interactions between polyphenols and chitosan. Likewise, Na and collaborators [45] demonstrated that films composed of chitosan and plant extracts significantly inhibited mesophilic and psychrotrophic bacteria, thereby extending the shelf life of

refrigerated shrimp. These findings support the hypothesis that multi-component films provide improved antimicrobial effectiveness compared to single-polymer systems.

Additionally, the reduced microbial growth observed in coated shrimp may also be related to the formation of a semi-permeable barrier that limits gas exchange and delays oxidative reactions. The presence of antioxidant phenolic compounds further contributes to preservation by inhibiting lipid oxidation, which otherwise generates substrates that can promote microbial growth [46]. Therefore, the combined antimicrobial and antioxidant effects of grape seed extract, together with the structural barrier provided by the polymeric blends, likely played a key role in maintaining microbiological quality during refrigerated storage.

Taken together, the results presented in Table 2 demonstrate that films incorporating grape seed extract, particularly when combined with chitosan (QFE), provided a multifactorial preservation mechanism involving antimicrobial activity, barrier effects, and antioxidant protection. These combined effects contributed to reduced microbial growth and improved shelf life of refrigerated shrimp, highlighting the potential of active polysaccharide-based films for seafood preservation in line.

4.2. Physicochemical Analysis of Coated Shrimp

4.2.1. Color and Luminosity

Maintaining visual color is critical for seafood products, as they are highly susceptible to biochemical and structural changes during refrigerated storage. Maintaining color is an important factor for shrimp quality, as it is directly related to the perception of freshness and product acceptance [47,48]. Even slight discoloration may lead to product rejection, resulting in commercial losses despite acceptable microbiological quality [49]. Therefore, preserving color stability is directly related to marketability and consumer acceptance [23,50].

The visual appearance of shrimp samples (Figure 1) showed that coated treatments maintained a more uniform coloration throughout storage compared to the control. Samples coated with formulations containing grape seed extract, particularly QE, FE, and QFE, exhibited less surface whitening and dehydration after six days of storage. These observations are consistent with the instrumental color measurements presented in Figures 2 and 3, indicating that edible films contributed to maintaining visual quality.

Luminosity (L*) values (Figure 2) ranged approximately from 38 to 47 during storage, with all treatments showing a slight decrease at day 3 followed by stabilization or a mild increase at day 6. The reduction in L* values observed at day 3 was more pronounced for films without extract, such as F and QF, indicating increased surface dullness. In contrast, treatments containing grape seed extract (QE, FE, and QFE) exhibited smaller variations in luminosity, suggesting improved color stability. Films with lower L* values generally present higher opacity, which reduces light transmission and protects photosensitive pigments from degradation [51]. This protective effect is particularly relevant for shrimp, whose characteristic coloration is attributed to carotenoids such as astaxanthin, which are highly susceptible to oxidation and bleaching during storage.

The a* coordinate (Figure 3) represents the red–green axis and provides information about redness intensity. The control sample exhibited negative values throughout storage (approximately -2 to -3), indicating a loss of reddish coloration. In contrast, coated samples showed positive a* values, particularly those containing grape seed extract. At day 3, treatments QE, FE, and QF reached values close to 4–4.5, demonstrating enhanced redness compared to the control. Although a slight reduction was observed at day 6, extract-containing treatments maintained higher a* values than films without extract. This behavior may be attributed to both the intrinsic color of phenolic compounds and their

antioxidant capacity, which protects carotenoids from oxidative degradation. Transparent starch-based films tend to allow greater light transmission, which may accelerate pigment oxidation and explain the lower a* values observed in some treatments [52].

The b* coordinate (Figure 3) reflects the yellow–blue axis and is also associated with pigment stability. The control exhibited a marked increase in b* values from approximately −3 at day 0 to positive values at day 6, indicating color degradation and development of yellowish tones typical of spoilage. In contrast, coated samples showed higher initial b* values (approximately 5–9) but smaller variations during storage, suggesting better stability of chromatic characteristics. Treatments containing grape seed extract, particularly QE and FE, exhibited peak values at day 3 followed by slight decreases at day 6, indicating controlled pigment modification rather than oxidative deterioration.

The incorporation of grape seed extract modified the optical properties of the films, producing darker and more colored films. This behavior was particularly evident in QE and QFE treatments, which showed more stable L*, a*, and b* values throughout storage. The darker appearance may be related to interactions between phenolic compounds and the polysaccharide matrix, which increase light scattering and optical density. Similar findings were reported by [20], who observed reduced luminosity and increased red-yellow tones in polysaccharide films enriched with plant extracts.

Additionally, the preservation of shrimp coloration may be attributed to the barrier properties of the polysaccharide films. These films form a semi-permeable layer that reduces oxygen diffusion and moisture loss, both of which accelerate pigment degradation. The antioxidant activity of grape seed phenolics also inhibits lipid oxidation, which is closely associated with discoloration in seafood products [46,53]. The combined effect of antioxidant protection and reduced oxygen exposure likely contributed to the maintenance of visual quality.

Therefore, the results obtained from visual analysis (Figure 1) and instrumental color measurements (Figures 2 and 3) demonstrate that films enriched with grape seed extract improved color stability of refrigerated shrimp. The reduced variation in luminosity, maintenance of positive a* values, and stabilization of b* coordinates indicate that the combined barrier properties of the polysaccharide matrix and the antioxidant activity of phenolic compounds minimized pigment oxidation. These findings highlight the potential of polysaccharide-based active films as sustainable strategies for preserving visual quality and extending the commercial shelf life of shrimp.

4.2.2. pH

The pH values of shrimp samples coated with different polymeric formulations during refrigerated storage are presented in Table 3. On day 0, all treatments exhibited near-neutral pH values ranging from 6.63 to 6.88, which is typical of fresh shrimp and indicates minimal post-mortem biochemical degradation [54,55]. Fresh crustaceans generally present pH values between 6.5 and 7.0 due to the limited glycogen reserves available for post-mortem lactic acid formation [56]. The slightly lower initial pH observed in the chitosan treatment (Q = 6.634) can be attributed to the solubilization of chitosan in acetic acid, which is commonly used during film preparation and contributes to mild acidification [57,58].

During storage, a slight reduction in pH was observed for most treatments at day 3. For example, the control decreased from 6.866 to 6.836, while Q decreased from 6.634 to 6.540 and QE from 6.814 to 6.624. This reduction may be associated with the formation of acidic compounds, such as lactic acid, as well as the dissolution of CO_2 produced during early microbial metabolism [57]. Such behavior is typical during the initial storage period of seafood and is considered an indicator of physicochemical quality changes [59]. A more pronounced decrease was observed for QFE (5.414), suggesting stronger acidification

likely related to synergistic effects between chitosan, starch, and phenolic compounds. The acidic nature of phenolic compounds present in grape seed extract may also contribute to this reduction.

By day 6, most treatments showed stabilization or slight increases in pH values. The control decreased to 6.630, whereas Q and QE maintained values close to their initial levels (6.508 and 6.608, respectively). Similarly, QF and QFE showed recovery to 6.732 and 6.678, respectively. This increase in pH during later storage stages is typically associated with the production of alkaline compounds such as volatile amines, trimethylamine, and ammonia generated by microbial and enzymatic degradation of nitrogenous compounds [60,61]. However, the magnitude of increase observed in coated samples was limited, indicating inhibition of spoilage reactions.

Treatments containing chitosan and/or grape seed extract exhibited more stable pH values throughout storage compared to the control. Chitosan is known for its antimicrobial properties, which reduce microbial growth and consequently limit the formation of alkaline spoilage metabolites [62]. In addition, phenolic compounds present in grape seed extract exert antioxidant and antimicrobial effects, contributing to the inhibition of biochemical deterioration. The combined treatment QFE, despite showing an initial drop, maintained pH within acceptable limits at day 6, suggesting effective control of spoilage reactions.

From a microbiological standpoint, pH values between 6.6 and 7.5 favor the growth of a wide range of spoilage microorganisms [61]. The relatively small variation observed across treatments (approximately 0.2–0.4 units for most samples) indicates limited biochemical changes during storage. Such stability suggests that the polysaccharide films acted as semi-permeable barriers, reducing oxygen diffusion and microbial proliferation. Similar findings were reported by Kho-Danazary and collaborators [59], who observed reduced pH fluctuations in shrimp coated with polysaccharide-based films enriched with plant extracts.

Thus, the results demonstrate that edible films, particularly those containing chitosan and grape seed extract, contributed to maintaining pH stability during refrigerated storage. The limited variation observed indicates reduced formation of acidic and alkaline degradation compounds, suggesting delayed spoilage and improved physicochemical stability. These findings reinforce the potential of polysaccharide-based active films as effective strategies for preserving shrimp quality.

4.3. Total Flavonoid and Phenolic Compounds

The grape seed extract used in this study exhibited a high content of total phenolic compounds (17.09 g GAE) and a quercetin concentration of 0.73 g L^{-1}, confirming its potential as a source of bioactive molecules for active film formulations. These values are consistent with those reported by Kupe and collaborators [63], who demonstrated that grape seeds contain significantly higher concentrations of phenolic compounds than grape skins and pulp due to their richness in flavonoids, proanthocyanidins, catechins, and phenolic acids. The predominance of these compounds is particularly relevant for food preservation applications, as they are widely recognized for their strong antioxidant and antimicrobial properties.

Phenolic compounds exert antioxidant activity primarily through hydrogen atom transfer and single-electron transfer mechanisms, neutralizing free radicals and interrupting oxidative chain reactions [46,64]. In seafood products such as shrimp, lipid oxidation is one of the main causes of quality deterioration, leading to off-flavors, discoloration, and textural changes during storage [65–67]. Therefore, the relatively high phenolic content observed in the grape seed extract suggests a strong potential to delay oxidative processes. This hypothesis is supported by the improved color stability observed in coated samples

discussed in Section 4.2.1, where treatments containing grape seed extract exhibited reduced variations in L*, a*, and b* values, indicating protection against pigment oxidation.

Flavonoids, particularly quercetin, also contribute to oxidative stability through metal chelation [68–70]. Transition metals such as Fe^{2+} and Cu^{2+} catalyze the formation of reactive oxygen species via Fenton-type reactions, accelerating lipid peroxidation. Quercetin can chelate these metal ions, reducing their catalytic activity and limiting oxidative degradation [71]. The quercetin concentration identified in the extract (0.73 g L^{-1}) therefore represents an important functional component contributing to the antioxidant performance of the films.

Beyond antioxidant activity, phenolic compounds exhibit antimicrobial effects through multiple mechanisms [72–74]. These include disruption of microbial cell membranes, alteration of permeability, inhibition of extracellular enzymes, and interference with nucleic acid synthesis [40]. Such antimicrobial activity is particularly relevant for shrimp preservation, as microbial growth contributes to pH increase, production of volatile amines, and overall spoilage. The relatively stable pH values observed in coated samples (Section 4.2.2) suggest that the phenolic compounds, together with chitosan, contributed to inhibiting microbial proliferation during refrigerated storage.

The incorporation of phenolic-rich extracts into polysaccharide-based films may also promote controlled release of bioactive compounds. The interaction between phenolic molecules and polymeric matrices such as chitosan and starch can slow diffusion rates, allowing gradual migration of antioxidants and antimicrobials to the food surface [20,51]. This sustained release mechanism enhances the functional performance of edible films by maintaining protective activity throughout storage. Such behavior may explain the prolonged color stability and limited physicochemical changes observed in shrimp samples coated with extract-containing formulations.

Additionally, synergistic effects between phenolic compounds and polysaccharides should be considered. Chitosan possesses inherent antimicrobial activity due to its cationic nature, which allows interaction with negatively charged microbial cell membranes [75–77]. When combined with phenolic compounds, this effect may be enhanced, leading to improved preservation efficiency. Similarly, starch-based matrices provide structural integrity and barrier properties, reducing oxygen diffusion and complementing the antioxidant activity of phenolics. This synergy between the polymeric matrix and bioactive compounds aligns with the improved physicochemical stability observed in coated shrimp samples.

Thus, the elevated levels of total phenolics and flavonoids detected in the grape seed extract support its functional role in improving shrimp stability during refrigerated storage. The combination of antioxidant activity, metal chelation, antimicrobial effects, and controlled release from polysaccharide matrices likely contributed to the preservation of color, stabilization of pH, and delayed deterioration observed in this study. These findings highlight the potential of grape seed extract as a natural bioactive ingredient in polysaccharide-based films, reinforcing its applicability in sustainable food preservation strategies.

4.4. Characterization of Polymer Blends and Films

4.4.1. Barrier and Solubility Properties

Variations in film thickness are commonly associated with the casting method and formulation composition, since solution viscosity, total solids content, and drying conditions directly influence the final film structure [78,79]. In the present study (Table 4), film thickness ranged from 0.047 to 0.078 mm, with significant differences among formulations, as determined by ANOVA in a split-plot design followed by Tukey's test ($p < 0.05$). The cassava starch film (F) exhibited the lowest thickness (0.0472 mm), indicating the formation

of a thinner and possibly more compact structure. In contrast, chitosan-containing films (Q, QE, and QF) presented higher thickness values, ranging from 0.0748 to 0.0779 mm. This behavior can be attributed to the higher viscosity of chitosan solutions and their greater solid content, which typically results in thicker films after solvent evaporation [80,81].

The incorporation of grape seed extract slightly modified thickness values depending on the polymeric matrix. For example, QE (0.0779 mm) and QF (0.0779 mm) showed similar thicknesses, while FE exhibited an intermediate value (0.0645 mm). These variations may be associated with interactions between phenolic compounds and polymer chains, which can promote structural rearrangements and alter film formation during drying. Phenolic compounds may also induce phase heterogeneity or act as plasticizing agents, influencing film microstructure and thickness [20,51].

Water vapor permeability (WVP) is a critical parameter for food packaging applications, as moisture transfer plays a central role in microbial growth and physicochemical deterioration [82]. Lower WVP values indicate better barrier performance, reducing water migration between the product and the environment [83–86]. In this study, the starch-based film (F) exhibited the lowest WVP value (36.64 $g/m^2 \cdot h$), indicating superior barrier properties compared to other formulations. This result suggests the formation of a more compact polymeric network, likely due to starch gelatinization and hydrogen bonding between amylose and amylopectin chains, which reduces free volume and limits moisture diffusion.

In contrast, the chitosan film (Q) presented the highest WVP value (62.18 $g/m^2 \cdot h$), demonstrating greater permeability to water vapor. Although chitosan is often described as forming cohesive films, its structure contains hydroxyl and amino groups capable of interacting with water molecules via hydrogen bonding. These interactions increase moisture adsorption and diffusion, resulting in higher permeability [86–88]. The hydrophilic nature of chitosan therefore contributes to increased water uptake and reduced barrier performance.

The incorporation of grape seed extract influenced WVP depending on the polymeric matrix. For chitosan films, the addition of extract reduced WVP from 62.18 $g/m^2 \cdot h$ (Q) to 48.82 $g/m^2 \cdot h$ (QE), suggesting that phenolic compounds improved barrier properties, possibly by promoting additional intermolecular interactions and reducing free volume. However, in starch-based films, the addition of extract increased WVP from 36.64 $g/m^2 \cdot h$ (F) to 51.18 $g/m^2 \cdot h$ (FE). This behavior indicates that phenolic compounds may disrupt hydrogen bonding between starch chains, creating microvoids that facilitate moisture diffusion. Similar matrix-dependent effects have been reported by Versino and García [89], who demonstrated that plant extracts can either improve or impair barrier properties depending on polymer composition.

Blended films showed intermediate WVP values, with QFE and QF presenting 57.66 and 49.90 $g/m^2 \cdot h$, respectively. These results indicate that combining chitosan and starch partially balanced the hydrophilic nature of chitosan and the compact structure of starch. Such behavior suggests synergistic interactions between polymers, leading to modified structural organization and moderate barrier performance.

Solubility results also reflected differences in polymer composition. The chitosan film (Q) showed the lowest solubility (16.85%), followed by QF (20.26%), indicating stronger intermolecular interactions and reduced water sensitivity. In contrast, the QE formulation exhibited the highest solubility (61.65%), demonstrating that the addition of grape seed extract significantly increased water sensitivity. This increase may be attributed to the presence of low-molecular-weight phenolic compounds that disrupt polymer–polymer interactions and increase chain mobility, facilitating water penetration. Similar effects have been observed in polysaccharide films enriched with plant extracts, where phenolic compounds act as plasticizers [20,51].

Starch-based films (F and FE) presented intermediate solubility values (34.44% and 31.80%, respectively), indicating moderate resistance to water dissolution. The slight reduction observed for FE suggests that some phenolic–starch interactions may have enhanced matrix cohesion. Meanwhile, the blended QFE film exhibited solubility of 32.95%, reinforcing the hypothesis that polymer blending improves structural stability and reduces excessive solubility.

Thereby, the results demonstrate that both polymer type and grape seed extract incorporation significantly influenced thickness, solubility, and barrier properties. Starch-based films provided superior moisture barrier performance, while chitosan contributed to lower solubility and structural integrity. The addition of grape seed extract altered intermolecular interactions within the polymeric matrices, affecting both WVP and solubility. These findings highlight the importance of optimizing polysaccharide-based formulations to balance barrier efficiency and functional bioactivity, supporting the development of active biodegradable films for food preservation.

4.4.2. Optical Properties

The optical properties of polymeric films are critical for food packaging applications, as they influence both product visibility and protection against light-induced deterioration. Luminosity (L*) is directly associated with film transparency, with higher values indicating greater light transmission and visual clarity [90]. As shown in Figure 5, L* values ranged from approximately 78 to 88, indicating that all films presented relatively high brightness. Starch-based films (F and FE) exhibited the highest luminosity values (≈86–87), followed by QF, whereas the QE formulation presented the lowest value (≈78), demonstrating reduced transparency. The high L* values observed for starch films can be attributed to the homogeneous structure formed during starch gelatinization, which promotes uniform light transmission and minimizes light scattering [86,91]. This transparency is advantageous for fresh seafood products such as shrimp, where visual inspection by consumers is an important quality indicator.

The incorporation of grape seed extract modified the optical properties of the films. The QE formulation showed a noticeable reduction in luminosity compared to Q, indicating that phenolic compounds influenced light transmission. This behavior may be associated with interactions between polyphenols and the polymeric matrix, which create microstructural heterogeneities that scatter incident light [92]. Additionally, the intrinsic coloration of grape seed extract, rich in flavonoids and tannins, contributes to darker films. Similar reductions in luminosity have been reported in polysaccharide-based films enriched with plant extracts, where the presence of colored phenolics modifies optical characteristics [20].

Opacity values (Figure 5) ranged from approximately 33 to 37%, with statistically significant differences among treatments. Chitosan-based films (Q and QE) exhibited the highest opacity values (≈36–37%), while QF presented the lowest opacity (≈33%). Starch-based films showed intermediate behavior. The slight increase in opacity observed for extract-containing films suggests that phenolic compounds promoted greater light scattering within the matrix. This effect may be attributed to differences in refractive index between polymer chains and dispersed phenolic molecules, which enhance optical heterogeneity [51]. Although the changes in opacity were moderate, they may still contribute to improved light barrier properties.

The color coordinates of the films (Figure 4) further confirm the influence of extract incorporation. The a* coordinate ranged from negative values for Q (≈−2) and QF (≈−1.5) to positive values for QE (≈4.8) and QFE (≈2.0), indicating a shift toward reddish tones after addition of grape seed extract. This shift is directly related to the presence of anthocyanin-like pigments and condensed tannins in grape seed phenolics. Similarly, the b* coordinate

showed a marked decrease after extract incorporation, with Q exhibiting the highest value (≈30), while F and FE presented values close to 1–3. These results indicate that extract incorporation altered the chromatic profile of the films, producing darker and more colored matrices. Comparable findings were reported by Talón and collaborators [93], who observed increased coloration in starch films containing plant-derived phenolics.

From a functional perspective, increased opacity and reduced luminosity may be advantageous for food preservation. Light exposure accelerates lipid oxidation and pigment degradation in seafood, leading to discoloration and quality loss [46]. Therefore, films with moderate opacity, such as QE and QFE, may provide improved protection against photooxidative processes. This protective effect complements the antioxidant activity of phenolic compounds, creating a synergistic mechanism that enhances product stability.

Thus, the results demonstrate that both polymer type and grape seed extract incorporation significantly influenced the optical properties of the films. Starch-based formulations exhibited higher luminosity and transparency, while extract-enriched films showed increased coloration and slightly higher opacity. These modifications indicate a trade-off between transparency and protective functionality. However, the moderate reduction in transparency combined with improved light barrier properties suggests that polysaccharide-based films enriched with grape seed extract are promising materials for active food packaging, particularly for light-sensitive products such as refrigerated shrimp.

4.4.3. Mechanical Properties

Mechanical properties are essential for evaluating the applicability of polysaccharide-based films as food films, since adequate tensile strength, elasticity, and flexibility are required to withstand handling and storage conditions. The tensile strength, Young's modulus, and elongation at break of the produced films are presented in Figure 6. These parameters reflect the structural integrity and cohesion of the polymeric matrices formed by chitosan, cassava starch, and grape seed extract.

Cassava starch films are known for forming smooth and homogeneous structures; however, they often exhibit brittle behavior due to the predominance of hydrogen bonding between starch chains, which limits resistance to mechanical stress [94]. In the present study, the starch film (F) showed intermediate tensile strength (≈18 MPa) compared to other formulations (Figure 6A), confirming the relatively fragile nature of starch-based systems. Nevertheless, this formulation exhibited the highest Young's modulus (≈3900 MPa; Figure 6B), indicating a rigid and less deformable structure. High modulus values are typically associated with reduced chain mobility and stronger intermolecular interactions within starch matrices [91]. This rigidity also explains the higher elongation observed for F (≈2.5%; Figure 6C), which was significantly greater than the other treatments, suggesting that starch films possess limited but measurable flexibility.

Chitosan films (Q), in contrast, exhibited the lowest tensile strength (≈13 MPa) and Young's modulus (≈600 MPa), indicating reduced resistance and stiffness. Although chitosan is widely recognized for good film-forming ability, its mechanical performance depends on molecular weight, degree of deacetylation, and plasticizer concentration [87]. The relatively low mechanical resistance observed here may be associated with increased hydrophilicity and reduced intermolecular cohesion within the chitosan matrix. Similar results have been reported for chitosan-based films, which often display lower tensile strength compared to starch-based systems [95].

The incorporation of grape seed extract significantly influenced mechanical behavior. The QE formulation exhibited the highest tensile strength (≈37 MPa), demonstrating that phenolic compounds reinforced the chitosan matrix. This increase may be attributed to hydrogen bonding and electrostatic interactions between polyphenols and chitosan amino

groups, which improve intermolecular cohesion and reduce structural defects [20,51]. However, QE presented a moderate Young's modulus (≈900 MPa), suggesting that extract incorporation improved resistance without excessively increasing rigidity. The elongation values remained low, indicating that the reinforcement effect did not significantly enhance flexibility.

Blended films (QF and QFE) exhibited intermediate tensile strength values (≈28 and ≈23 MPa, respectively), indicating synergistic interactions between starch and chitosan (Figure 6A). These blends combined the rigidity of starch with the cohesive properties of chitosan, resulting in improved mechanical performance compared to single-polymer systems. Such behavior is commonly observed in polysaccharide blends, where hydrogen bonding between hydroxyl groups of starch and amino groups of chitosan leads to interpenetrating polymer networks [87,96]. These interactions enhance structural integrity and reduce crack propagation, improving film resistance.

Young's modulus values for QF and QFE (≈1000–1200 MPa) were higher than those of chitosan alone but lower than those of starch films, confirming the intermediate stiffness of blended systems (Figure 6B). This balance between rigidity and flexibility is desirable for food films, as excessively rigid films may fracture, whereas very flexible films may lack structural stability. The elongation at break values for blended films remained low (≈0.2–0.3%), indicating limited extensibility but sufficient cohesion for film applications (Figure 6C).

The mechanical behavior observed suggests that phenolic compounds may act either as reinforcing or plasticizing agents depending on their interaction with the polymer matrix. In QE, phenolics enhanced tensile strength, whereas in blended systems, they slightly reduced modulus compared to pure starch films, indicating increased chain mobility. Such dual effects have been widely reported in polysaccharide films enriched with plant extracts, where phenolics modify intermolecular interactions and influence mechanical properties [51,93].

Therefore, the results demonstrate that polymer composition significantly influenced mechanical performance. Starch films exhibited high rigidity but moderate resistance, chitosan films showed lower mechanical strength, and blended systems presented improved balance between tensile strength and stiffness. The addition of grape seed extract enhanced tensile strength, particularly in chitosan-based films, indicating reinforcing interactions between phenolic compounds and the polymer network. These findings highlight the synergistic effect of combining polysaccharides and bioactive extracts, supporting the development of mechanically stable biodegradable films suitable for shrimp preservation. Such optimization of mechanical properties is essential for practical application and aligns with the design of functional polysaccharide-based biomaterials proposed.

4.4.4. Scanning Electron Microscopy (SEM)

Scanning electron microscopy provided important insights into the microstructural organization of the films and helped explain the mechanical behavior previously discussed. The cassava starch film (F) exhibited a relatively continuous and homogeneous matrix, with no visible starch granules (Figure 7A), confirming complete gelatinization during film formation. This structural uniformity is commonly associated with improved chain mobility and flexibility, which is consistent with the higher elongation at break observed for F in the mechanical analysis (Figure 6C). However, despite its elasticity, the presence of localized roughness and subtle discontinuities along the cross-section suggests limited intermolecular cohesion, which may contribute to the moderate tensile strength and reduced Young's modulus of this formulation. Similar structural features have been reported for gelatinized

starch films, where hydrogen-bond-dominated interactions lead to flexible yet mechanically fragile networks [81,92,97,98].

In contrast, the cassava starch film containing grape seed extract (FE) displayed pronounced internal voids and microcavities distributed throughout the cross-section (Figure 7A). These structural defects likely originated from phase separation and solvent evaporation during drying, resulting in reduced cohesion within the polymer matrix. The presence of these voids explains the brittle behavior and poor mechanical performance observed for FE, particularly the lower tensile strength and reduced structural resistance compared to blended systems. Phenolic compounds present in grape seed extract may disrupt starch–starch hydrogen bonding, weakening intermolecular interactions and promoting microphase separation, as reported for phenolic-loaded polysaccharide films [99–102].

Films containing chitosan (Q and QE) exhibited denser and more compact morphologies (Figure 7B), reflecting stronger intermolecular interactions within the polymeric network. Chitosan is known to form rigid matrices due to extensive hydrogen bonding between amino and hydroxyl groups, which increases structural cohesion and reduces porosity [103]. However, the relatively smooth but compact structure observed in Q is consistent with its lower elongation at break and more rigid mechanical behavior. The addition of grape seed extract in QE resulted in slight structural heterogeneity, indicating partial miscibility between chitosan and phenolic compounds. Such microstructural changes may act as stress concentrators, influencing the moderate mechanical performance observed for QE.

The blended films containing both cassava starch and chitosan (QF and QFE) showed multilayered and more organized structures (Figure 7C), suggesting the formation of interpenetrating polymer networks. These blends exhibited reduced void formation compared to FE and improved matrix continuity, indicating enhanced compatibility between components. The presence of both polymers likely promoted intermolecular interactions between hydroxyl groups of starch and amino groups of chitosan, leading to improved structural integrity. This microstructural organization is consistent with the higher tensile strength and balanced mechanical performance observed for QF and QFE in Figure 6. Additionally, the QFE formulation displayed a slightly more heterogeneous morphology compared to QF, suggesting that the grape seed extract influenced polymer packing and may have acted as a plasticizing agent, modifying chain mobility and intermolecular spacing.

Overall, SEM analysis confirms that microstructural organization directly influences the mechanical properties of the films. Homogeneous and continuous matrices, as observed in blended systems, resulted in improved structural resistance, whereas the presence of voids and phase separation led to brittle behavior. Although partial miscibility between components was observed, the polymer blends formed cohesive structures suitable for use as biodegradable preservation films. These findings reinforce the synergistic interaction between cassava starch and chitosan and highlight the role of grape seed extract in modulating the microstructure and performance of the developed films.

5. Conclusions

The developed films effectively preserved peeled shrimp under refrigeration for up to six days, reducing microbial growth, maintaining pH stability, and minimizing color changes. The results were consistent with expectations for biodegradable polymer systems, with no deterioration in quality parameters; instead, the films delayed spoilage compared to the control. Film properties were strongly influenced by formulation. Cassava starch–chitosan blends showed improved mechanical performance and cohesive microstructures, while the incorporation of grape seed extract modulated barrier and solubility properties,

indicating a functional balance between water resistance and bioactivity. Although the barrier properties remain lower than those of conventional synthetic materials, the films demonstrated relevant functional improvements within the context of biodegradable films. Thus, these formulations show potential for extending the shelf life of refrigerated shrimp. Future studies should focus on optimizing water resistance, evaluating scalability and sensory acceptance, and exploring combined preservation strategies for industrial application.

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