



BIOPOLIMER FILM ADDITED WITH JAMBOLAN FRUITS EXTRACT (*SYZYGIUM CUMINI* L.) AND ZnO NANOPARTICLES: CHARACTERIZATION AND APPLICATION

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ABSTRACT

Objectives: This study developed and characterized bifunctional (active and intelligent) packaging films using sodium alginate, jambolan fruit extract, and ZnO nanoparticles. The films were analyzed for their physicochemical, mechanical, optical, and antimicrobial properties.

Theoretical Framework: The introduction highlights the importance of packaging to protect food and extend its shelf life. The trend towards active and intelligent packaging, which monitors product conditions in real-time using food-interacting ingredients, is emphasized. The use of natural pH indicators, such as plant pigments, is mentioned as a sensitive and low-cost solution for detecting food damage. The jambolan fruit (*Syzygium cumini* L.) is presented as a rich but underutilized source of anthocyanins, hydrolyzable tannins, and flavonoids.

Method: Sodium alginate films were prepared with aqueous jambolan extract (EAJ) and ZnO nanoparticles (nanoZnO). The films' properties were analyzed, including total phenolic content (TPC), antioxidant capacity (%AC) using the DPPH* method, and physicochemical and mechanical properties (moisture, solubility, water vapor permeability, swelling, tensile strength, and elongation). Antimicrobial activity was tested against bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Results and Discussion: The results showed that the addition of jambolan extract and nanoZnO altered the films' moisture, solubility, and swelling, while also increasing their light permeability. The films demonstrated high antioxidant capacity and significant antimicrobial activity against.

Research Implications: The results indicate that the alginate films with jambolan extract and nanoZnO are promising for developing active and intelligent packaging, especially for preserving high-moisture foods. The films' antioxidant and antimicrobial properties can extend the shelf life of perishable products. The pH study suggests that these films can be used as freshness sensors, changing color as food quality deteriorates. *Staphylococcus aureus* in vitro. Furthermore, the films changed their effective color as a function of pH, confirming their potential for smart food packaging.

Originality/Value: This study is original in investigating the combination of jambolan extract and ZnO nanoparticles in an alginate matrix to create packaging with dual properties (active and intelligent). The research validates the use of an underutilized plant byproduct (jambolan) as a source of bioactive compounds, aligning with the trend of more sustainable and efficient packaging. The detailed analysis of the physicochemical,

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morphological, mechanical, and antimicrobial properties provides a solid foundation for future applications, including the creation of multilayer films for the preservation of refrigerated fish by-products.

Keywords: Antimicrobials, Antioxidants, Anthocyanins, Biopolymers, Active and Intelligent Packaging, Food Packaging.

EXTRATO DE FRUTOS (*SYZYGIIUM CUMINI* L.) E NANOPARTÍCULAS DE ZnO: CARACTERIZAÇÃO E APLICAÇÃO

RESUMO

Objetivos: Este estudo desenvolveu e caracterizou filmes de embalagem bifuncionais (ativos e inteligentes) utilizando alginato de sódio, extrato de jambolão e nanopartículas de ZnO. Os filmes foram analisados quanto às suas propriedades físico-químicas, mecânicas, ópticas e antimicrobianas.

Referência Teórica: A introdução destaca a importância da embalagem para proteger os alimentos e prolongar sua vida útil. A tendência para embalagens ativas e inteligentes, que monitoram as condições do produto em tempo real utilizando ingredientes que interagem com os alimentos, é enfatizada. O uso de indicadores naturais de pH, como pigmentos vegetais, é mencionado como uma solução sensível e de baixo custo para a detecção de danos em alimentos. O jambolão (*Syzygium cumini* L.) é apresentado como uma fonte rica, porém subutilizada, de antocianinas, taninos hidrolisáveis e flavonoides.

Método: Filmes de alginato de sódio foram preparados com extrato aquoso de jambolão (EAJ) e nanopartículas de ZnO (nanoZnO). As propriedades dos filmes foram analisadas, incluindo o teor total de fenólicos (TPC), a capacidade antioxidante (%CA) pelo método DPPH* e as propriedades físico-químicas e mecânicas (umidade, solubilidade, permeabilidade ao vapor d'água, intumescimento, resistência à tração e alongamento). A atividade antimicrobiana foi testada contra bactérias como *Staphylococcus aureus*, *Escherichia coli* e *Pseudomonas aeruginosa*.

Resultados e Discussão: Os resultados mostraram que a adição de extrato de jambolão e nanoZnO alterou a umidade, a solubilidade e o intumescimento dos filmes, além de aumentar sua permeabilidade à luz. Os filmes demonstraram alta capacidade antioxidante e atividade antimicrobiana significativa contra...

Implicações da Pesquisa: Os resultados indicam que os filmes de alginato com extrato de jambolão e nanoZnO são promissores para o desenvolvimento de embalagens ativas e inteligentes, especialmente para a conservação de alimentos com alto teor de umidade. As propriedades antioxidantes e antimicrobianas dos filmes podem prolongar a vida útil de produtos perecíveis. O estudo de pH sugere que esses filmes podem ser usados como sensores de frescor, mudando de cor conforme a qualidade dos alimentos se deteriora. *Staphylococcus aureus* in vitro. Além disso, os filmes mudaram sua cor efetiva em função do pH, confirmando seu potencial para embalagens inteligentes de alimentos.

Originalidade/Valor: Este estudo é original ao investigar a combinação de extrato de jambolão e nanopartículas de ZnO em uma matriz de alginato para criar embalagens com propriedades duplas (ativa e inteligente). A pesquisa valida o uso de um subproduto vegetal subutilizado (jambolan) como fonte de compostos bioativos, alinhando-se à tendência de embalagens mais sustentáveis e eficientes. A análise detalhada das propriedades físico-químicas, morfológicas, mecânicas e antimicrobianas fornece uma base sólida para aplicações futuras, incluindo a criação de filmes multicamadas para a preservação de subprodutos de pescado refrigerados.

Palavras-chave: Antimicrobianos, Antioxidantes, Antocianinas, Biopolímeros, Embalagens Ativas e Inteligentes, Embalagens de Alimentos.

EXTRATO DE FRUTOS (*SYZYGIIUM CUMINI* L.) E NANOPARTÍCULAS DE ZnO: CARACTERIZAÇÃO E APLICAÇÃO

RESUMEN

Objetivos: Este estudio desarrolló y caracterizó películas de envasado bifuncionales (activas e inteligentes) utilizando alginato de sodio, extracto de jambolán y nanopartículas de ZnO. Se analizaron las películas para determinar sus propiedades fisicoquímicas, mecánicas, ópticas y antimicrobianas.



Marco teórico: La introducción destaca la importancia del envasado para proteger los alimentos y prolongar su vida útil. Se enfatiza la tendencia hacia envases activos e inteligentes, que monitorizan las condiciones del producto en tiempo real utilizando ingredientes que interactúan con los alimentos. El uso de indicadores naturales de pH, como pigmentos vegetales, se menciona como una solución sensible y económica para detectar daños en los alimentos. El jambolán (*Syzygium cumini* L.) se presenta como una fuente rica, pero subutilizada, de antocianinas, taninos hidrolizables y flavonoides.

Método: Se prepararon películas de alginato de sodio con extracto acuoso de jambolán (EAJ) y nanopartículas de ZnO (nanoZnO). Se analizaron las propiedades de las películas, incluyendo el contenido fenólico total (CFT), la capacidad antioxidante (%CA) mediante el método DPPH*, y las propiedades fisicoquímicas y mecánicas (humedad, solubilidad, permeabilidad al vapor de agua, hinchamiento, resistencia a la tracción y elongación). Se evaluó la actividad antimicrobiana contra bacterias como *Staphylococcus aureus*, *Escherichia coli* y *Pseudomonas aeruginosa*.

Resultados y discusión: Los resultados mostraron que la adición de extracto de jambolán y nanoZnO alteró la humedad, la solubilidad y el hinchamiento de las películas, a la vez que aumentó su permeabilidad a la luz. Las películas demostraron una alta capacidad antioxidante y una significativa actividad antimicrobiana.

Implicaciones de la investigación: Los resultados indican que las películas de alginato con extracto de jambolán y nanoZnO son prometedoras para el desarrollo de envases activos e inteligentes, especialmente para la conservación de alimentos con alto contenido de humedad. Las propiedades antioxidantes y antimicrobianas de las películas pueden prolongar la vida útil de los productos perecederos. El estudio de pH sugiere que estas películas pueden utilizarse como sensores de frescura, cambiando de color a medida que se deteriora la calidad de los alimentos.

Staphylococcus aureus in vitro. Además, las películas cambiaron su color efectivo en función del pH, lo que confirma su potencial para el envasado inteligente de alimentos.

Originalidad/Valor: Este estudio es original al investigar la combinación de extracto de jambolán y nanopartículas de ZnO en una matriz de alginato para crear envases con propiedades duales (activas e inteligentes). La investigación valida el uso de un subproducto vegetal infrautilizado (*jambolán*) como fuente de compuestos bioactivos, en línea con la tendencia hacia envases más sostenibles y eficientes. El análisis detallado de las propiedades fisicoquímicas, morfológicas, mecánicas y antimicrobianas proporciona una base sólida para futuras aplicaciones, incluyendo la creación de películas multicapa para la conservación de subproductos de pescado refrigerados.

Palabras clave: Antimicrobianos, Antioxidantes, Antocianinas, Biopolímeros, Envases Activos e Inteligentes, Envases para Alimentos.

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1 INTRODUCTION

Food packaging protects products during transport, display, storage, and consumption. Driven by consumer safety and quality concerns [1], new packaging uses diverse materials and techniques to extend shelf life and reduce spoilage. Effective food packaging offers convenience, protection, containment, and communication [2]. Active and intelligent packaging systems monitor and report product conditions in real-time achieved by incorporating food-interacting ingredients that enhance safety by preventing deterioration [3]



Intelligent systems with visible pH indicators offer advantages, enabling sensitive and low-cost detection of minor food damage. Natural pH indicators change color with acids or bases [4]. This has facilitated the development of intelligent biofilms with natural pigments like anthocyanins, betalains, and curcuminoids, exhibiting varied pH responses and mechanical properties [5] to monitor food quality; [6].

Anthocyanins in plants provide color and health benefits. The global market, mainly driven by food and beverages, is expected to grow from \$338 million in 2023 to \$435 million in 2028 at a 5.15% CAGR. Key companies include Kalsec, Sensient, Givaudan, ADM, and Novozymesb [7]. Given industrial demand, exploring anthocyanin sources and their incorporation into new packaging biofilms is crucial [8]. Jambolan fruit (*Syzygium cumini* L.) is rich in anthocyanins, hydrolysable tannins, and flavonoids, yet underutilized despite high tree productivity.

This study aimed to develop dual-functional (active and intelligent) packaging by assessing alginate biopolymers infused with jambolan extract and nanoZnO. It evaluated their mechanical, optical, physicochemical, and morphological barrier properties, as well as antioxidant and antimicrobial activities, for potential food packaging applications.

2 Material and Methods

2.1 MATERIAL

To prepare the jambolan fruit extracts, ethanol p.a., 37% acetic acid, and deionized distilled water (DDW) were used. The main formulation of the biopolymers included sodium alginate, glycerol, and ZnO nanoparticles, purchased respectively from DINÂMICA®, Vetec Química Fina®, and provided by the Laboratory of Devices and Nanostructures (LDN) at the Federal University of Pernambuco (UFPE). Other reagents used for quantifying total monomeric anthocyanins, total phenolic compounds, antioxidant capacity, or evaluating the solubility of the biopolymers were obtained from Vetec Química Fina®.

2.2 COLLECTION, CLEANING AND EXTRACTION OF SYZYGIUM CUMINI (L.) FRUITS

After harvest, ripe jambolan fruits were processed at the IFAL-MD Food Technology Lab. They were washed, disinfected with hypochlorite and ethanol, rinsed with sterile water, dried, pitted, and frozen at -24°C in sterile bags. To obtain extracts, 200g of pulp was blended



with 600mL of distilled water (DDW), hydroethanol (EHJ), or acidified hydroethanol (EHAcJ). The mixtures were agitated (100 rpm, 35°C, dark, 80 min), centrifuged (10,000 rpm, 10 min), vacuum filtered, and stored at 4°C in amber bottles [9].

2.3 CHARACTERIZATION OF EXTRACTS

2.3.1 Total phenolic compounds (TPC) and antioxidant capacity (%AC)

The total phenolic content (TPC) was measured using the Folin-Ciocalteu method with gallic acid standards, expressed as mg gallic acid equivalents per

50 gram of fruit. The DPPH radical scavenging activity was evaluated by adding extract samples to DPPH solution and measuring absorbance at 517 nm to determine antioxidant capacity [10] [11].

2.3.2 Stability study during storage

The stability of phenolic compounds and antioxidant action during storage was carried out according to the methodology adapted from [12]. The samples were placed in amber glass containers and stored under refrigeration for 120 days. Every 30 days, aliquots of each extract were removed for CFT and antioxidant capacity analyses, as described in item 2.3.2.

2.3.3 Preparation of the biopolymeric film with aqueous jambolan extract (EAJ)

The films were prepared using the casting method with three concentrations of EAJ (5%, 10%, 20%) with or without nanoZnO, following the specified conditions. Alginate (1.5%) was dissolved in DDW, mixed with glycerol and EAJ, then poured into Petri dishes, cross-linked with CaCl, dried for 72 hours at 45.5°C, and stored in a desiccator for analysis (Supplementary Material 5).

2.3.4 AC of biopolymeric films with EAJ

The %AC of films to scavenge the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH*) was evaluated as described by [11], with some adaptations. An aliquot of 0.1 mL of the solution extracted from the films was added to 2.9 mL of the DPPH* radical solution (6×10^{-5} mol.L⁻¹), and its absorbance was recorded at



70 517 nm (Shimadzu spectrophotometer UV-1650PC), until reaching the plateau. The %AC of each mixture was calculated using equation 2, according to item 2.3.2.

2.3.5 Antimicrobial activity of biopolymeric films with EAJ

Antimicrobial activity was tested using the agar diffusion method against *E. coli*, *P. aeruginosa*, and *S. aureus*. Bacteria were cultured, inoculated on Mueller Hinton agar, and discs with antibiotics or films (with/without nanoZnO and different EAJ) were placed on the plates. After 24 hours, inhibition zones were measured to assess effectiveness, including contact inhibition under the films [13].

2.4 PHYSICOCHEMICAL AND MECHANICAL PROPERTIES OF BIOPOLYMERS WITH EAJ

2.4.1 Solubility, moisture and Water vapor permeability (WVP)

Solubility tests in DDW, NaCl, phosphate buffered saline (PBS) and Ringer's saline solution (RSS) were carried out according to the modified method of [14].

Biopolymer discs (20 cm diameter) were stored in a silica desiccator for 7 days, then weighed. They were immersed in 50 mL of solvent for 24 hours at 30°C, dried on filter paper, and weighed again.

Moisture content was determined by gravimetric method. The films were dried in an oven (model TE-393/2; Tecnal, Brazil), at $105 \pm 3^\circ\text{C}$ for 24 h. Humidity was calculated considering the mass loss of the samples and the equation, in which "X" is the moisture content of the films (%), "f1" is the initial mass and "f2" is the final mass of the films [15].

WVP was measured gravimetrically (modified ASTM 969) at 25°C using a 75% RH gradient (CaCl inside cell, NaCl solution outside). Film mass increase in permeation cells was tracked for 72 hours (triplicate) to calculate WVP. [16].

$$WVP = (Ma.T)/(A.pt.p) \quad (1)$$

"M a" represents the mass in grams of water that permeated the film; "T" is the film thickness in mm; "A" is the permeation area in m^2 ; "pt" is the permeation time; and " Δp " is the



difference in water vapor pressure on both sides of the film (kPa). The value of 1.7535 kPa at 20 °C established saturation of water vapor pressure.

2.4.2 Swelling

Film swelling was measured by recording the initial mass (DS) of dried samples (2.5 cm diameter). Samples were immersed in DDW until saturation (1-2 min), excess water was removed, and swollen mass (SS) was measured. Maximum water absorption content (WAC) was calculated using an equation [17].

$$WAC = (SS - DS)100/DS \quad (2)$$

2.4.3 Tensile strength and percentage of deformation at rupture

Tensile strength and elongation were tested per ASTM D882-10 using DL-500 MF-EMIC®. Samples (7.5 x 2.5 cm) with 30 mm gauge length were pulled at 0.5 mm/s; tests were done in triplicate.

2.4.4 Opacity and visible light barrier

Film opacity was measured at 600 nm and light barrier at 280 nm using a Shimadzu UV-1650PC spectrophotometer, following established methods. Samples (7.0 x 1.5 cm) were tested in triplicate [18].

2.4.5 Fourier transform infrared (FTIR) spectroscopy of biopolymers with EAJ

FTIR spectra of all dried films were recorded at 25°C using a Spectrum GX FTIR spectrometer (Perkin Elmer). The method followed Pimonpan Kaewprachu et al. (2015). Each spectrum was obtained with 64 scans at 4cm⁻¹ resolution, between 600 and 4000cm⁻¹, using ambient air as reference [19].



2.4.6 Statistical analysis

Experimental data were analyzed as means and standard deviations from triplicates. ANOVA and Duncan tests were performed using StatSoft v12.0 at 5% significance.

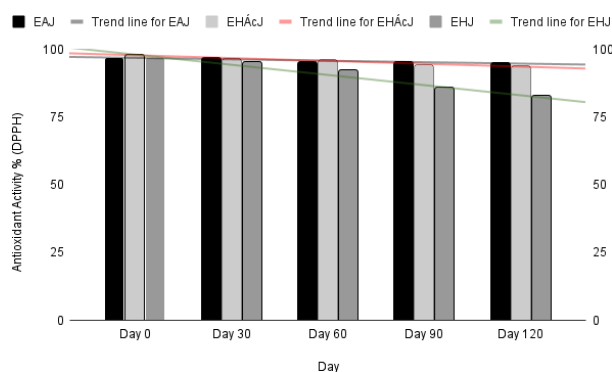
3 RESULTS AND DISCUSSIONS

3.1 TPC CONTENT AND AC% OF JAMBOLAN EXTRACTS

EHJ's TMA content (118.5 mg/100g) is consistent with other research, though variations exist due to environmental and methodological factors. The TPC of the extracts (146-159 mg GAE/g) was high, and EAJ showed good TPC stability. Aqueous extraction of jambolan leaves improved TPC yield quickly, with levels remaining stable over extended time and [20] [21].

Figure 1

Stability of antioxidant activity of different jambolan extracts. EAJ= Jambolan Aqueous Extract; EHAcJ= Jambolan Acidified Hydroethanolic Extract; EHJ= Jambolan Hydroethanolic Extract. Values are presented as mean and standard deverbativo.



Conversely, EHJ showed the most significant decrease in TFC after 90 days. This is lower than the TFC results for EHJ (pulp) reported by Azima et al. (2017) and for jaboticaba peel extract by Marsiglia et al. (2023) [22]. The DPPH* assay, a common method for assessing antioxidant capacity, showed that %CA remained high after 120 days for EAJ and EHAcJ, with no significant reduction. However, EHJ's %CA slightly decreased over the same period (from 96.86% to 82.71%). This suggests that while the antioxidant capacity of EAJ and EHAcJ was stable over the 120-day storage, EHJ's antioxidant components were somewhat less stable [23].

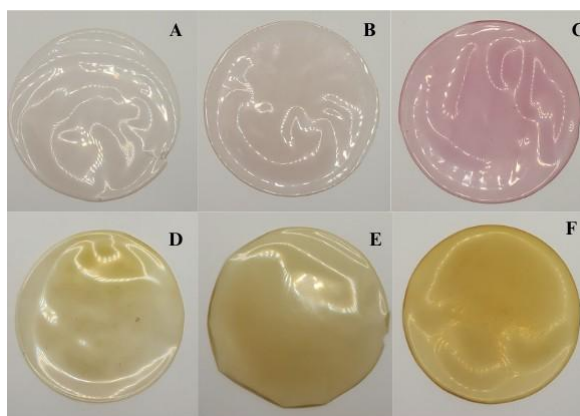


3.2 ANALYSIS OF BIOPOLYMERIC FILMS CONTAINING OR NOT NANOZNO AND EAJ

According to the data obtained on the extracts, it was defined that films with and without nanoZnO would be produced with three concentrations of EAJ, due to its compatibility for application in food, good solubility in biopolymers and good stability. The films showed good homogeneity, without fractures and without bubbles. The intensification of color with increasing EAJ concentration was visually noticeable (Figure 5).

Figure 2

Biopolymeric films produced with alginate and additives with: A) 5% EAJ, B) 10% EAJ; C) 20% EAJ; D) 5% EAJ and nanoZnO; E) 10% EAJ and nanoZnO; F) 20% EAJ and nanoZnO.



3.3 MECHANICAL PROPERTIES OF BIOPOLYMERS WITH EAJ: HUMIDITY, WATER VAPOR PERMEABILITY, FILM SWELLING, TENSILE STRENGTH AND DEFORMATION (ELONGATION) AT RUPTURE

Moisture, and swelling are fundamental to food packaging. Each type of food product requires materials with specific properties to guarantee its quality from production, during storage and distribution, to consumption. Supplementary Material 1 shows the effects of different concentrations of jambolan aqueous extract with and without nanoZnO on the properties of alginate films. This research aligns with Fredrick Nwude Eze et al. (2022), who found increased humidity in chitosan films with black rice extract due to potential hydrogen bonds between the extract, chitosan hydroxyl groups, and water [24].



The swelling index couldn't be evaluated in the control film without nanoZnO and films with 5% and 10% EAJ (F1, F2) due to alginate's high hydrophilicity, which can be reduced by higher EAJ concentrations and nanoZnO. Film F3 (203.19%) showed increased swelling, possibly due to higher EAJ concentration increasing alginate matrix porosity and water retention [25]. Biopolymers with nanoZnO (F0, F4, F5, F6) swelled significantly more than those without, potentially because nanoZnO strengthens biopolymer-water interactions [26].

Tensile strength (RT) and deformation at rupture (DR) ensure packaging safety by indicating its resistance and flexibility. RT reflects the film's mechanical strength, while DR shows its ability to stretch before breaking (Supplementary Material 4) [27] [28].

Adding nano ZnO significantly increased the tensile strength (RT) of the biopolymers from 28.06 MPa to 62.06 MPa, due to hydrogen bonding and a more compact structure, as shown in previous studies. Conversely, incorporating EAJ decreased the RT of films F1, F2, and F3 to 1.57, 1.39, and 1.18 MPa, respectively. Similar reductions in RT with extract addition have been observed in other polysaccharide films, such as chitosan with purple eggplant extract and mulberry extract [29] [30] [31]. The insertion of nanoZnO (Table 3), however, significantly increased the RT of the films with EAJ (F4, F5, and F6), to values of 58.01, 59.47 and 60.32 MPa, respectively. All formulations with EAJ showed higher elongation at break, with or without nanoZnO, which is promising for food packaging. Films without nanoZnO had lower elongation, likely due to uneven distribution. Adding phenolic compounds can also reduce flexibility by hindering chain interactions, as seen in other biocomposites with anthocyanin-rich extracts

3.4 SOLUBILITY IN WATER, NaCl, PBS AND RINGER'S SALINE SOLUTION (RSS)

Film solubility, the percentage of remaining dry matter after 24 hours in a solvent, depends on the biofilm's components and their water affinity. Hydrophilic sodium alginate films (Control, F0-F3) were fully soluble in water, NaCl, PBS, and RSS, indicating high degradability. Only less soluble films are in Supplementary Material 2. NanoZnO-containing films (F4-F6) showed reduced solubility in these solutions, especially with more EAJ, decreasing it significantly (to 0.02%). This is likely due to enhanced film matrix cohesion from nanoZnO and EAJ, limiting water interaction. [33] [34] [35] [36].

NanoZnO/EAJ significantly reduced the solubility of F4, F5, and F6 films in various solutions. This is because the nanocomposite caused the alginate structure to become more compact, limiting water and salt entry. Higher salt concentrations, like in RSS, further



decreased solubility. The low solubility of F5 and F6 makes them suitable for preserving high-water-content products and as carriers for antimicrobial dressings, especially in body fluid-like environments [37] [38] [37] [39].

3.5 OPACITY, UV LIGHT TRANSMISSION OF BIOPOLYMERS

In food packaging, the transparency of the material is an advantage because allows the food industry to present the best product to the consumer. On the other hand, packaging components must protect products from the negative effects of UV light [40]. The light transmittance of the Fc, F0, F1, F2, F3, F4, F5 and F6 films was measured at 280 nm, and the opacity at 600 nm, as shown in Table 5.

All films containing EAJ (except F1) showed increased opacity compared to those without EAJ. Biopolymers F3 and F6, with higher EAJ levels, were more opaque. Similar results were found in studies with sodium alginate films incorporating red onion and butterfly pea extracts. Darker, more opaque packaging is typically used for fatty foods to extend shelf life by reducing light exposure and preventing lipid oxidation [41] [42].

Means followed by the same letters in the same column do not differ from each other using the Duncan test ($p < 0,05$). Regarding permeability to UV light, films F1, F2, F3, F5 and F6 presented a greater protection barrier to UV light (Supplementary Material 3). Anthocyanins and other phenolics, present in EAJ films, offer protection against oxidative processes favored by UV radiation [43]. Similar behavior was obtained in CMC [34] and starch films [33] incorporated into anthocyanin-rich extracts. Color, as well as transparency, are also very important for the appearance of biopolymeric films, which in turn can influence consumer acceptance of packaged products [44] [38].

Incorporating nanoZnO significantly increased the yellow color (b^*) and made the films darker, increasing the color difference (ΔE^*) and opacity, especially with the addition of EAJ. These characteristics can be used to reduce nutrient losses, discoloration and off-flavor in foods when exposed to visible and ultraviolet light (Table5) [45] [4].

3.6 ANTIOXIDANT POTENTIAL WITH EAJ

Control films (with/without nanoZnO) had lower antioxidant capacity (%AC). Despite low TPC retention, %AC was present, indicating sufficient retained phenolics. Over 10% EAJ decreased %AC in films without nanoZnO, while nanoZnO films showed poorer antioxidant



performance. Similar %AC increases with phenolic extracts were seen in gellan gum and CMC films [46], with the latter plateauing above 30% extract. NanoZnO reduced antioxidant activity in PVC films with ascorbic acid possibly by interfering with electron transfer in DPPH assays [47] (Table 6).

Table 1

Content of percentage of antioxidant capacity (%AC) of alginate films incorporated with different concentrations of EAJ.

Films	DPPH (% AC)	Phenolic retention (%)
Fc	1.30 ± 0.06^g	–
F0	0.59 ± 0.04^h	–
F1	63.56 ± 2.05^c	4.17 ± 0.32^a
F2	91.47 ± 2.80^a	3.78 ± 0.11^b
F3	68.21 ± 3.28^b	1.28 ± 0.12^c
Films	DPPH (% AC)	Phenolic retention (%)
F4	13.31 ± 1.18^f	1.32 ± 0.09^c
F5	22.64 ± 0.84^e	1.42 ± 0.08^c
F6	56.78 ± 1.32^d	0.44 ± 0.02^d

Means followed by the same letters in the same column do not differ from each other using the Duncan test ($p < 0,05$).

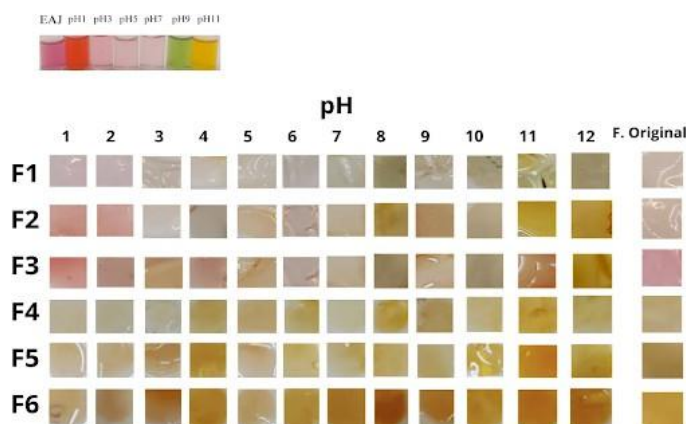
3.7 SENSITIVITY OF BIOPOLYMERIC FILMS WITH EAJ TO PH

EAJ films' color change (pH 1-12) was studied for food freshness indication. Without nanoZnO, films showed clearer color shifts (red/pink to yellow) due to anthocyanins, especially with higher EAJ content, similar to jambolan extract. Significant visual color change ($\Delta E > 12$) suggests potential as pH indicators, consistent with other studies. NanoZnO hindered this color-changing ability (Figure 6).



Figure 3

Color changes of sodium alginate films incorporated with aqueous extract of jambolan (EAJ) at different pH. F1=5% EAJ; F2=10% EAJ; F3=20% EAJ; F4=5% EAJ + nanoZnO; F5=10% EAJ+nanoZnO; F6=20% EAJ+nanoZnO.



At low pH (<2), red flavylum cations dominate. Between pH 2-4, unstable quinoid bases exist. Around pH 6, loss of another proton forms anionic quinoid bases or colorless carbinol pseudo-bases (Filipini et al., 2020). High pH (>11) turns anthocyanins yellow (Filipini et al., 2020). Too little anthocyanin gives weak color change, too much alters its inherent color. The films' pH color response closely mirrored EAJ's, suggesting near-ideal extract loading (Kan et al., 2019). Thus, EAJ-functionalized sodium alginate films can change color with pH, showing promise as smart packaging pH sensors [46] [46] [48].

3.8 FT-IR ANALYSIS

Fourier transform infrared spectroscopy (FTIR) is a powerful and versatile tool for evaluating the molecular composition of samples, and, in the case of the films studied, for confirming the components used in their preparation and visualizing probable changes in molecular interactions and functional groups during preparation, both on its surface and inside (Supplementary Material 6 and 7).

FTIR analysis confirmed the components and revealed molecular changes in the films. Alginate films showed characteristic bands at 3277 cm⁻¹ (-OH), 2927 cm⁻¹ (C-H), 1600 cm⁻¹ (COO asymmetric stretch), 1415 cm⁻¹ (COO symmetric stretch), 1026 cm⁻¹ (COC stretch), and 945 cm⁻¹ (Na-O vibration) (Boonsiriwit et al., 2021; Koshy et al., 2022; Santos et al., 2022). The 3277 cm⁻¹ peak, from-OH groups in alginate and glycerol, increased with EAJ addition, especially at higher concentrations (F2, F3) (Supplementary Material 6) [33]; [41]. The 1600



cm¹ peak in the control film (F0) was linked to water in alginate (Song et al., 2021), and shifted with EAJ addition. Unlike some studies, higher extract concentration didn't increase peak size, with F3 (20% EAJ) showing reduced absorbance compared to F2 (10% EAJ) [49] [50]. FTIR showed nanoZnO broad- ened and weakened the -OH peak, indicating interaction. Alginate structure remained intact with nanoZnO and EAJ addition. EAJ and nanoZnO char- acteristic bands were present, confirming dispersion. Peak intensity changed with EAJ concentration, suggesting varying interactions between anthocyanins, polysaccharides, and nanoZnO, consistent with other studies on phenolic-rich films (Supplementary Material 7) [32] [29].

3.9 ANTIMICROBIAL ACTIVITY OF BIOPOLYMERS

Antimicrobial testing shows that while none of the films inhibited the Gram- negative *Pseudomonas*, films with both nanoZnO and EAJ (F4, F5, F6) did inhibit the Gram-positive bacteria tested (Supplementary Material 8). This aligns with previous research (Indumathi et al., 2019) indicating nanoZnO's ability to inhibit *S. aureus* [51]. The anti-*S.aureus* effect of the F4-F6 films (Figure 23) may be related to the combined presence of nanoZnO with the EAJ CFT, that is, the EAJ may have enhanced the antibacterial action of nanoZnO, generally related to the release of Zn +2 in the film matrix, while the antimicrobial mechanism of phenolic compounds is related to their partially hydrophilic nature, which assists in the interaction with the cytoplasmic membrane and bacterial lipopolysaccharides, causing instability and cell death [29].

3.10 FINAL CONSIDERATIONS AND PERSPECTIVES

The jambolan extracts (EAJ, EHAcJ, EHJ) were rich in phenolics and an- thocyanins, absorbed UV light intensely, and showed high antioxidant capacity (>90% DPPH). Incorporating EAJ into alginate films boosted antioxidant ca- pacity (up to 92% in F2). Films without nanoZnO were highly water-soluble, suggesting use in soluble sachets and rapid environmental degradation. NanoZnO films had low solubility, suitable for high-moisture foods. Film color varied with nanoZnO (pink/purple vs. yellow/green). FTIR confirmed the presence and integrity of added compounds, and EAJ improved film cohesiveness, mechanical, and barrier properties. EAJ-enriched biopolymers were pH-sensitive, indicating potential for freshness monitoring. Formulations with 20% EAJ (F3, F6) showed good pH indication, low water solubility, and antioxidant/antimicrobial activity against *S. aureus*, making them suitable for



multilayer biopolymers for food preservation and freshness indication. Future research will focus on multilayer film preparation, microstructure and thermal properties, bioactive compound migration, and active packaging application for refrigerated fish by-products compared to synthetic films.

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