Antibacterial activity and physicochemical characterization of bioplastic films based on cassava (*Manihot esculenta* Crantz) starch and rosemary (*Salvia rosmarinus*) essential oil

Actividad antibacteriana y caracterización fisicoquímica de láminas bioplásticas basadas en almidón de yuca (*Manihot esculenta* Crantz) y aceite esencial de romero (*Salvia rosmarinus*)

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Abstract

Bioplastics composed of renewable sources and antimicrobial components are desirable in food packaging. This study prepared bioplastic films with cassava starch and rosemary essential oil using a casting methodology. Film antibacterial activity, water vapour transmission (*Wvt*), mechanical resistance, and microstructure were measured after exposure to pathogenic bacteria such as *Salmonella enterica, Escherichia coli, Staphylococcus aureus, and Bacillus cereus.* Antibacterial activity was evidenced against the pathogens evaluated except for *B. cereus*. The films showed average values of *Wvt* 3.6988 (10^{-14} g/Pa s m), tensile strength 8.90 MPa, young modulus 1679.72 MPa, and elongation at break 4.33%. Film microstructure showed good adhesion to bioplastic components in the matrix. Bioplastics of cassava starch and rosemary oil constitute potential food packaging solutions mainly for fruits, egg-based products or chicken.

Keywords
polymers • packaging • bacteria • water vapour

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Resumen

Los bioplásticos elaborados a partir de fuentes renovables y componentes antimicrobianos son deseables en el empacado de alimentos. Por tanto, se prepararon láminas bioplásticas con almidón de yuca y aceite esencial de romero usando el método de vaciado en placa. Se midió la actividad antibacteriana, transmisión de vapor de agua (*Tva*), resistencia mecánica y microestructura de láminas bioplásticas. Las láminas fueron expuestas a bacterias patógenas como *Salmonella enterica, Escherichia coli, Staphylococcus aureus y Bacillus cereus*. Se evidenció actividad antibacteriana para los patógenos evaluados excepto para *B. cereus*. Las láminas evidenciaron valores promedio de *Tva* 3,6988 (10^{-14} g/Pa s m), esfuerzo a tensión 8,90 MPa, módulo de young 1679,72 MPa y deformación a la rotura 4,33%. Su microestructura evidenció buena adhesión entre los componentes de la matriz bioplástica. Estos resultados muestran el potencial de los bioplásticos de almidón de yuca y aceite esencial de romero para el empacado de alimentos, principalmente de frutas o productos elaborados con huevo o pollo.

Palabras clave

polímeros • empaques • bacterias • vapor de agua

Introduction

The production of bioplastics from renewable sources is a field of research, development, and innovation of great interest worldwide (58). Bioplastics have increased from 2.4 million tons in 2021 to 7.5 million tons in 2023 (21). Applications include the packaging industry, agriculture/horticulture, consumer electronics, automobile, consumer goods, and household appliances. Package manufacturing, where rigid and flexible materials are required, is the most representative market segment (12, 23).

Bioplastics can be totally or partially obtained from natural sources (32). Fossil raw materials are generally not biodegradable. However, exceptions such as polycaprolactone can be used to make bioplastics. Polysaccharides, proteins, and fatty acids are renewable raw materials commonly used to manufacture bioplastics. Cellulose, starch, pectin, alginate, soy, wheat gluten, and gelatin are used alone or mixed with fossil polymers such as polyethylene or polypropylene (15, 46). Starch is a polysaccharide frequently used in bioplastics due to availability, costs, and biodegradable and renewable characteristics (58). Among bioplastics, starch-based bioplastics are the most widely traded (21). However, some disadvantages, mainly related to polarity, limit some applications (36, 58). Bioplastic food packaging must overcome the “polarity challenge” that implies high deterioration risks (48).

Active compounds increase biopolymers functionality for active food packaging (25). Food packaging with antimicrobial components has a positive impact on shelf life of packaged products (43, 46). These components are generally compatible with the natural raw materials used to produce bioplastic. Many studies have incorporated essential oils and plant extracts in polymer matrices to obtain bioplastics (10, 22, 25, 29, 42, 52, 57). Nevertheless, very few studies measure antimicrobial effects of rosemary oil incorporated in bioplastic films on more than three strains of bacteria or fungi. In fact, the composition of the essential oil may vary according to the place of origin affecting both bioplastic antimicrobial and physicochemical properties. To the best of our knowledge, no study has simultaneously evaluated the influence against Gram-negative bacteria (*E. coli* and *Salmonella sp.*) and, Gram-positive bacteria (*S. aureus* and *B. cereus*).

Bioplastic mechanical properties, stability against moisture and antimicrobial characteristics determine their applications. This study aimed to determine the antibacterial activity against *E. coli, S. enterica*, *S. aureus,* and *B. cereus*, physical-chemical and mechanical properties of a bioplastic film made with cassava starch and rosemary essential oil.

Materials and methods

Materials

Cassava starch (*Manihot esculenta* Crantz) was purchased from Tecnas S. A. (Cali, Colombia). Rosemary (*Salvia rosmarinus*) oil was purchased at the local market (Cali, Colombia). Food-grade glycerol was purchased from Merck (Burlington, MA, USA). All chemicals were reagent grade and purchased from Merck (Burlington, MA, USA). The American Type Culture Collection (ATCC) of the Universidad de San Buenaventura Cali (Colombia) provided bacteria. Two Gram-positive bacteria, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 15579 and two Gram-negative bacteria, *Salmonella enterica* ATCC 13314 and *Escherichia coli* ATCC 10798, were evaluated. This study was conducted at the University of San Buenaventura Cali, in Cali, Colombia.

Rosemary essential oil extraction

Rosemary leaves were placed in distilled water (mass/volume ratio 1/12). The essential oil was extracted in a hydrodistillation system for 4 hours at 100°C and stored refrigerated.

Film preparation

Cassava starch (CS) films were produced by the casting method (47) from forming suspensions (FSs). The FSs were prepared by dissolving 3 g of CS, 120 mg of rosemary essential oil, 83 mg of tween-80, and 0.75 g of glycerol in 100 mL of distilled water with heating (75 ± 5°C) and magnetic stirring. The FSs were dehydrated by convective drying at 40°C until obtaining films with 10% humidity, optimized formulation from a previous study (39) with a central composite design. The optimized formulation was validated with error values ranging from - 3.31 to 10.61%.

Antibacterial properties

Film antimicrobial activity was evaluated against Gram-negative bacteria (*E. coli* and *S. enterica*) and Gram-positive bacteria (*S. aureus* and *B. cereus*) using the disc diffusion method (54).

Mueller-Hinton agar (Sigma-Aldrich) was used to inoculate the bacteria. Then, a foil disc was placed in the center of the Petri dishes, and incubated at 37 ± 2°C for 24 hours. A calibrator (Mitutoyo, Japan) was used to measure the halo around the disc, determining inhibition percentage with Equation 1:

$$\%\, inhibition = \frac{halo\ diameter}{colony\ diameter}\ x\ 100 \tag{1}$$

Five repetitions were made for each bacteria. Chloramphenicol (Colmed, International) was used as a positive control at 100 ppn (parts-per notation).

Statistical Analysis

ANOVA and Fisher's LSD determined significant differences among treatments. Minitab 19 software was used to analyze variance with a significance level of 5%.

Rosemary essential oil

A Gas Chromatograph (AT 6890 Series Plus, Agilent Technologies, Palo Alto, California, USA) coupled to a mass selective detector (Agilent Technologies, MSD 5975 Inert XL) determined the chemical composition of rosemary essential oil operated in the full radio frequency sweep. The column was DB-5MS (J & M Scientific, Folsom, CA, and USA) [5% -phenyl-poly (dimethylsiloxane), 60mm x 0.25mm x 0.25µm]. Injection was done in Split mode (30:1) with a volume of 2µL.

Water vapour transmission

The water vapour transmission *Wvt* was measured gravimetrically following the ASTM E96-05 standard methodology (16). We used glass permeation cells filled with silica gel (0% RH). Films with a diameter of 80 mm were bonded with liquid silicone in the circular mouth of each cell. Cells were stored in airtight containers with a saturated sodium chloride solution (73 ± 2% RH) at 25°C. Weight variation in the permeation cell was plotted against time. Slopes were calculated by linear regression. The *Wvt* (g/Pa s m) was calculated by equation 2:

$$Wvt = \frac{WVTR}{PxRH} x\, l \qquad (2)$$

where:

WVTR = water vapour transmission rate, calculated as the ratio between the slope of the straight line (g/s) and the permeation cell area (m^2)
P = saturation vapour pressure of water (Pa)
RH = relative humidity in the airtight container
l = mean film thickness (m). Analyses were conducted in triplicate.

Mechanical properties

A texturometer (EZ-Test L, Shimadzu, Japan) equipped with Trapezium X software conducted the test following the ASTM D882-10 standard (55). The films were cut in a rectangular shape of 20 mm wide and 100 mm long and stored for a week at 50% RH. The initial gauge was 65 mm long and test speed was 50 mm/min, using a load cell of 500 N. Tensile strength *Ts*, young modulus *Ym,* and elongation at break Eb were measured. Tests were performed ten times and the average was reported.

Scanning electron microscopy

Film surface morphology was analyzed at 20Kv scanning electron microscopy (SEM) (Jeol JSM-6490LV, USA) with backscattered electrons obtaining surface and cross-section images. Samples 5 mm wide and 5 mm long were coated with gold in a vacuum chamber (Denton Vacuum, Desk IV, USA). Images were captured at 500 and 2000 increases.

Results and discussion

Antibacterial properties

Inhibition percentages shown in table 1 indicate bioplastic films showed higher inhibition against *E. coli, S. aureus* than against *S. enterica*.

Table 1. Antibacterial inhibition percentages of bioplastic films.
Tabla 1. Porcentaje de inihibición antibacteriana de las láminas bioplásticas.

Bacteria	Inhibition (%)
E. coli	28.78 ± 0.45 [c]
S. enterica	18.48 ± 0.28 [b]
S. aureus	27.51 ± 0.75 [c]
B. cereus	0.00 ± 0.00 [a]

Different letters in the same column indicate significant differences ($p<0.05$).
Letras diferentes en la misma columna indican diferencias significativas ($p<0,05$).

The antibacterial activity of rosemary essential oil depends on ketones and monoterpene hydrocarbons that affect cell membrane permeability (5). This oil has proven antibacterial effects against *E. coli* (5, 18, 27, 30, 31), *Salmonella* (2, 33), and *S. aureus* strains (5, 6, 18, 27).

As shown in table 1, page 129, *B. Cereus* was not inhibited, probably given to bacterial rapid mutation and adaptation to different media, reaching quick resistance against antimicrobial agents (14, 53). Unlike *E. Coli, S. enterica*, and *S. aureus*, *B. cereus* is a sporulated bacterium, a mechanism that reinforces cell wall protection via environmental isolation and prevention of inhibitory interactions (40). It also generates highly resistant biofilms hindering its elimination (19).

Table 2 shows how rosemary oil incorporated in the film is mainly composed of *β*-Mircene (27.8 g/100 g), Camphor (23.9 g/100 g), and 1.8-Cineol (16.2 g/100 g). *β*-Mircene is an antibacterial monoterpene against *S. aureus, E. coli, Pseudomonas aeruginosa,* and *Proteus vulgaris* (3). Camphor is a terpenoid that affects lipoproteins and lipopolysaccharides present in bacteria cell walls, particularly gram-negative ones, generating lysis and subsequent cell death (6, 59). The third main component, 1,8-Cineol (9), is an oxygenated monoterpene (26, 35) widely used as inhibitory agent of food pathogens (9, 37). Even though many compounds have antimicrobial capacity (3), microorganisms develop defence and resistance mechanisms such as biofilms, a conglomeration of different cells allowing group protection from external factors (38). However, 1.8-Cineol inhibits biofilm formation in *S. aureus* through inhibitory agents affecting cell wall (34).

Table 2. Rosemary oil composition.

Tabla 2. Composición del aceite de romero.

Compound	Concentration (g/100 g)
Triciclene	0.2
α-Tujene	1.0
α-Pinene	4.5
Canfene	5.5
Sabinene	0.1
β-Pinene	4.6
β-Myrcene	27.8
α-Felandrene	<0.1
α-Terpinene	<0.1
p-Cimene	1.5
Limonene	2.1
1,8-Cyneol	16.2
trans-β-Ocimene	0.1
ϒ-Terpinene	0.3
*c*is-Sabinene Hydrate	0.3
Terpinolene	0.2
6,7-Epoxymyrcene	0.1
Linalool	0.6
trans-Sabinene Hydrate	0.2
Camphor	23.9
Borneol	0.8
$C_{10}H_{16}O$ (M^+ 152)	0.2
Terpinen-4-ol	0.7
α-Terpineol	1.1
Verbenone	0.3
Bornyl Acetate	3.4
α-Copaene	0.1
trans-β-Cariofilene	2.2
α-Humulene	0.7
ϒ-Muurolene	0.2
δ-Cadinene	0.2
Caryophyllene oxide	0.9

Water vapour transmission

Table 3 shows an experimental average *Wvt* of 3.6988 (10^{-14} g/Pa s m), lower than for other studies under similar manufacturing conditions. Considering that minimum *Wvt* values allow low vapour exchange between the food and the surrounding atmosphere, bioplastics for the food packaging industry should have low *Wvt* values for a longer shelf life (17).

Table 3. Physiochemical and mechanical characteristics of bioplastic films.

Tabla 3. Caracterización fisicoquímica y mecánica de las láminas bioplásticas.

Characteristic	Average values
Thickness (mm)	0.079± 0.002
Wvt (10^{-14} g/Pa s m)	3.6988 ± 0.158
Ym (MPa)	1679.72 ± 17.295
Eb (%)	4.33 ± 0.152
Ts (MPa)	8.90 ± 0.199

Wvt values of 3.11 to 8.72 (10^{-11} g Pa s m) were reported in anchovy (*Coccinia abyssinica*) starch films with cellulose nanocrystals and rosemary essential oil (27); from 2.95 to 2.7 (10^{-10} g/Pa s m) in films of polyvinyl alcohol, corn starch and cardanol oil (56); from 5.8 to 11 (10^{-10} g/Pa s m) in cassava starch films with rosemary extract (47); 4.16 to 5.27 (10^{-11} g/Pa s m) in modified cassava starch films (13); 5.8 to 12.5 (10^{-10} g/Pa s m) in cassava starch films with rosemary nanoparticles (20) and 3.9 to 8.2 (10^{-11} g/Pa s m) in biodegradable films of cassava starch with nanoclays (50). In the food industry, cellophane polymer derived from cellulose is used as wrapping film in the confectionery industry with a *Wvt* of 8.44 (10^{-11} g/Pa s m) (20). Considering conventional films, our bioplastic obtained good values.

The *Wvt* values obtained are related to film composition. The starch/tween 80 ratio constitutes a relevant factor since when its concentration allows for a continuous network, this polysorbate acts as water vapour transmission barrier (7). The network keeps the surfactant molecules dispersed, promoting a balance between the hydrophobic and hydrophilic phases and reducing *Wvt*. An excessive concentration of tween 80 will enhance the plasticizer effect, increasing the free volume inside the bioplastic structure and increasing *Wvt* (8). In addition, when starch and glycerol proportions increase, *Wvt* values may as well increase. Both starch and glycerol behave as polar components stimulating OH bonds with water molecules. Instead, the interaction between starch and rosemary oil limits the amount of water absorbed by the film (27) with covalent bonds that reduce OH groups and consequently decrease *Wvt* (49). The equilibrium among bioplastic components promoted low water values for food packaging.

Mechanical properties

Mechanical properties define bioplastic usage in food packaging. Tensile strengths and Young's modulus relate to mechanical tensile strength, while elongation at break defines ductility.

Table 3 shows an average tensile strength of 8.9 MPa, and Young's modulus of 1679.72 MPa, both higher than those reported in similar studies. Biofilms made from anchovy starch (*Coccinia abyssinica*) with cellulose nanocrystals and rosemary essential oil evidenced Ts values of 9.42 to 23.44 MPa (27). Bioplastic films made of modified starch with soybean oil oligomers reported *Ts values* of 3.35 MPa (58), while other ones made from cassava starch showed *Ts* values from 0.1 to 1.07 MPa and *Ym values* from 0.07 to 0.50 MPa (11). Films with essential oils had *Ts values* from 3 to 14 MPa (20), and bioplastic films of cassava starch with cinnamon essential oil showed Ts values ranging from 1.05 to 3.75 MPa (51). Plantain starch films had Ts values from 2.4 to 12.4 MPa and *Ym* values from 55.6 to 1482.2 MPa (41).

Bioplastic components define final mechanical properties while their concentration affects moisture gain. Starch is the major film component affecting mechanical resistance, forming hydrogen bridges with water and promoting adsorption. Water acts as a plasticizer agent, increasing mobility of polymer structure and, thus, decreasing mechanical resistance. On the other hand, the oil-starch bonds promote structural stiffness and increase polymer mechanical strength (41). However, excessive apolar components could reduce cohesion of starch binding forces and consequently, mechanical strength (24, 27).

Table 3 (page 131), shows average elongation at break (*Eb*) of 4.33%. In other studies, *Eb* values were higher, indicating low flexibility of our films. Biofilms made from anchovy (*Coccinia abyssinica*) starch with cellulose nanocrystals and rosemary essential oil reported *Eb* values between 27.71 and 73.91% (27). Others made of starch with soybean oil showed *Eb* of 58.32% (58); while films made of *Dioscorea hispida* Dennst starch and natural antimicrobial agents from turmeric extract showed *Eb* of 30.24% (28). Films made of cassava starch with cinnamon essential oil had *Eb* values between 128 and 264%; others made of corn starch with essential oils had Eb values from 30 to 170% (20). In bioplastic films made of cassava starch with cinnamon, cloves, and oregano essential oils, *Eb* values ranged between 8 and 17% (1), while films of rice starch with oregano essential oil, showed *Eb values* between 83.5% and 108.8% (45).

Bioplastic low flexibility is related to intra-structure free volume. Molecular movement of the polymer is directly proportional to intern free volume. Based on the above, we state that molecular adhesion in the assessed bioplastic matrix was high, and films had low free volume. Components promoting molecular mobility are glycerol, behaving as a plasticizer, and tween 80, a surfactant. Surfactants increase free volume into adjacent starch chains generating a flexible structure (44).

Scanning electron microscopy

Figure 1, and figure 2 (page 133), show flm cross-section and surface micrographs obtained by scanning electron microscopy. A smooth and homogeneous surface on both sides of the film indicate mixing and forming processes that allow whole matrix integration and adhesion. This indicates good bioplastic functionality for food packaging. The appropriate linkage of matrix components directly affects mechanical strength and stability against moisture. In the first case, a more compact structure could have high resistance and lower deformation or breakage capacity. Researchers stated that the finely distributed structure shown in the cross-section of corn starch films through SEM justified a better physical-mechanical behaviour and even better antibacterial response than other films without this characteristic (20).

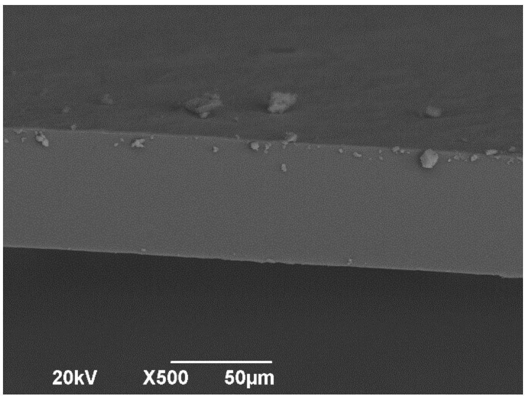

Figure 1. Scanning electron microscopic image of a cross-section of bioplastic film.

Figura 1. Imagen de microscopía electrónica de barrido de la sección transversal de la lámina bioplástica.

Structural integrity leads to good mechanical properties such as tensile strength and deformation, given by a high intermolecular interaction, components entanglement, and a continuous phase in the polymer matrix (45). Thus, interfacial interactions between mixture components and the essential oil are improved. On the other hand, when intermolecular linkage is high, the film has less porosity and empty spaces. Thus, bioplastics could have better stability against moisture.

In addition, no oil droplets were observed on film surface. In this regard, oil droplets may cause discontinuity, resulting in a cracked structure (4).

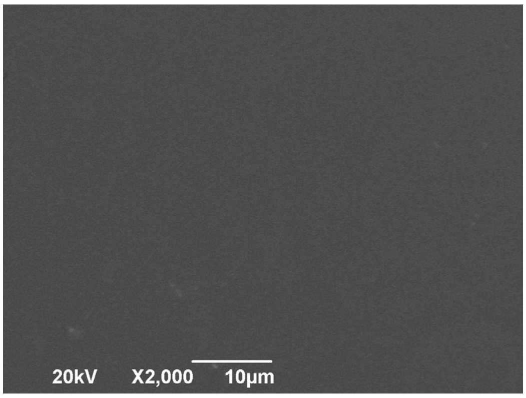

Figure 2. Scanning electron microscopic image of the surface of bioplastic film.

Figura 2. Imagen de microscopía electrónica de barrido de la superficie de la lámina bioplástica.

Conclusions

Bioplastic films based on cassava starch showed antimicrobial activity against *S. enterica, E. coli, and S. aureus,* low permeability to water vapour, good mechanical resistance, and high homogeneity in the surface and internal structure, indicating appropriate component linkage. These bioplastics constitute alternatives for packaging of susceptible foods. In this regard, these films could be used in packaging of fresh fruits or dairy products such as cheeses, where a high vapour barrier is required.

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