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Original article

Extraction of biopolymer from Cape gooseberry (*Physalis peruviana* L.) calyx residue for bioplastic synthesis

Extracción de biopolímero del residuo de cáliz de uvilla (*Physalis peruviana* L.) para síntesis de bioplásticos

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ABSTRACT: Unlike synthetic polymers, natural polymers (biopolymers) are obtained through processes with a low environmental footprint and, due to their inherent biodegradability, represent an ideal alternative for the production of single-use items such as plates, cups, and straws. The calyx of the Cape gooseberry (*Physalis peruviana* L.), an agricultural residue, is/ exceptionally rich in structural polysaccharides (cellulose, pectin, and starch), /making it a renewable and low-cost raw material for bioplastification. This study focuses on the development and methodological optimization of an efficient protocol for the extraction and purification of a biopolymer from this residue. The plant material was collected through sampling of natural populations in two Andean ecosystems, from which four distinct extraction experiments were implemented: the "ice-water" method, Soxhlet extraction, cellulose extraction, and a combined cellulose-starch method. The results allowed the establishment of a reliable protocol for obtaining a biopolymer from *Physalis peruviana* L., confirming it as a sustainable source with potential to replace petrochemical materials and to reduce both agro-industrial waste and plastic pollution.

Key words: Starch, biodegradable cellulose, natural polymer.

RESUMEN: A diferencia de los polímeros sintéticos, los polímeros naturales (biopolímeros) se obtienen mediante procesos de baja huella ambiental y, gracias a su inherente susceptibilidad a la biodegradación, son una alternativa ideal para la elaboración de productos de un solo uso como platos, vasos y sorbetes. El cáliz de uvilla *(Physalis peruviana* L.*)*, un residuo agrícola, es excepcionalmente rico en polisacáridos estructurales (celulosa, pectina y almidón), lo que lo posiciona como una materia prima renovable y de bajo costo para la bioplastificación. Este estudio aborda el desarrollo y la optimización metodológica de un protocolo eficiente para la extracción y purificación de un biopolímero a partir de este residuo. El material vegetal se obtuvo mediante muestreo de poblaciones naturales en dos ecosistemas andinos, a partir del cual se implementaron cuatro ensayos experimentales distintos de extracción: método de "hielo-agua", Soxhlet, celulosa, y método combinado de celulosa y almidón. Los resultados permitieron establecer un protocolo confiable para obtener un biopolímero a partir de *Physalis peruviana* L.*,* siendo una fuente sostenible con potencial para reemplazar materiales petroquímicos y reducir tanto los residuos agroindustriales como la contaminación plástica.

Palabras Clave: Almidón, biodegradable celulosa, polímero natural.

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INTRODUCTION

The persistent environmental pollution caused by synthetic polymers, mostly derived from petrochemical compounds and resistant to biodegradation (1), has positioned biopolymers as a technological alternative. These biologically sourced macromolecules hold fundamental ecological importance due to their inherent biodegradability, enabling their mineralization in the environment within a significantly shorter time frame (2). This characteristic is key, as it allows their application in high-consumption, short-life materials such as plates, cups, and straws, ensuring that these single-use items do not persist after disposal (3).

Polymer chemistry focuses on the study of these organic macromolecules resulting from the polymerization of monomeric units, linked through covalent bonds (4). The molecular architecture of these compounds is the primary factor determining their wide spectrum of physicochemical, thermal, and mechanical properties. Historically, production has centered on synthetic polymers, mostly derived from petrochemical compounds (5).

This problem is exacerbated by the inefficiency of recycling protocols, which often involve prohibitive energy and logistical costs in recovery, purification, and reprocessing phases. This dynamic has accelerated climate change and generated health risks, framing the urgent need for sustainable solutions (6).

In response to this crisis, research has focused on the valorization of biopolymers, as they are macromolecules of biological origin or synthesized from renewable raw materials and are proposed as the most viable sustainable alternative (7). Ecologically, their advantage lies in the mineralization of the polymer chain, a process mediated by microbial extracellular enzymes that generates harmless products (water, carbon dioxide, and methane), in contrast to petrochemical plastics (1). This property is crucial, as it enables the development of materials intended for short or single uses (such as plates, cups, and straws) that do not persist in the environment after disposal, thus closing the product life cycle.

Biopolymers are classified into main groups: those derived from biomass (such as starch, cellulose, and chitin derivatives), those synthesized from bio-derived monomers, and those synthesized by microorganisms (polyhydroxyalkanoates - PHA) (8). Biopolymers obtained from agro-industrial residues are particularly attractive, offering low acquisition costs and promoting a circular economy model.

Despite the promise of plant-based biopolymers, conventional extraction methods present significant limitations in yield, purity, and reproducibility (9). This technological barrier underscores the need to develop and optimize specific isolation protocols. From this perspective, this research focuses on non-traditional plant sources, such as *Physalis peruviana* L., an Andean species whose composition suggests high biotechnological potential (10). Optimizing the extraction process in this species constitutes an essential step for the valorization of Andean phytogenetic resources and the promotion of clean technologies applied to biopolymer production (11).

Therefore, the objective of the present research is to design and standardize an extraction protocol for biopolymers from the calyx of *P. peruviana* L., with the purpose of evaluating the yield and potential application of this renewable raw material in the development of biodegradable materials with technological projection, such as packaging and single-use utensils.

MATERIALS AND METHODS

Study area

The plant material of *P. peruviana* L. was collected in two Ecuadorian Andean ecosystems, reflecting a sampling design crucial for assessing ecogeographic variability. Samples were obtained in Pelileo (Tungurahua, 2600 m.a.s.l.), characterized by a cold climate, and in Carigan-Tenería (Loja, 1850 m.a.s.l.), with a temperate sub-humid regime. These contrasting conditions (average temperatures of 16-22 °C) allow the study of environmental influence on the yield and structural properties of the biopolymer extracted, mainly from the calyx of the species.

Collection

Biological sampling of *P. peruviana* L. was carried out in natural populations using 5 × 5 m quadrat plots. Collection focused on calyces detached at the base of the plants, selecting only those with structural integrity and chromatic tones ranging from yellowish-green to light brown, corresponding to different maturation stages. Specimens showing any signs of physical deterioration or disintegration were rigorously excluded. In the laboratory, the calyx was manually separated from the fruit and dried at 32 °C for one week. Subsequently, the dried material was processed. For assays 1, 2, and 3, crushed calyx was used, while assay 4 employed whole calyx, following protocols reviewed in the literature.

Experimental Design

The methodology focused on obtaining a biopolymer from the calyx of goldenberry (*Physalis peruviana* L.) through four main assays designed to extract its key components: resins (Assays 1 and 2) and cellulose (Assays 3 and 4). The assays used dried material processed in two forms: crushed (Assays 1, 2, and 3) and whole (Assay 4), aiming to apply different extraction protocols reviewed in the literature.

For resin extraction, two distinct methods were applied, based on the principle that low temperatures or the use of organic solvents facilitate the separation of the active compound (12).

Assay 1 (ice-water): The method relied on resin fragilization induced by cold. Two hundred grams of crushed calyx were agitated in cold water (0-15 °C) for 45 minutes to facilitate separation. Although part of the resin remained retained in the fibers upon hydration, an efficiency of 90 % was achieved. The resin obtained was dried with filter paper for one hour (12).

Assay 2 (Soxhlet extraction): Resin was extracted using a Soxhlet apparatus with ethanol as the solvent for three days. The extract was concentrated in a rotary evaporator, and the resin obtained was treated with an acid bath to reduce its sticky consistency and facilitate handling (13).

Assay 3 (cellulose extraction): Twenty-five grams of crushed calyx were subjected to a four-stage process to obtain high-purity cellulose (14-16):

1. Initial Alkaline Hydrolysis: Treatment with 150 mL of 10 % NaOH at 150 °C for one hour to remove waxes, pectins, and resins.
2. Mild Acid Hydrolysis: Application of 150 mL of 0.4 % H_2SO_4 for one hour, followed by washing with distilled water until neutral pH.
3. Chlorination: Use of 250 mL of 3.5 % NaClO in a water bath at 30 °C until reaching pH 9.2, followed by washing until neutral pH.
4. Final Alkaline Extraction and Bleaching: Extraction with 150 mL of 20 % NaOH for one hour, followed by bleaching with 0.5 % NaClO for one hour.

The cellulose obtained was air-dried for 24 hours and then oven-dried at 60 °C for another 24 hours.

For Assay 3, 0.25 g of extracted cellulose were mixed with 3 mL of water, 0.5 mL of glycerin, and 0.5 mL of vinegar. Based on the results, three sub-assays were performed:

- Assay 3.1: 0.25 g cellulose + 0.25 g chitosan.
- Assay 3.2: 0.25 g cellulose + 0.25 g starch.
- Assay 3.3: 0.25 g cellulose (sieved at 0.17 mm) + 0.50 g starch.

Each mixture was stirred at 200 rpm and 100 °C for 30 minutes, then oven-dried at 50 °C for 24 hours, following the protocol (15).

Assay 4 (cellulose-starch): This protocol was based on modifications of previous references (14,16), using 6 g of whole dried calyx. The process consisted of:

1. Alkaline/Stabilizing Treatment: 150 mL of 10 % NaOH and 30 mL of 1 % Na_2SO_3 (to preserve polymers) at 100 °C for 3 hours under agitation. The solid material was filtered and washed until a crystalline color was obtained.
2. Prolonged Bleaching: The sample was treated with 0.5 % NaClO in a water bath at 50 °C under agitation, following a cycle of 4 hours, then 2 hours, and finally 24 hours, with intermediate washes.

The solid material was ground with 100 mL of water, filtered, and used for biopolymer formulation. Two grams of extracted cellulose were combined with 6 mL of water, 0.25 g of starch, 1 mL of acetic acid, and 5 drops of glycerin. This mixture was stirred at 100 °C and 300 rpm for 30 minutes. The viscous solution was poured into a plastic Petri dish and oven-dried at 45 °C for 24 hours, followed by ambient drying for 48 hours.

Evaluation of transparency and biodegradability of biopolymers

Treatments that showed signs of polymerization and a partially transparent appearance were selected for transparency and biodegradability evaluation.

Transparency

For transparency assessment, biopolymer samples were sectioned into films of 2.5 cm × 1 cm, obtaining five replicates per assay. Each film was microscopically analyzed through three observations in different surface areas to determine optical homogeneity. Transparency was rated using a Likert scale: 1 (completely opaque), 2 (slightly opaque), 3 (semi-transparent), 4 (transparent), and 5 (completely transparent) (17).

Biodegradability

To evaluate photo-induced degradation of biopolymers, samples were exposed to ultraviolet (UV) radiation at a wavelength of 365 nm for six days. Initial and final weights of each sample were recorded to determine mass variation associated with the degradation process (18).

Statistical Analysis

Data analysis was performed using SPSS software, version 27. Comparison of the variables Transparency (Likert scale) and Weight Loss (Biodegradability) among the five independent assays (E3, E3.1, E3.2, E3.3, E4) was conducted using the Kruskal-Wallis test. Statistically significant differences ($p < 0.05$) were subsequently evaluated through pairwise comparisons with Bonferroni correction, in order to determine the optimal protocol in terms of optical properties and degradation.

RESULTS AND DISCUSSION

In Assays 1 and 2, the objective was to formulate biopolymers from plant resin. In Assay 1, the material obtained exhibited a brittle and powdery texture with a light green coloration, indicating incomplete polymerization and low structural cohesion. In contrast, Assay 2 produced a biopolymer with a sticky consistency and dark green tone, demonstrating greater retention of resinous compounds and a less efficient drying process (Figure 1).

In Assay 3, based on cellulose extraction, a biopolymer with an intense yellow coloration was obtained, presenting an opaque mass without evidence of effective polymerization. Regarding Sub-Assays 3.1 and 3.2, both exhibited a soft and moist texture and retained a yellowish tone even after 24 hours of oven drying, without achieving the formation of a consolidated polymeric matrix. In contrast, Sub-Assay 3.3 revealed the most favorable results, displaying a more uniform structure, whitish coloration, smooth and glossy surface, and a degree of transparency comparable to that of a conventional biopolymer (Figure 2).

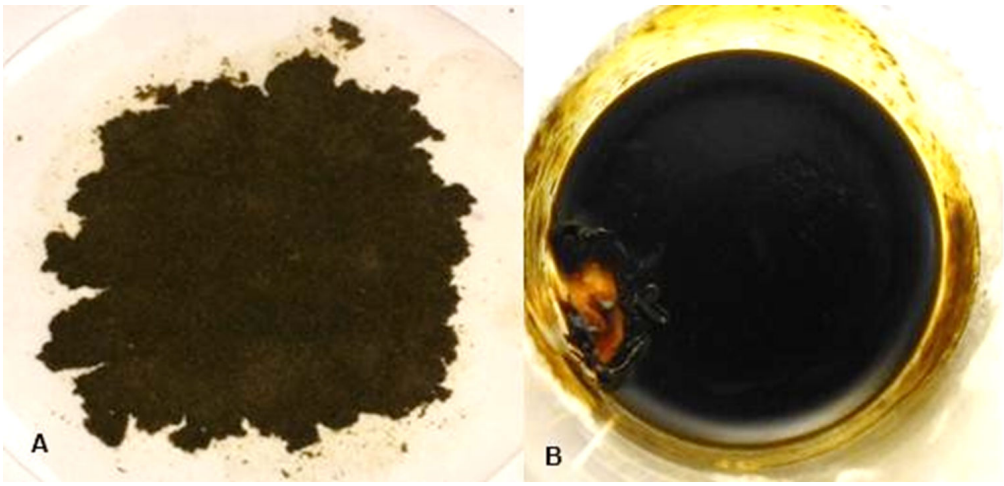

Figure 1. (A) Biopolymer obtained through “Ice-Water” extraction; (B) Biopolymer obtained through Soxhlet extraction

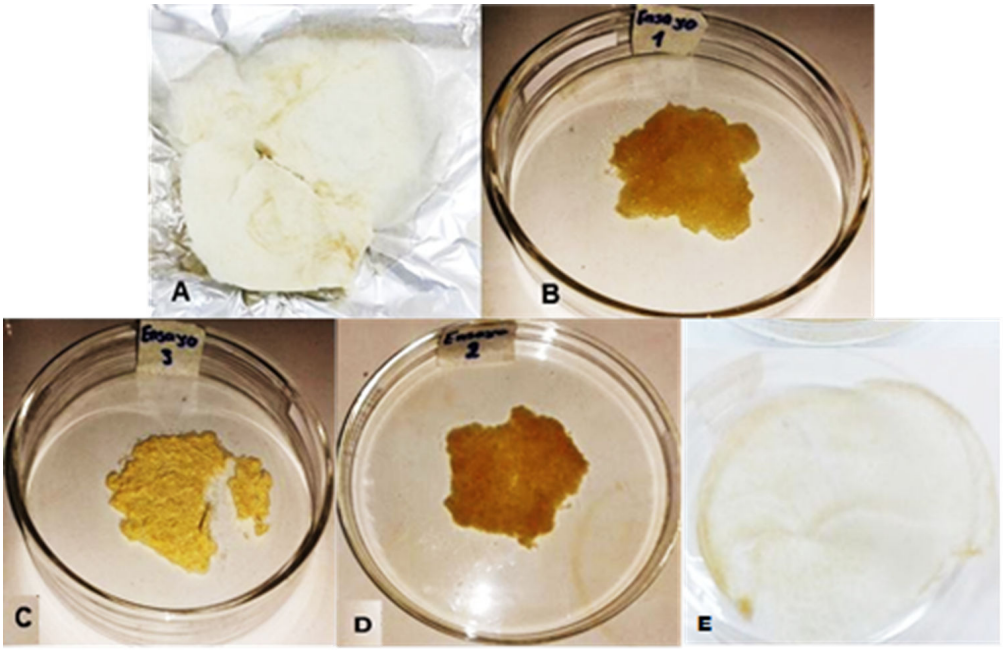

Figure 2. (A) Extracted cellulose; (B) Assay 3 - Cellulose biopolymer; (C) Assay 3.1 - Cellulose biopolymer with chitosan; (D) Assay 3.2 - Cellulose biopolymer with 0.25 g starch; (E) Assay 3.3 -mer with 0.50 g starch Cellulose biopolymer

In Assay 4, a whitish film with a tendency toward transparency surface with evident gloss. These properties remained stable and consistent both under accelerated and during ambient was obtained, characterized by a smooth, homogeneous oven-drying conditions drying (Figure 3).

The transparency variations among the different assays produced from resin performed. Films (Assays 1 and 2) exhibited a completely opaque appearance, indicating low light transmission through the material. Similarly, the biopolymer obtained solely from cellulose cellulose-chitos (Assay 3) and then blend (Assay 3.1) also showed null transparency, evidencing a compact aspect with no visible light passage. Assay 3.2, mixed with 0.25 g of starch, reached the “slightly opaque” level on the Likert scale. In contrast, Assay 3.3, with 0.50 g of starch, generated a positive effect on the optical property of the material, achieving a high degree of transparency and being rated as “transparent” on the Likert scale. Likewise, the sample corresponding to Assay 4, composed of cellulose, with a homogeneous and transparent and starch, displayed similar visual characteristics surface. The evidenced statistically Kruskal-Wallis test significant differences in transparency values among the biopolymers across the different assays ($H = 74.000$; $df = 4$; $p < 0.001$). These results highlight variations in transparency depending on the assay type (Table 1).

Table 1. Comparison of assays using the Kruskal-Wallis test

Summary of Kruskal-Wallis test for independent samples	
Total N	75
Test Statistic	74.000[a]
Degrees of Freedom (df)	4
Asymptotic Significance (two-tailed)	<0.001

*$p < 0.05$ indicates significant differences in transparency among the assays

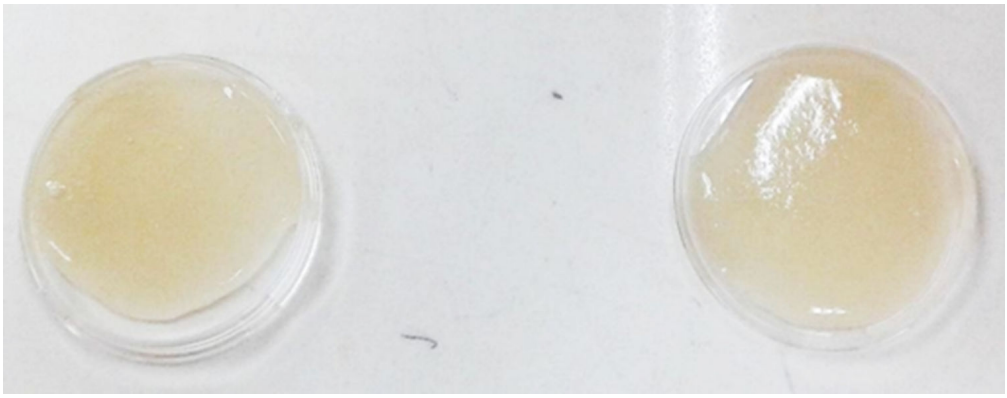

Figure 3. Cellulose-starch biopolymer

Pairwise comparisons showed that conditions in E3.3 and E4 induced the most significant changes in biopolymer transparency compared to E3 and E3.1 (adjusted p = 0.000), indicating that these are the most effective conditions optical property for modifying this. However, the transparencyE3.3 and E4 resulting was statistically identical (adjusted p = 1.000). Similarly, conditions E3 and E3.1 equivalent transparency the strong influence also resulted in (adjusted p = 1.000). Data analysis (Table 2) confirmed of conditions E3.3, E4, and E3.2 on the final transparency of the biopolymer.

Assays 1 and 2 produced materials with brittle and sticky textures, respectively, illustrating the challenges in controlling polymerization to achieve the ideal characteristics of a functional biopolymer. An optimal biopolymer should exhibit not only adequate structural integrity (avoiding the brittleness observed in Assay 1) but also nonadhesive surface properties and dimensional stability (unlike the stickiness of Assay 2). These properties are intrinsically linked to the degree of polymerization and crosslinking. The brittleness and dark coloration of Assay 1 correlate with incomplete

Table 2. Pairwise comparison of assays using the Kruskal-Wallis test

Pairwise Comparisons of Assays					
Muestra 1- Muestra 2	**Test Statistic**	**Std. Error**	**Std. Test Statistic**	**Sig.**	**Adjusted Significance[a]**
E3-E3.1	.000	7.398	.000	1.000	1.000
E3-E3.2	-22.500	7.398	-3.041	.002	.024
E3-E3.3	-45.000	7.398	-6.083	.000	.000
E3-E4	-45.000	7.398	-6.083	.000	.000
E3.1-E3.2	-22.500	7.398	-3.041	.002	.024
E3.1-E3.3	-45.000	7.398	-6.083	.000	.000
E3.1-E4	-45.000	7.398	-6.083	.000	.000
E3.2-E3.3	-22.500	7.398	-3.041	.002	.024
E3.2-E4	-22.500	7.398	-3.041	.002	.024
E3.3-E4	.000	7.398	.000	1.000	1.000
The significance level is 0.50					

Total weight loss (biodegradability) after 6 days of UV exposure was compared among the five independent assays (E3, E3.1, E3.2, E3.3, E4). The overall analysis yielded a statistically significant result: H (4) = 19.075, with an asymptotic significance of p<0.001. This indicates significant differences in the median weight loss (biodegradability) among the assay conditions applied to the biopolymer, demonstrating a meaningful effect of UV radiation on the material's biodegradability (Table 3).

Table 3. Kruskal-Wallis test for total weight loss of the biopolymer

Test Statistic [a,b]	
	TOTAL LOSS
H (Kruskal-Wallis test)	19.075
Degrees of freedom (df)	4
Asymptotic significance (.	.001

p < 0.05 indicates significant differences in biodegradability among the assays

polymerization or low molecular weight, which compromise the material's mechanical strength and toughness (19).

Results from the cellulose extraction and processing assays reveal a dependence on formulation conditions to obtain characteristics consistent with a biopolymer. Assay 3, which produced an opaque mass with an intense yellow color and no evidence of effective polymerization, represents a failure in the isolation process. Opacity in cellulosic biopolymers is often due to residual impurities (such as lignin or hemicellulose) or a high degree of fiber aggregation (microfibrils or nanofibrils) that causes significant light scattering (21).

In sub-assays 3.1 and 3.2, the persistence of a soft, moist texture after 24 hours of drying suggests high hygroscopicity associated with a disordered polymeric structure unable to consolidate a stable matrix. Cellulose, the main component of the biopolymer, is characterized by its ability to form intra- and interchain hydrogen bonds that are critical for maintaining cohesion and structural rigidity.

However, when the polymer network is more amorphous, the number or stability of these bonds decreases, favoring water penetration and retention between chains. This behavior explains the observed moist texture and indicates lower crystallinity of the cellulosic matrix and, therefore, reduced structural stability of the obtained material (22).

By contrast, sub-assay 3.3 achieved the most promising characteristics: a uniform structure, whitish color, smooth and glossy surface, and a degree of transparency comparable to that of a conventional biopolymer. This favorable outcome indicates that the extraction and/or film-formation process reached a high cellulose purity (whitish appearance) and a homogeneous structural arrangement. Transparency in cellulose is maximized when fibers are dispersed at the nanoscale (nanocellulose) or when a uniform amorphous structure is achieved that minimizes visible light scattering (23). The success of sub-assay 3.3 in producing a smooth, uniform, and transparent matrix suggests a molecular structure that balances integrity for application with potential bioaccessibility for final degradation (24).

Assay 4 represents a significant success in optimizing the biopolymer formulation, yielding a film with ideal optical and morphological characteristics: a whitish hue tending toward transparency, a smooth, homogeneous surface with evident gloss-properties that are highly desirable in high-performance bioplastics derived from cellulose for packaging applications (25). The stability and consistency of these properties, even under accelerated oven drying and ambient drying conditions, are particularly relevant because they demonstrate superior control of residual moisture and prevent additive migration or matrix retrogradation, factors that commonly compromise final product quality and long-term performance (26).

The results reveal that film transparency is strongly influenced by formulation, with significant differences between assays ($p < 0.001$). Pure cellulose formulations (E3) and cellulose-chitosan blends (E3.1) were completely opaque, suggesting high crystallinity or molecular aggregation that scatters light (27). In contrast, the incorporation of starch at optimal levels (E3.3 with 0.50 g and E4) produced a positive and statistically identical effect (adjusted $p = 1.000$), achieving a high degree of transparency and a homogeneous surface. This finding aligns with current research in biopolymers, where the addition of well-dispersed polysaccharides or plasticizers is key to reducing surface roughness and matrix heterogeneity, thereby improving light transmission and approaching the optical properties of conventional plastics (28). Achieving transparency in these films, which are also biodegradable due to their cellulose-starch composition, represents an important step toward creating sustainable packaging that can compete functionally with conventional materials (29).

Analysis of total weight loss (biodegradability) after 6 days of UV exposure demonstrated a statistically significant difference among the tested formulations ($p < 0.001$), indicating that biopolymer composition critically affects stability. UV radiation acts as a catalyst for photodegradation, initiating chain scission in polysaccharide polymers such as cellulose and starch (30). The observed variations in median weight loss confirm that specific components (E3, E3.1, E3.2, E3.3, E4) modulate the film's susceptibility to degradation. For sustainable development, it is essential that formulations balance rapid biodegradability with adequate functional stability under solar exposure (31).

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CONCLUSIONS

- The total opacity observed in films made solely from resin (Assays 1 and 2) suggests the formation of a highly disordered polymeric structure. This finding rules out the usefulness of this matrix for bioplastic industry applications that require transparency.

- The transparency of the cellulose-starch biopolymer is strongly determined by its formulation; specifically, the incorporation of starch at optimal levels (Assays E3.3 and E4) proved to be the most effective factor for achieving transparency degrees competitive with other market biopolymers.
- Biopolymer composition was key to its susceptibility to UV-induced degradation. Exposure confirmed that the photo-degradation mechanism is modulated by the presence of different additives (chitosan and starch) in the formulations.
- Based on the evaluation of optical properties, formulations E3.3 (cellulose with 0.50 g starch) and E4 (cellulose and starch) are proposed as the most suitable and promising for biopolymer production. These represent a viable option for manufacturing single-use items such as plates, cups, and straws.

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