Development and Characterization of Reinforced Flexible Packaging Based on Amazonian Cassava Starch Through Flat Sheet Extrusion

Johanna Garavito , Sofía Castellanos-González , Clara P. Peña-Venegas , Diego Castellanos *

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Article

Development and Characterization of Reinforced Flexible Packaging Based on Amazonian Cassava Starch Through Flat Sheet Extrusion

Short title: Reinforced flexible packaging from Amazonian cassava starch

Johanna Garavito [1,2], Sofía Castellanos [1], Clara P. Peña-Venegas [2] and Diego A. Castellanos [1,*]

[1] Food Packaging and Shelf Life Laboratory, Instituto de Ciencia y Tecnología de Alimentos, Universidad Nacional de Colombia, Carrera 30 Número 45 - 03, 111321, Bogotá, Colombia

[2] Instituto Amazónico de Investigaciones Científicas – SINCHI, Avenida Vásquez Cobo Calle 15/16, 910001, Leticia, Colombia

* Correspondence: dacastellanose@unal.edu.co; Tel.: +601-3165000 (ext. 19220)

Abstract

Thermoplastic starch (TPS) can be a sustainable alternative to petrochemical plastics for flexible packaging, especially in rainforests and tropical regions where native starch sources such as cassava are abundant. However, one problem preventing TPS packaging from widespread use is its susceptibility to moisture. This study evaluated TPS formulations based on Amazonian cassava starch reinforced with plantain leaf fibers, beeswax, and low-density polyethylene (LDPE) particles. The plastic compounds were extruded to obtain pellets and then films at 120-130 °C. The resulting films were then cut and heat-sealed to obtain flexible packaging. Different properties of the TPS packages were evaluated, such as mechanical strength, water vapor transmission (WVTR), color, infrared spectrum (FT-IR), and moisture adsorption. The results showed that the formulation with beeswax (2% w/w), plantain leaves powder (1% w/w), and LDPE powder (2% w/w) had a higher tensile strength (5.99 MPa) and moisture barrier (WVTR = 366.6 g m^{-2} d^{-1}) compared to the control formulation only with plasticizers (glycerol and water) but without reinforcements (0.48 MPa and 1486.6 g m^{-2} d^{-1}, respectively). Film with only beeswax (4% w/w) and plantain leaves powder (2.5% w/w) had a tensile strength = 5.53 MPa and WVTR = 716.8 g m^{-2} d^{-1}, although higher moisture adsorption compared to the film with LDPE. In both cases, homogeneous and heat-sealable bags were obtained. The reinforced TPS films can be used to reduce the environmental impact generated by single-use packaging applications, especially in food commercialization.

Keywords: thermoplastic starch; pelletization; grounded plantain leaf; beeswax; moisture stability

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Practical Applications

This study aimed to investigate packaging alternatives for replacing waste plastics in regions such as the Amazon, using locally sourced natural materials. In these areas, cassava cultivation is widespread among small producers and indigenous communities, so a bag based on this polymer is proposed, reinforced with beeswax, dried and ground banana leaves, and powdered low-density polyethylene to provide better mechanical and moisture resistance. This type of flexible biopackaging can be used for retail in small and large food stores.

1. Introduction

Using starch as a plastic material for packaging has gained significant attention recently due to the increased concern about the environmental impact of traditional polymers (Asgher et al., 2020; Cazón et al., 2017; Dutta & Sit, 2024). Thermoplastic starch (TPS) can become a sustainable and viable

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alternative to replace petroleum-derived plastics, especially in the production of single-use flexible packaging, mainly considering its high biodegradation rate at ambient conditions and low requirements for home composting (Bartolucci et al., 2023; Garavito et al., 2024; Polman et al., 2021). Replacing low-sustainability petrochemical plastics is imperative for the planet's environmental balance. In places of high biodiversity, such as the Amazon, the incidence of pollution has become alarming, as the presence of plastics has already been reported in waters (de Souza et al., 2023; Schmidt et al., 2019), alluvial soils (Lucas-Solis et al., 2021), fish (Chota-Macuyama and Mendoza, 2020; Urbansky et al., 2020), and terrestrial animals such as ants (Lenoir et al., 2016) and bats (Correia et al., 2022). Furthermore, this contamination has been associated with the proliferation of insects that carry human diseases (Soares-Pinheiro et al. 2017), suggesting that the problem could be more serious than previously considered.

There are practical problems in replacing traditional materials with more sustainable ones, such as starch. Raw materials are available for significant production (Gonçalves et al., 2024) and obtaining packages with appropriate functional characteristics. As for the first, in regions such as the Colombian Amazon, part of the population has small crops of products such as cassava, from which sufficient surpluses can be generated to obtain thermoplastic starch without affecting the food security in the region (Canales & Trujillo, 2023; Rosenthal & Ort, 2012; Vilpoux et al., 2017). Since it comes from small farms in broad geographical areas, different varieties of cassava are used according to the possibilities of each small producer (Orjuela-Baquero et al., 2016). Likewise, starch extraction is mainly done by hand, which is why there can be much heterogeneity in the raw material obtained. This implies the need for prior characterization of the starch in packaging to determine its composition, amylose/amylopectin ratio, and moisture content (Chisenga et al., 2019; Rashwan et al., 2024).

Several authors have explored using starch for flexible packaging, such as bags and pouches (Iacovone et al., 2023; Kim et al., 2020). However, some starch characteristics are unsuitable, especially its low mechanical strength and high sensitivity to moisture (Onyeaka et al., 2022; Singh et al., 2022). For the production of flexible packaging, TPS has a water vapor transmission rate (WVTR) of 1104.0 to 1120.8 g m^{-2} d^{-1} (Żołek-Tryznowska & Kałuża, 2021), tensile strength of 1-5 MPa and Young's modulus near 3-22 MPa (Betancur-D'Ambrosio et al., 2023; Méité et al., 2022), and moisture absorption ranges from 0.035 to 0.091 g g^{-1}, depending on the starch source and the type of plasticizer considering that the latter increases active site availability by exposing hydrophilic hydroxyl groups (Mali et al., 2005; Sirbu et al., 2024). Additionally, relative humidity plays a key role in water uptake and the film's structural stability (Mali et al., 2005), which limits its usefulness in the packaging of food and high-moisture products, or under high humidity and temperature conditions (Fazeli et al., 2018; Zhang et al., 2014).

One option for improving the qualities of thermoplastic starch in the manufacture of flexible packaging is its reinforcement and/or compounding with other materials (Cataño et al., 2023). Currently, many commercial flexible packages that include starch do so using formulations with high amounts of different polymers such as polylactic acid (PLA), polybutylene adipate terephthalate (PBAT), and low-density polyethylene (LDPE) (He et al., 2024; Lackner et al., 2021; Zhong & Xin, 2023). Regarding reinforcement materials, using different fillers for plastic compounding, such as cellulose fibers, clays, metal oxides, waxes, and agro-industrial waste, has been explored (Mansour et al., 2020; Pérez-Pacheco et al., 2024). These materials are included at micro and nanoscopic scales to obtain a highly homogeneous dispersion using appropriate plasticizing, cross-linking, and emulsifying additives (Stelescu et al., 2024).

Several studies have been developed to improve the properties of TPS using reinforcing materials. Siriwong et al. (2024) investigated using cellulose fibers extracted from sugarcane bagasse as reinforcement in starch films, obtaining a significant improvement in the water vapor barrier due to forming an interconnected network between starch and cellulose fibers. Jeencham et al. (2024) developed reinforced TPS films reinforced with microcrystalline cellulose, obtaining a tensile strength of 3.21–11.18 MPa and Young's modulus of 112.12–488.89 MPa, respectively. For their part,

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Marta et al. (2022) utilized chemically modified starch nanocrystals to enhance bioplastics, improving tensile strength, thermal stability, and biodegradability while reducing water vapor permeability. However, excessive concentrations led to aggregation, diminishing overall performance and effectiveness. Jumaidin et al. (2021) incorporated heat-treated *plantain leaf* fibers into thermoplastic cassava starch (TPS), enhancing the biocomposites' thermal stability, mechanical properties, and sustainability, with optimal performance at 40 wt.% fiber content. Finally, González et al. (2023) plasticized starch nanocomposited films reinforced with graphene (G) and graphene oxide (GO), prepared through solvent casting using Salvia extracts as surfactants, exhibited notable enhancements in mechanical properties and electrical conductivity, reaching values up to 9.0×10^{-4} S m^{-1} after GO reduction. The studies emphasized the importance of selecting appropriate additives, such as compatibility or functionalization agents, and ensuring thorough mixing of the components to achieve a homogeneous plastic matrix with well-dispersed reinforcing elements, resulting in superior mechanical strength and barrier properties following casting or extrusion processes.

Considering the above, in this study, cassava starch from Amazonian regions was used to obtain flexible packaging through flat sheet extrusion and reinforced with *plantain leaves* microfibers, beeswax, and LDPE microparticles to increase its stability at high relative humidity and temperature conditions typical of the Amazon region.

2. Materials and Methods

2.1. Starch

Raw cassava starch was collected from 10 indigenous communities near the so-called triple border between Brazil, Colombia, and Peru, and close to Leticia (Amazonas Department, Colombia, 25/38 °C and 76/90% RH). In this region, small-scale Indigenous producers cultivate cassava in small plots of land known as 'Chagras.' Samples of several polyclonal starch varieties from native species of the area were collected and mixed into a single final material. For this study, it was decided to proceed in this way, considering the low representativeness of each variety for possible future scaling up processing. After obtaining the raw mixture, bromatological measurements were conducted as indicated below.

Starch samples were extracted by hand, initially removing the tuber's peel and then grating the pulp. The grated pulp was then washed and left to rest, allowing the starch to settle, which was then filtered through a sheet of fine cloth to drain off excess moisture. The wet starch mass with a moisture content > 40% was then packaged in low-density polyethylene (LDPE) pouches (0.05 mm thick) and sent within 24-48 h to the facilities of the Food Packaging and Shelf-Life Laboratory of the National University of Colombia (Bogotá, Colombia), where it was kept at 4.0 ± 0.1 °C until conditioned.

2.2. Plasticizers and Additives

For the extruded film production tests, glycerol (Necardis S.A.S., Bogotá, Colombia; 99.8% purity) was used as a plasticizer, sorbitan monooleate (Span 80) supplied by Sigma-Aldrich (Ontario, Canada; 99.5% purity) was used as an emulsifier, and citric acid (Shandong Ensign Industry Co. Ltd., Shandong, China; 99.5% purity) was used as a cross-linking agent.

2.3. Reinforcing Compounds

For the inclusion tests with reinforcing components, latex extracted from the *Hevea brasiliensis* trees from the Guaviare region in Colombia, solid beeswax (Adbaquim S.A.S., Bogotá, Colombia), and sunflower oil (La Fabril S.A., Montecristi, Ecuador) were used. Likewise, low-density polyethylene (LDPE) (SABIC, Riyadh, Saudi Arabia; Ref. 1922N0) and plantain (*Musa paradisiaca*) leaves from small local crops near Leticia (Amazonas, Colombia) were also used. The leaves obtained for the study are usually discarded as waste from plantain production.

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2.4. Conditioning of Raw Materials

Before extrusion testing, starch was dried in a three-tray convection dehydrating oven (Comek, Bogotá, Colombia) at 40 ± 2 °C until obtaining a moisture content of 10.0 ± 2.0%. Moisture content was measured using a Mettler Toledo HR73 halogen moisture analyzer (Mettler-Toledo International Inc., Ohio, USA) (AOAC 925.10-1925). Once dried, starch was ground in a hammer mill pulverizer (Maquinaria & Soluciones Alimenticias S.A.S, Bogotá, Colombia) and sieved with a Number 40 mesh sieve down to an average particle size of <0.40 mm. Plantain leaves were dried down to a moisture of 7.7 ± 0.1% and then ground to a particle size < 0.15 mm. The LDPE was pulverized from blocks to a particle size of <0.40 mm. The latex was hydrolyzed by adjusting the pH to an optimal value of 3 with citric acid and incorporating hydrogen peroxide (PanReac Química SLU, Barcelona, Spain) at 5% (w/w) by continuous dripping while maintaining constant agitation for 24 hours at 40 °C ± 2 °C.

2.5. Bromatological Characterization of Amazonian Starch

Bromatological analyses were performed on the mixture of samples of polyclonal cassava starches collected from the local Amazon communities. Moisture content was determined using a Precisa EM 120-HR Moisture Analyzer (Precisa Gravimetrics AG, Moosmattstrasse, Dietikon, Switzerland) and following the AOAC method 945.15-1945. Ash content was determined using a Terrigeno Model D8 calciner (Compañia Terrigeno S.A.S., Medellin, Colombia) following the AOAC method 942.05-1990; ether extract was measured using the AOAC 920.29-1990 method, protein content was obtained by the AOAC 975.17-1975 method, and fiber and carbohydrates by the AOAC 991.43-1994 method. All the measurements were expressed as mean values ± standard deviation for 10 replicates.

2.6. Formulation and Premixing to Produce Films

After performing preliminary tests, five formulations were preselected and prepared with cassava starch as the main polymeric matrix (between 60.1-66.6% w/w), varying the inclusion and composition of additives and reinforcing components. Glycerol (23-25% w/w) and water (6.9-7.4% w/w) were included as plasticizers (Montilla--Buitrago et al., 2021; Zdanowicz & Sałasińska, 2023), citric acid (1% w/w) as a crosslinker (Gerezgiher & Szabó, 2022; Dudeja et al., 2023), polysorbate 80 (0-0.5%) as an emulsifier to stabilize the emulsion (Majeed et al., 2023), and sunflower oil, hydrolyzed beeswax latex, powdered plantain leaves and powdered LDPE (0-4% w/w each) as moisture barrier and mechanical strength reinforcements. The specific composition of each formulation is shown in Table 1. A control formulation was also included with a mixture of only starch, water, glycerol, and citric acid.

Table 1. Formulations evaluated for obtaining reinforced thermoplastic starch films and flexible packages.

Component	Formulation					
	Control	F1	F2	F3	F4	F5
Cassava starch	66.6%*	63%	63%	64.9%	60.1%	63.9%
Water	7.4%	7%	7%	6.9%	6.9%	7.1%
Glicerol	25%	25%	25%	25%	25%	23%
Sunflower oil	0%	4%	0%	0%	0%	0%
Beeswax	0%	0%	4%	2%	4%	2%
Hydrolyzed latex	0%	0%	0%	0.2%	0%	0%
Powdered plantain leaves	0%	0%	0%	0%	2.5%	1%
Powdered LDPE	0%	0%	0%	0%	0%	2%
Citric acid	1%	1%	1%	1%	1%	1%
Span 80	0%	0%	0%	0%	0.5%	0.5%

* The percentages indicated are on a w/w basis.

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Preliminary pellet, and film extrusion tests were performed, to establish the concentrations of each compound in the selected formulations, verifying that the formulation met minimum plasticizing and molding characteristics: 1) smooth flow through the extruder without blockages; 2) homogeneous pellets without granules or various visible solid phases; 3) reinforcing compounds perfectly mixed in the pellet and film without exudation or partial phase separation; 4) continuity of the film in the flat sheet extrusion without defects such as "fish eyes" formation, breakage or excessive adhesiveness.

Before starting the mixing process, the solid components (powdered plantain leaves, starch, and citric acid) were manually pre-mixed and homogenized to ensure uniform distribution. On the other hand, the beeswax was heated at 60 °C until completely melted, after which the impurities were filtered out while maintaining the temperature. At the same time, glycerol was also heated at 60 °C separately, after which it was mixed with beeswax and polysorbate 80 until fully integrated, forming a stable liquid mixture. This emulsion was then transferred to a new container, where the solid mixture was then gradually incorporated into the liquid phase, providing complete integration. For formulation F5, pulverized LDPE was added last and thoroughly homogenized to ensure even dispersion throughout the mixture.

2.7. Extrusion-Pelletization

Initially, 1 kg of a mixture of each of the formulations to be evaluated was prepared, besides the control. These were left to rest for 24 hours in closed containers at room temperature to facilitate the complete diffusion of the plasticizers throughout the material. The mixtures were fed into a Bimek BK30 extruder (Bimek S.A., Bogotá, Colombia), with a 2-inch diameter screw and a L/D ratio = 25. The extruder consists of 4 heating zones, which were set at 120-125-130-135 °C from the feeding zone to the die. 120 °C for the feeding zone, 125 °C at the compression zone, 130 °C in the dosing zone, and 135 °C at the die. The screw speed was set to 60 rpm to ensure adequate plasticization, preventing the mixture from becoming sandy or excessively liquid according to preliminary tests carried out for the fine-tuning of the process and based on other extrusion studies for TPS (Białasz & Garbacz, 2019; H. Cheng et al., 2021; Pushpadass et al., 2009). The extruded strands were cooled by a stream of dry air before cutting, allowing adequate solidification for pelletization. Finally, the strands were cut into pellets with approximate dimensions of 3 ± 2 mm in diameter and 5 mm in length.

2.8. Film Extrusion

Another extruder of the same model and brand as the one used for the strands' extrusion was used to obtain the films, but equipped with a flat sheet die with an effective output length of 21.5 cm and an opening between lips of 1.04 mm. The pellets obtained for each formulation were fed into the extruder, which was adjusted to a screw speed of 60 rpm and with a temperature profile of 120-125-130-135 °C from the feeding zone to the die. At the exit of the extruder, the molten sheet was transferred to a calender equipped with water-cooled rollers, setting the pulling speed at 4 rpm and the opening of the rollers at 0.5 mm to ensure a uniform thickness. The calender's pulling speed was adjusted to obtain continuous films with thicknesses of 0.2-0.5 mm.

2.9. Making of Pouches

The films obtained from each formulation were initially cut to obtain 15 x 20 cm pouches from a single rectangular piece. After cutting, the piece was folded in half and heat-sealed in an impulse sealer type PFS-400 (Americas Maquinaria S.A.S., Bogotá, Colombia) at an approximate sealing temperature of 120 °C. The heat-sealing process started with a contact time of 3 seconds, sealing the lateral ends of the sheets to form a continuous bag.

Once the bags of each film formulation were obtained, characterization tests were performed to evaluate their physical and stability properties, thus determining the most favorable formulation obtained for flexible packaging.

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2.10. Film Characterization

Some physicochemical parameters were determined for the films obtained, as detailed below. Functional groups of the components present in the films were identified by modulated mid-infrared analysis with a LYZA 7000™ FTIR-spectrophotometer (Anton Paar GmbH, Graz, Austria) at room temperature and measuring absorbance in the region of 4200–500 cm^{-1} with the Diamond attenuated total reflectance (ATR) cell.

Microscopy images were obtained using a CM1000C Celestron Deluxe optical microscope (Celestron, LLC., Torrance, CA, USA) with a 50–1000x chromatic lens and 1000x magnification. Samples were sectioned for top and cross-sectional observation and mounted on uncoated slides. Environmental conditions during analysis were 14 ± 2 °C and 75 ± 5% RH. Illumination was provided from the bottom of the optical system, generating an inverted field that highlighted morphological details.

The film's thickness was determined with a Mitutoyo® Digimatic IP65 digital micrometer (Mitutoyo, Kawasaki, Japan) by measuring at three different points of the sample and recording the average value.

The samples' color was measured on the surface of the films using a 3nh YS3020 spectrophotometer-colorimeter (Shenzhen 3nh Technology Co., Ltd.), reporting the L*, a*, and b* coordinates of the CIELAB color space. A standard illuminant, "Daylight 65," and a 10° observer were considered.

The water vapor transmission rate (WVTR) and water vapor permeability coefficient, water vapor adsorption, and mechanical strength of the films were determined at 35 ± 2 °C and 75 ± 5% RH, simulating conditions like those expected in the Leticia area (Amazon, Colombia). In each case, the samples for each test were introduced into an ICH110 climatic cabinet (Memmert GmbH + Co.KG, Schwabach, Germany). The climate cabinet was maintained under forced air working conditions at maximum adjustable fan speed capacity to allow homogeneous conditions (Mahecha-Rubiano et al., 2024).

The water vapor permeability coefficient was determined following the ASTM E96/E96M-22 standard, based on gravimetric weight loss analysis (Water Method). Initially, distilled water (5 ± 0.05 g) was introduced into a 35 mm diameter aluminum cylindrical cell with a permeation area of 0.00096 m^2, and the test film was then placed on top of the cell. The weight loss of the evaporated water (permeated through the film) was recorded until a constant loss rate was reached, and with the differentials of lost weight, the calculation of the water vapor transmission rate (WVTR, in g m^{-2} d^{-1}) was performed. From this, the permeability coefficient of each material was estimated (in g mm m^{-2} atm^{-1} d^{-1}) considering the thickness and permeation area of the film (Garavito et al., 2022).

The sample's moisture (water vapor) adsorption was determined following a procedure similar to the ASTM D570 standard. Samples of 100 x 20 mm were used and preconditioned in an oven at 50 °C for 24 h before testing. Moisture adsorption was estimated based on the increase in sample weight relative to its initial weight while exposed to the controlled conditions (Pérez-Martínez et al., 2024). The moisture adsorption of the films was measured at 6 and 24 h after being placed in the climatic cabinet.

On the other hand, additional samples were introduced to determine the tensile strength based on the same storage conditions, taking measurements at 0, 6, and 24 h. In this way, the effect of high humidity and temperature conditions was evaluated on the mechanical properties of the films. Tensile strength was determined according to the ASTM D882 standard. For this, 100 x 20 mm specimens were prepared and placed in a Lonroy LR-C001 tensile machine (Dongguan Lonroy Equipment Co. Ltd., Dongguan, China), where they were stressed to failure at an elongation rate of 10 mm s^{-1}. Peak force (N), tensile strength (MPa), elongation at break (%), and Young's modulus (MPa) were recorded.

All measurements were made in triplicate, reporting each case's mean value and standard deviation.

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2.10. Statistical Analysis

A General Linear Model (GLM) ANOVA was performed at a 95% confidence level to compare the different formulations according to their property measurements. The statistical analysis was conducted using Minitab® Statistical Software 22.2.1 (State College, PA, USA: Minitab, LLC). The model included treatment, time, and interaction as fixed factors to assess their individual and combined effects on the response variable. Following the ANOVA, Tukey's HSD test method was applied to determine which means differ significantly from others within each factor level. The multiple comparisons were carried out separately for treatment, time, and the treatment × time interaction to ensure an accurate identification of significant differences.

3. Results and Discussion

3.1. Starch Characterization

Table 2 presents the physicochemical characterization of polyclonal starches extracted from ten regional farming communities. The table includes key parameters such as moisture content, ash content, protein levels, and carbohydrate composition, providing an overview of the average quality and variability of starches from diverse varieties and local farming practices.

Table 2. Physicochemical characterization of the mixture of raw polyclonal cassava starch obtained from Amazonian indigenous communities.

Parameter	Value (% w/w)
Moisture *	44.15 ± 0.27
Carbohydrates **	97.89 ± 0.72
Ashes **	0.29 ± 0.02
Ether Extract **	0.38 ± 0.02
Fiber **	0.13 ± 0.00
Protein **	1.31 ± 0.30

Standard deviation (SD) included for n = 10. * Moisture content on a total basis. ** Values on a dry basis.

When comparing the physicochemical parameters of the Amazonian cassava starch (Table 2) with the standards established by the FAO technical guides for the production and analysis of this product (Aristizábal and Sánchez, 2007), variations in different compositional characteristics can be observed. The moisture content (44.15 ± 0.27%) significantly exceeds the recommended commercial range for starch (10–13%), due to the local practice of vending high-moisture material for food preparations. This approximation makes the extracted starch highly susceptible to microbial deterioration and fermentation. Similarly, the ash content on a dry basis (0.29%) surpasses the suggested limit (<0.12%), pointing to potential mineral impurities or inefficiencies during the extraction process. The above corresponds to a higher fiber content (0.13% on a dry basis), which reflects artisanal filtration techniques, which are typically less effective than commercial methods. Regarding protein, a value of 1.21% (on a dry basis) was recorded as shown in Table 2, much higher than usual for cassava starch of 0,4% (Montagnac et al., 2009; Waterschoot et al., 2015). In this case, variations in protein content could significantly influence the flexibility and stability of starch-based materials. A higher protein content can enhance film elasticity by improving the tensile properties of the starch matrix (Jagannath et al., 2003), while lower protein levels indicate greater starch purity and could increase water permeation (Long et al., 2023).

Despite the differences with values reported for other cassava starches for the different components mentioned above, Amazonian starch maintains a high carbohydrate content on a dry basis, as shown in Table 1, aligned with the FAO range (92–98%) and evidencing that even rudimentary extraction methods can produce starch of satisfactory purity (Aristizábal and Sánchez, 2007). This degree of purity is sufficient for obtaining cassava starch films. Criollo-Feijoo et al. (2024) for example, prepared active films with starches extracted from cassava bagasse with a purity close

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to 83%, showing satisfactory results and only some differences in color and elongation compared to industrially extracted cassava starch. In this case, the starch molecules make possible the formation of strong, cohesive films through hydrogen bonding among their chains. Starch's two main components play distinct roles in determining the mechanical and structural properties of the films; amylose contributes to rigidity and strength, and amylopectin enhances flexibility (Garavito et al., 2024). Cassava starch is particularly valued for its excellent film-forming capacity, which is attributed to its amylose-to-amylopectin ratio of approximately 17/83% (w/w), in addition to offering other advantages such as a low gelatinization temperature, high gel stability, and high transparency (Cheng et al., 2021; Tan et al., 2024; Tappiban et al., 2020).

3.2. Conditioning of Starch, Formulation, and Pellet Formation

The raw cassava starch exhibited a high initial moisture content (44.15 ± 0.27% w/w), which made it necessary to dry the material to achieve appropriate moisture levels before extrusion, reaching values of 10,56 ± 1.01% (n=10) to facilitate uniform plasticization during pelletizing and flat sheet molding. Preliminary tests showed that moisture levels higher than 10–12% in the raw starch powder led to an unstable matrix with high water evaporation within the extruder, lump formation, and low fluidity. The lumps' formation resulted in an extruded material that was prone to fracture. These moisture levels correspond to values of 7-8% in the overall mixture, previous to the plasticization of the reinforced starch, and for this reason, the formulations shown in Table 1 were predefined under that range. The above can be related to increased starch gelatinization under high heat and pressure at high moisture levels (Liu et al., 2020). On the other hand, moisture contents above 8% (w/w) in the mixture before extrusion resulted in changes in the consistency of the resulting film, making the latter hard and brittle after evaporation of the additional moisture in the sample, which may be because part of this water is not effectively included in the structure of the solid dispersion obtained and can easily migrate to the outside due to humidity differences with the air.

Regarding the other components, the concentration ranges selected for the study and included in Table 1 are related to difficulties at the time of mixing, quality defects in the pellet or film, or the impossibility of obtaining a homogeneous structure during the extrusion. As for glycerol, preliminary tests showed that increasing its concentration to 30% (w/w) or higher resulted in an over-plasticized material with excessive flexibility and very adhesive after extrusion, similar to that reported by Mali et al. (2005). Initially, water-soluble Polysorbate 80 (Tween 80) was used as an emulsifying agent, but the beeswax did not homogenize well in the dispersion during mixing. This may be related to the proportion of beeswax itself or LDPE in the mixture, as these are nonpolar materials. The high-lipophilic emulsifier Span 80 was then tested, achieving better homogenization of the different formulations shown in Table 1. In this case, 0.5% (w/w) was sufficient to obtain good dispersion. Similar results have been reported by Ortega-Toro et al. (2014), producing starch films by solvent casting. Citric acid was maintained at 1% for all formulations because higher levels did not provide any apparent benefit in terms of homogeneity and mechanical strength.

In the case of sunflower oil, preliminary tests showed that quantities greater than 4% (w/w) resulted in difficulties with homogenization and retention in the dispersion, and in excessive migration of the acid after the film was obtained. On the other hand, for beeswax, quantities greater than 4% led to very brittle films. This can be related to the low affinity of these nonpolar substances with starch and plasticizers (water and glycerol) (Yang et al., 2024). A similar situation also occurred in the case of LDPE, where it was not possible to include quantities greater than 2%, which would have required the use of compatibilizing substances such as maleic anhydride (Alim et al., 2023). The high susceptibility to agglomeration of latex made its incorporation into the formulation difficult (F3), and it was not possible to add quantities greater than 0.2%. For the powered plantain leaves, on the other hand, the incorporation of quantities of 3% or higher resulted in very poor film homogeneity (formation of holes or "fish eyes") and poor mechanical resistance to pulling during calendering.

Previous tests enabled proper formulations and successful pellet production through extrusion (Figure 1). Control samples, composed solely of starch without reinforcements, resulted in crystalline,

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translucent, and slightly yellowish pellets, with a homogeneous cylindrical shape and smooth surface, indicating good internal cohesion and matrix stability. However, some plasticizers' migration and rapid moisture absorption were observed, which can affect mechanical stability over time (Ben et al., 2022). Formulation F1 containing sunflower oil, for its part, produced pellets with irregular shapes and a noticeably tacky surface, reflecting the high adhesiveness induced by this lipophilic agent, consistent with other studies indicating that vegetable oils can hinder processability due to poor compatibility with hydrophilic matrices (Jiménez et al., 2013; Mirzaaghaei et al., 2022). On the other hand, formulation F2, incorporating beeswax, yielded well-formed, firm, and dimensionally stable pellets, supporting findings that natural waxes enhance moisture resistance and hydrophobicity without compromising material flexibility (Diyana et al., 2021). F3, which included natural latex, exhibited clear signs of thermal incompatibility even at this early stage: the pellets displayed a whitish, rough outer layer, indicative of material degradation and phase separation, affecting the uniform distribution of latex within the matrix, a phenomenon also reported in systems with non-compatible polymers (Boonluksiri et al., 2023; Cai et al., 2022). For F4, which included plantain leaves, the pellets were dark, rough, and visibly homogeneous, reflecting good dispersion of lignocellulosic particles. This physical reinforcement in starch matrices enhances mechanical stability and reduces adhesiveness. (Linan et al., 2024; Ni et al., 2022). Finally, formulation F5, which combined LDPE with *plantain leaves*, yielded similar results to those of F4 in the pelleting stage, except that a less intense brown color was observed, more crystalline, and with rigid LDPE microparticles that reinforce the polymer structure (Sabetzadeh et al., 2015; Haque Sachcha et al., 2024).

Figure 1. Pellets obtained for the extrusion of different compounded TPS formulations.

3.3. Sheet Extrusion and Bag Formation

The obtained pellets were processed through flat sheet extrusion, where drastic differences were observed in the material behavior during calendering and winding, and heat sealing. Figure 2 shows images of the extruded films for each formulation and the control. In general, the absence of reinforcements and the inclusion of only oil resulted in very flexible and adhesive films. Meanwhile, including components such as latex and high amounts of plantain leaves, or LDPE powder, led to poor homogeneity and easy film breakage during calendering. The controlled formulation enabled

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the formation of a slightly translucent and initially flexible film, although this turned out to be very fragile during calendering and winding, with rapid degradation of its properties under environmental conditions. Over time, a high moisture loss was observed, making the samples increasingly stiff and brittle. Additionally, although the control film was heat-sealable, ambient moisture absorption weakened the seals, leading to layer separation. The sealing temperature used was 170 °C in this case, with a sealing time of 3 seconds to obtain an effective seal between films. Plasticizer migration and sensitivity to environmental moisture are inherent challenges in pure starch matrices, negatively impacting the mechanical strength and stability of the resulting films (Surendren et al., 2022).

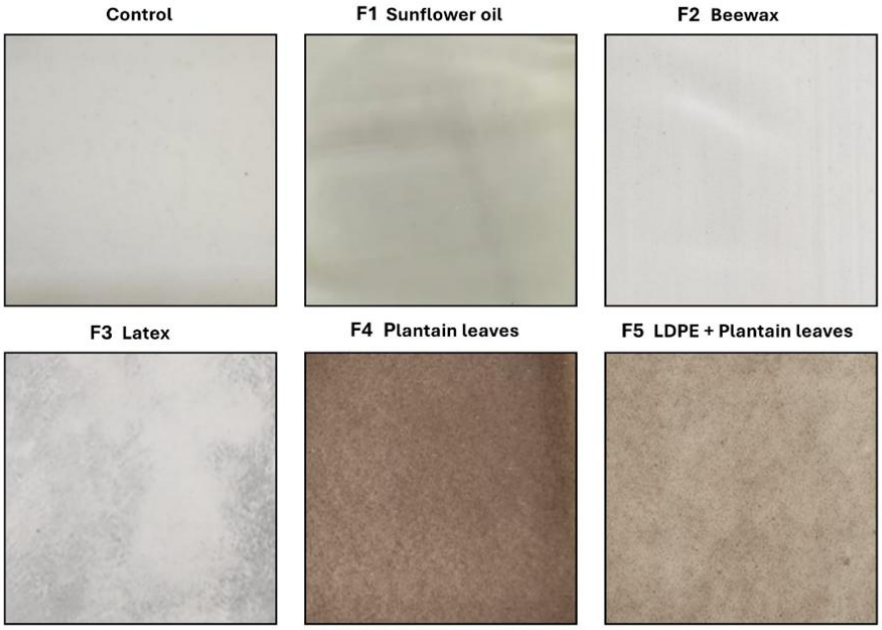

Figure 2. Images of films extruded by flat sheet extrusion for different compounded TPS formulations.

The formulation containing vegetable oil (F1) exhibited good continuity and initial elasticity after calendering. However, the obtained films showed excessive adhesiveness and sheet fusion during winding. This prevented heat-sealing tests, as it was impossible to unwind the rolls or achieve proper cuts which corresponds to what was reported by Zhang et al., (2021), who observed similar issues in films containing vegetable oils due to plasticizer migration and the inherent adhesive properties of these fatty agents. In contrast, the formulation with beeswax (F2) eliminated adhesiveness and significantly improved stability during calendered and winding, highlighting the hydrophobic and stabilizing properties of natural waxes in biopolymer matrices as reported by (Cheng et al., 2023; Diyana et al., 2021). However, this formulation had to be discarded later because of the lack of mechanical resistance during bag formation. The latex-based formulation (F3) exhibited high incompatibility between components and evident fragility, with non-homogeneous areas and a rough surface texture. The seals obtained after heat-sealing with this formulation were weak and easily disintegrated during bag forming, limiting their viability. This behavior could be attributed to the thermal degradation of latex and a change in its molecular structure at high temperatures, such as those used in extrusion (Haque et al., 2020).

On the other hand, Formulation F4, incorporating beeswax and grounded plantain leaves, showed good calendering stability and heat-sealing performance (Figure 3 above). The bag prototypes achieved consistent cuts and side seals, although with some imperfections due to some agglomeration of the plantain leaf particles. Meanwhile, formulation F5, which combined the grounded plantain leaves with the LDPE powder, produced films with superior plastic properties, better homogeneity, and no adhesion issues during winding, as shown in Figure 3 (above). Regarding

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heat sealing, it was possible to obtain strong and durable side seals for F4 and F5 films, maintaining integrity under handling without delamination or weakening (Figure 3). For these formulations, the sealing temperature was also 170 °C with sealing times of 5-6 seconds.

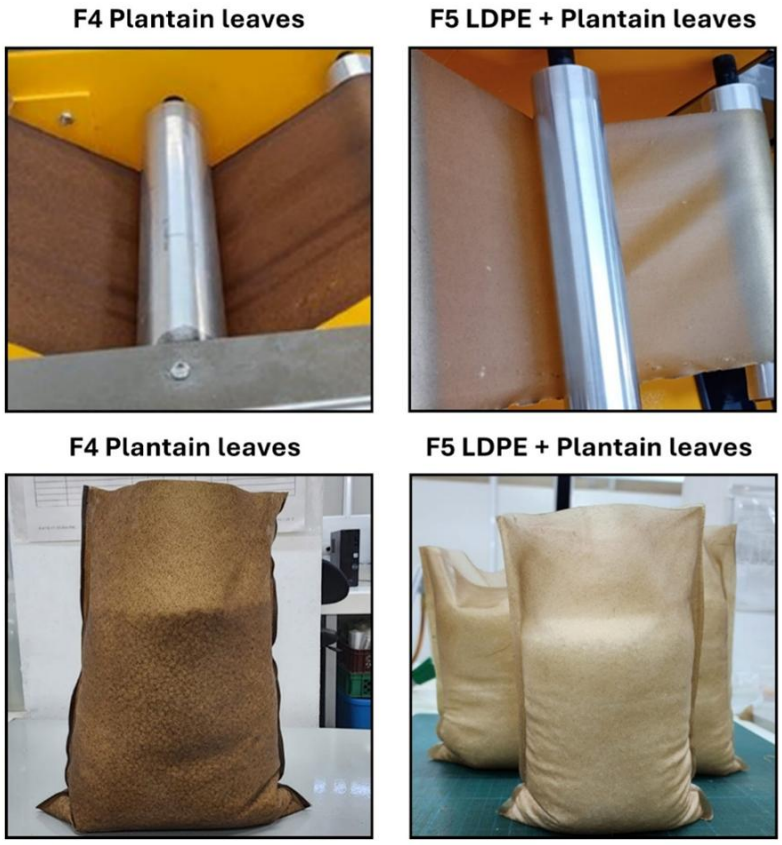

Figure 3. Calendering process for film obtention and heat-sealed bags of TPS formulations with grounded plantain leaves (F4) and LDPE powder + grounded plantain leaves (F5).

After the bags were formed and heat-sealed, a small seal capacity test was performed, introducing approximately 1 kg of starch pellets over 24 h to determine if the seals held properly. For formulations F4 and F5, the seals were maintained, preserving the bag structure with the contained material, as shown in Figure 3. F5 heat-sealed bags retained their structure and seals better after one day with contained material. In the case of the control formulation, there was rapid weakening and separation of the seals due to moisture absorption as previously mentioned. The findings achieved align with recent studies indicating that small amounts of synthetic polymers like LDPE can enhance biopolymers' processability and mechanical properties without compromising their environmental integrity (Aleksanyan et al., 2021; Nwuzor et al., 2023; Stelescu, Oprea, Motelica, et al., 2024).

3.4. Film Characterization

Considering the best characteristics of homogeneity and stability during the process of extrusion (pellets and flat sheet formation) of formulations F4 and F5, the characterization tests were carried out for the films obtained from these two formulations in addition to the control formulation as a comparison criterion. Formulations F1 and F3 were discharged since those formulations exhibited significant defects such as fragility, adhesiveness, or thermal incompatibility as described above. Formulation F2 was also discarded because it resulted in a very flexible (too stretched in the calender) and adhesive sheet.

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3.4.1. Infrared Spectroscopy (IR) of Films

The infrared spectroscopic analysis (Figure 4) confirmed that cassava starch remains the dominant structural component in all films, with a consistent main peak of around 995 cm^{-1}, corresponding to the C-O-C stretching of starch (Salazar-Sánchez et al., 2022). While additives such as beeswax, grounded plantain leaves, and LDPE introduce minor variations in specific regions, including C-H (2919 cm^{-1}) and O-H (3270-3275 cm^{-1}) stretching, these differences remain subtle due to their low concentrations. The film obtained with the F5 formulation exhibited a more pronounced impact in the C-H region, reflecting the polyethylene contribution. At the same time, vibrations in the 500-600 cm^{-1} range indicated interactions between starch chains and additives (García-Ramón et al., 2021; Pourfarzad et al., 2021). In any case, the spectra obtained for the two formulations and the control are very similar, which suggests that additives and reinforcing materials did not significantly alter the structure of the starch matrix. This may be of special interest at the time of the film's biodegradation after use.

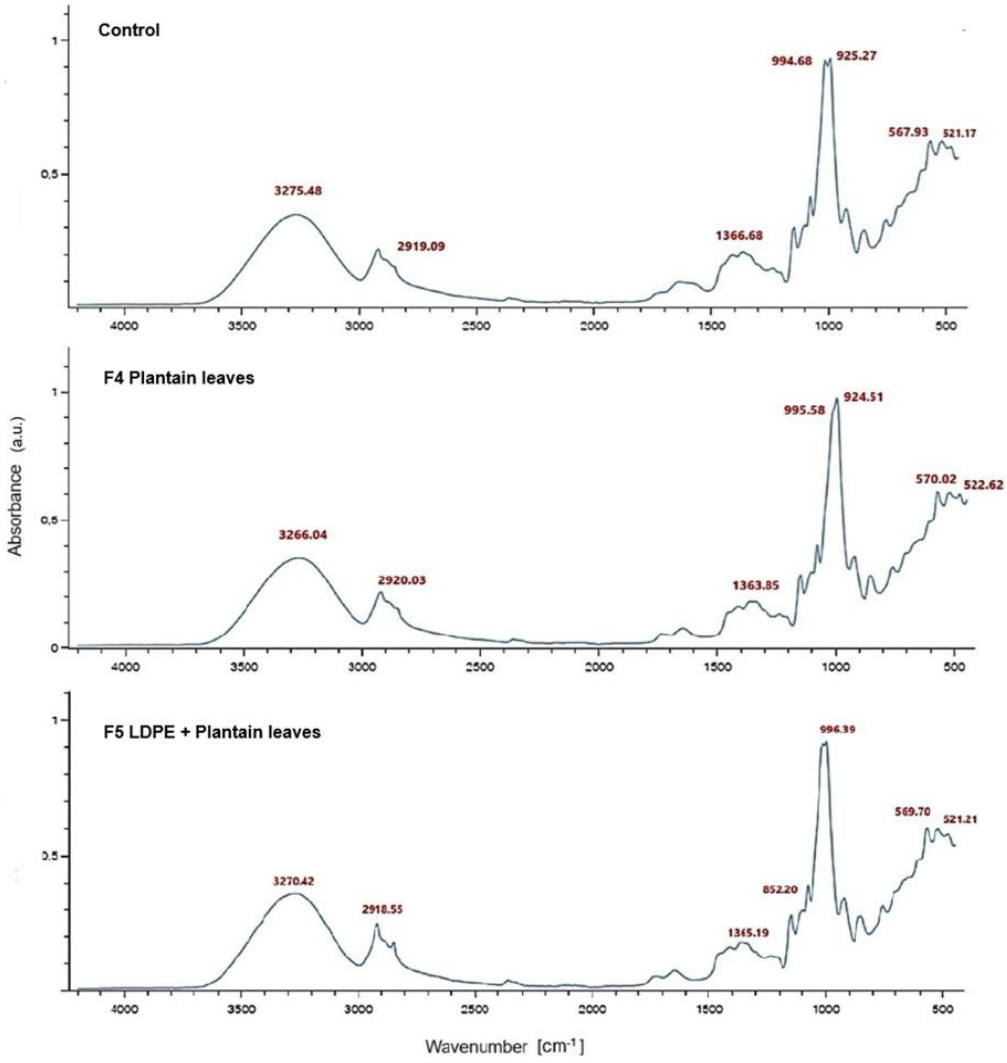

Figure 4. Infrared spectroscopy (absorbance) of compounded TPS films with different formulations: control, grounded plantain leaves (F4), and LDPE powder + grounded plantain leaves (F5).

3.4.2. Microscopy

Microscopic observations showed differences in the structure and properties between the control formulations, F4, and F5 (Figure 5). In the control formulation, the structure is homogeneous, although porous, with a uniform phase distribution. This porosity, although not visible to the naked

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eye, negatively affects the mechanical properties and water resistance, as it allows moisture penetration, compromising the durability and functionality of the material. For the F4 formulation, including ground plantain leaves and beeswax, created a more complex texture. The dark spots observed in the image can be attributed to the structural red formed by the plantain leaves particles, which are arranged on the surface of the films. This arrangement not only generates a solid brown color but also improves the material's structure by reinforcing the matrix and providing a certain degree of mechanical resistance. Beeswax, by forming a denser, more hydrophobic surface layer, helps reduce water permeability, although the uneven distribution of plantain leaf particles can create a more heterogeneous structure, affecting its internal cohesion. For the F5 formulation, LDPE powder, along with the grounded plantain leaves, produced a significant change in the structure. The larger whitish dots observed in the image can be attributed to the distribution of LDPE, which integrates into the matrix, improving the homogeneity of the material. The combination of these fillers reduced the porosity of the film compared to the control, as the distribution of LDPE and the outer layer of plantain leaves leads to a more uniform structure on the surface with fewer spaces compared to the control. These results correspond to the mechanical tests and moisture absorption shown below, where more favorable results (greater tensile strength and moisture barrier) were obtained for the F5 formulation with powered LDPE and grounded plantain leaves.

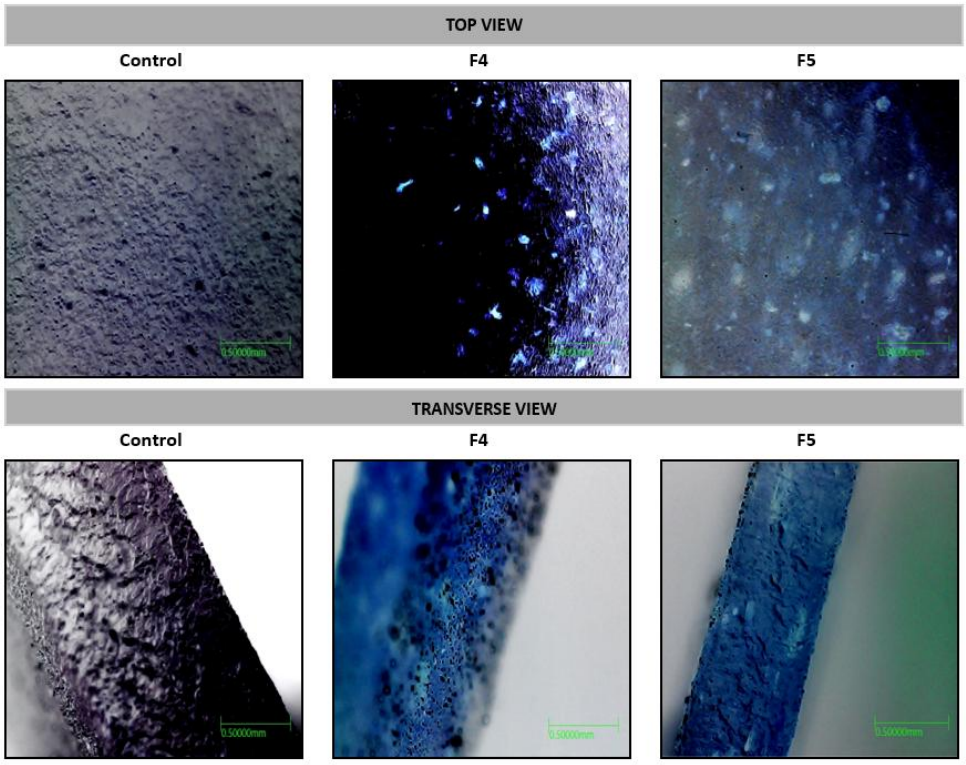

Figure 5. Micrographs of compounded TPS films with different formulations: control, grounded plantain leaves (F4), and LDPE powder + grounded plantain leaves (F5).

3.4.3. Thickness and Color

Film thickness obtained for the control samples and the formulations 4 and 5 is shown in Table 3. The thickness that could be achieved was directly related to the cohesion and tensile strength of each formulation evaluated. In all tests, an attempt was made to gradually accelerate the calendering speed while keeping the film uniform and without breaks. The control film exhibited the most significant thickness due to its lower mechanical strength, which restricted the calendering speed up to 2 ± 0.5 rpm. Incorporating the beeswax and powdered plantain leaves in F4, enhanced mechanical resistance, increasing the calendering speed up to 3 ± 0.5 rpm without a significant thickness reduction compared to the Control. However, adding LDPE microparticles in F5 further improved

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mechanical properties, enabling a higher calendering speed of 4 ± 0.5 rpm and resulting in a substantial thickness reduction compared to the Control. The F5 formulation showed greater mechanical resistance and greater cohesion in its structure, such that it can withstand greater pulling forces during calendering, obtaining a lower thickness.

Table 3. Color, thickness, water vapor transmission rate, and water adsorption of compounded TPS films with different formulations: control, grounded plantain leaves (F4), and LDPE powder + grounded plantain leaves (F5).

Property	Control	F4	F5
Thickness (mm)	0.32 ± 0.04a	0.30 ±0.02a	0.26 ± 0.02a
Lightness (L*)	39.49± 0.91a	28.25 ± 0.21b	33.94± 0.73c
Chromatic coordinate a*	0.24 ± 0.04a	4.09 ± 0.22b	3.06 ± 0.02c
Chromatic coordinate b*	2.12 ± 0.16a	7.91 ± 0.68b	9.74 ± 0.37c
WVTR (g m^{-2} d^{-1})*	1487 ± 287a	716 ± 184b	367 ± 79c
Q_{H2O} (g mm m^{-2} atm^{-1} d^{-1})	24845 ± 7980a	13366 ± 4585b	5095 ± 1499b
Water adsorption (g g^{-1})			
6 h	0.48 ± 0.01Aa	0.17 ± 0.02Ab	0.10 ± 0.02Ac
24 h	0.66 ± 0.01Ba	0.30 ± 0.01Bb	0.11 ± 0.02Ac

*WVTR, Q_{H2O} and water adsorption were measured at 35 °C and 75% RH. Different lowercase letters in the same row indicate significant differences ($p<0.05$) between treatments ($p<0.05$) by Tukey's HSD test. For the moisture adsorption, different uppercase letters in the same column indicate significant differences between treatments ($p<0.05$) for each measurement time. SD included for n = 3.

The color analysis of the extruded films reveals differences among the formulations, reflecting the direct influence of additives on the starch matrix. The control sample (without reinforcement materials), with the highest lightness (L* = 39.49), appears whitish-transparent due to the absence of colorant components. F4 exhibits the lowest lightness (L* = 28.25) and the highest values for the chromatic coordinates a* (4.09) and b* (7.91), resulting in a dark brown color intensified by the combination of beeswax and a higher concentration of pulverized plantain leaves, which contribute warm reddish and yellowish tones. In contrast, for the F5 films, a more balanced color was observed, presenting a lighter brown shade (L* = 33.94), lower red intensity (a* = 3.06), and a moderately higher yellow hue (b* = 9.74). This behavior can be attributed to a reduced proportion of plantain leaves and the predominance of beeswax. The F5 formulation resulted in the most homogeneous and uniform color for the film obtained which corresponds with literature findings that highlight how adjustments in natural additive proportions optimize both color and optical properties in biopolymers (Aziz et al., 2024; Ibáñez-García et al., 2024).

3.4.4. Permeability and Adsorption of Water Vapor

The results in Table 3 indicate significant differences in moisture permeation among the films with the different formulations, with the control showing the highest WVTR and Q_{H_2O}. The sample corresponding to formulation F4 showed a moderate reduction in moisture permeation, which can be attributed to the presence of beeswax and lignocellulosic particles with more hydrophobic characteristics (Liu et al., 2022; Tibolla et al., 2020). Likewise, F5 film exhibited a significant reduction in permeation, approximately a quarter of the control, which can be explained by the presence of LDPE and its higher hydrophobicity. The results correspond to those reported by Savadekar & Mhaske (2012); Malmir et al. (2018), and Raj et al. (2004) who evaluated the inclusion of reinforcing components such as nano-cellulose fibers, PBHV, and LDPE, reducing water vapor permeation to WVTR values of 0.024; 100, and 14,9 - 75,4 g m^{-2} $d^{-1,}$ respectively (at 0% RH; 37.8 °C, and 35% RH; 38 °C and 90% HR, respectively).

For moisture adsorption, significant differences were observed between the formulations, and also changes over time, specifically for the control and F4 films. The control sample showed the

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highest water vapor adsorption with values of 0.48 g g^{-1} at 6 h and 0.66 g g^{-1} at 24 h at the test conditions (38 °C and 80% RH). For this film, significant material degradation was also observed as moisture adsorption occurred, confirming its poor applicability in humid environments due to the high hydrophilicity of the raw starch because of the high presence of hydroxyl groups (Thakur et al., 2019; Wang et al., 2020). Film F4 showed a moisture adsorption (approximately 40%) compared to the control which can be explained by considering the greater hydrophobicity conferred by the beeswax and lignocellulosic particles as previously mentioned (De Carvalho et al., 2021; Hafila et al., 2022). However, less structural degradation was observed in the film after 24 h due to the conditions evaluated. This structural degradation in the case of the control and F4 films can also be related to the increase in the moisture adsorption rate over time, which is related to the increase in the hydrophilicity of the structure as the colloidal dispersion between the starch particles and the reinforcement materials breaks down and loses cohesion due to the adsorbed moisture. On the other hand, sample F5 showed the lowest moisture adsorption (0.1 g g^{-1}) and also greater structural stability under the conditions evaluated, with no signs of disintegration and a balance in the adsorption rate after 24 h of testing. In this case, the presence of LDPE resulted in increased resistance to moisture, given its low chemical affinity and solubility (Alim et al., 2023). Likewise, the greater stability and lower moisture adsorption in F5 can also be attributed to the lower glycerol content compared to the control and F4, considering the high chemical affinity of glycerol itself for water (Mali et al., 2005).

3.4.5. Mechanical Properties

The mechanical properties analysis revealed significant differences among the control, F4, and F5 formulations along the time at the test conditions (35 °C and 75% HR) as shown in Figure 6. The control formulation exhibited a rapid decline in its mechanical strength within the first few hours, stabilizing at very low levels due to the starch's high moisture adsorption, which weakened its structure. Conversely, F4 demonstrated more stability, attributed to the presence of beeswax and dried plantain leaves particles, which acted as partial moisture barriers. Likewise, it had a more balanced behavior in the mechanical properties evaluated with higher peak force, rigidity (Young's modulus), and mechanical resistance than the control film without reinforcements, and higher flexibility (elongation at break) than the F5 formulation with LDPE powder (Figure 6). On the other hand, this last formulation had the highest initial values of strength properties (tensile strength of 5.8 MPa and Young's modulus of 56 MPa), displaying significantly more gradual and lower reduction of these characteristics over time. This improvement was due to incorporating pulverized LDPE, which reinforced the polymer matrix by providing superior hydrophobic and mechanical properties. Similar findings have been reported in previous studies (Kaboorani et al., 2021; Zeraatpishe & Hassanajili, 2023), indicating that small amounts of synthetic polymers enhance the moisture resistance of biopolymers. Regarding elongation, the control formulation initially exhibited the highest flexibility but quickly lost cohesion, highlighting its fragility under humid conditions. While flexibility is desirable in flexible packaging, excessive elongation can lead to significant deformations under load, compromising functionality. In any case, for the three formulations evaluated in this test, the decline in their mechanical properties was evident and corresponded to the increase in the amount of moisture adsorbed over time (Table 3). This trend, common in moisture-absorbing biopolymers such as cassava starch, reduces structural stiffness, making them less suitable for prolonged stress exposure (Judawisastra et al., 2017). Thanks to its hydrophobic components, F4 maintained a balanced performance, with moderate elongation and lower mechanical strength decreasing along the test, which can be attributed to the function of beeswax and the powdered fiber of plantain leaves in providing a more cohesive structure and a greater barrier to moisture adsorption. This increase in strength is more evident for F5 with the inclusion of LDPE in addition to the other reinforcement components already mentioned. This formulation resulted in moderate elongation, preventing excessive flexibility, a significantly higher Young's modulus, indicating greater deformation resistance while maintaining the required flexibility. Likewise, the F5 film retained a higher part of its initial mechanical resistance throughout the test at high humidity and

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temperature compared to the other formulations, as shown in Figure 6. All the above correspond with what was seen during the production of the heat-sealed bags, where the bags corresponding to formulations 4 and 5 contained at least 1 kg of material for 24 h (Figure 3). These results were similar to those obtained by other authors for TPS films reinforced with cellulose nanofibers or LDPE (Hietala et al., 2013; Inceoglu & Menceloglu, 2013). In these studies, tensile strength of 8,3 - 11,8 MPa and elongation to break of 8,9 - 55,2% were obtained, achieving an increase in mechanical resistance compared to unreinforced films.

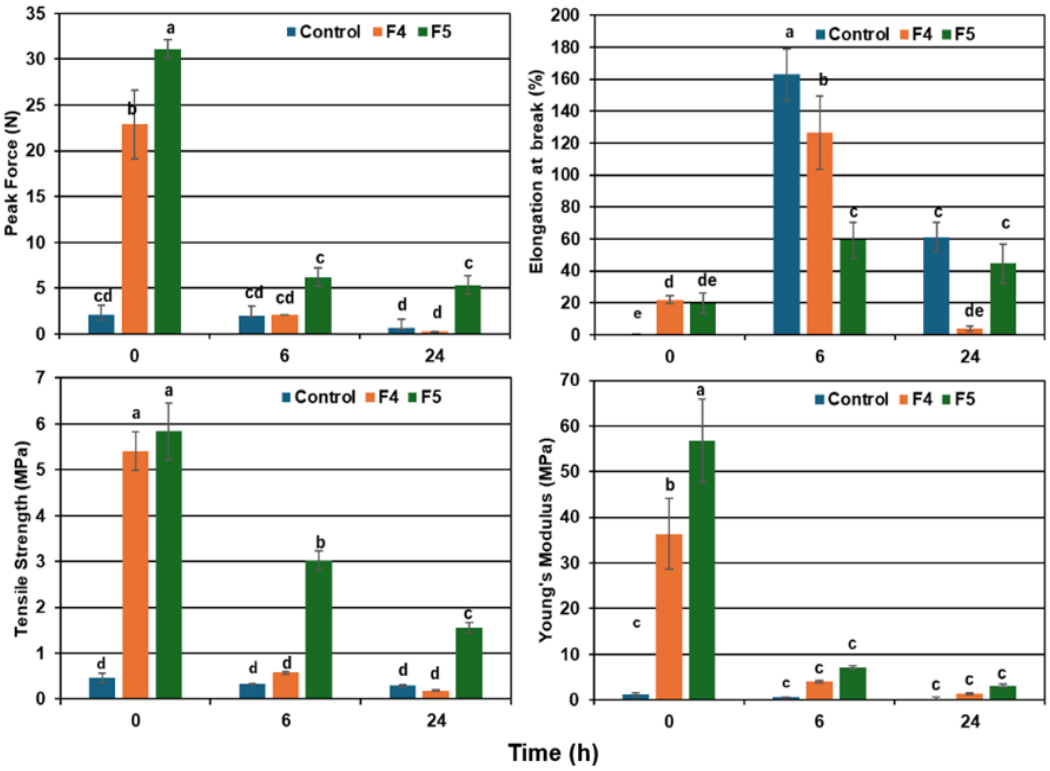

Figure 6. Evolution of mechanical Properties of TPS films with different formulations: control, grounded plantain leaves (F4), and LDPE powder + grounded plantain leaves (F5) at 35 °C and 75% RH. Lowercase letters indicate significant differences between treatments ($p < 0.05$) by Tukey's HSD test. SD included for n = 3.

Overall, the results showed that the starch obtained from the different small indigenous Amazonian producers could be used as a mixture to obtain single and reinforced TPS films, confirming the viability of diversifying raw material sources without compromising product quality. Such diversification facilitates large-scale biodegradable packaging production and encourages active local community participation in the supply chain, promoting economic development within cassava-producing regions.

On the other hand, the results showed that TPS formulations that included reinforcing compounds such as beeswax, grounded plantain leaf fibers, and LDPE powder led to films with improved barrier and resistance to moisture, lower moisture adsorption, and improved tensile strength compared to unreinforced TPS films. The results showed acceptable performance in heat sealing and load capacity for bags made with TPS formulations with the reinforcements under the climatic conditions typical of humid tropical areas such as the Amazon, as previously described. This is a promising result for these regions, as the reinforced bags could replace single-use flexible packaging with short service lives. The results obtained can be a basis for exploring the inclusion of biodegradable polymers (polybutylene adipate terephthalate - PBAT, Polybutylene succinate - PBS) as an alternative to the powdered LDPE, thus having bags with higher biodegradability/compostability after their final disposal.

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5. Conclusions

Cassava starch obtained from small indigenous Amazonian plantations can be successfully conditioned and used to make flexible packaging by extrusion. The cassava starch-based formulations analyzed in this study displayed distinct behaviors in terms of mechanical properties, moisture resistance, infrared spectroscopy, and optical characteristics. A direct relationship was observed between the use of reinforcing materials and the functionality and obtainability of the films and flexible pouches by heat sealing.

The control formulation, which contained only TPS without reinforcements, was notable for its structural fragility, high adhesiveness, and susceptibility to moisture, making it unsuitable for practical applications. Using ground plantain leaf particles and beeswax showed improvements in strength and functionality, although their susceptibility to moisture remains significant. Including LDPE powder in small quantities (2% w/w) with a balanced wax ratio and a lower content of ground plantain leaves presented the best overall performance, combining mechanical stability, greater moisture resistance, uniformity in particle and color distribution, and improved heat sealability. These characteristics demonstrate that this reinforced TPS formulation can be a good alternative for flexible packaging based on biodegradable materials that can be used in the retail trade of products such as food in places like the Amazon with high humidity and temperature environmental conditions.

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Data Availability Statement: The datasets and supplementary material generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors have no conflict of interest to disclose.

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