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Research Article

Biodegradable Cassava Starch-Based Films Formulated with Coconut Oil for Sustainable Food Packaging

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Abstract: A large volume of plastic waste is disposed of in the environment and in landfills worldwide. To remediate this serious environmental issue, sustainable alternatives to substitute traditional fossil fuel-derived plastics are necessary. In this regard, starch-degradable films are a promising avenue for promoting sustainability in the food industry due to starch's low cost, non-toxicity, availability and desirable film-forming capacities. However, starch-based films may have challenging water vapor barrier properties and mechanical resistance. To address this issue, in this study, we investigated the use of coconut oil (CO) to improve the performance of biodegradable cassava starch-based films. Different formulations (glycerol content and CO emulsion) were tested and the developed films were assessed regarding their morphology, mechanical properties, water permeability and biodegradability. The best formulation (COF-1) was achieved using 8.70 g of glycerol and 0.5 g of CO emulsion and showed better homogeneity and uniformity and improved mechanical properties. COF-1 showed a maximum tensile strength of 8.12 ± 1.99 MPa, almost three times higher than the control cassava starch-based film (CSF) produced without CO. The water vapor permeability (WVP) of COF-1 was 4.14 ± 0.56 $g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$, almost one-third lower compared to commercial cellophane. The incorporation of CO emulsion in the polymer matrix had a positive impact on the mechanical properties and showed good results of barrier and morphological properties. Overall, our work shows that enhanced starch-degradable films formulated with CO are potential solutions to conventional plastics, contributing to a cleaner environment and a more sustainable future.

Keywords: plastic substitute, coconut oil, environmentally friendly, ecological solutions, biodegradability

1. Introduction

The incorrect disposal and accumulation of plastics and other packaging materials in terrestrial and aquatic environments are major environmental problems [1]. Around 400 million tons of plastics are produced worldwide, generating a substantial volume of waste that ends up either in the environment or in landfills [2]. Replacing petrochemical-derived plastics with other materials that offer similar protective properties and good packaging

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properties, but with less environmental impact upon disposal is currently a challenge for industry [3]. Developing biodegradable films using natural, abundant and inexpensive biopolymers to provide the industry with smart, cleaner choices for the future is a sensible and much needed technology [4-5].

In this regard, starch has been extensively tested to produce filmogenic matrices, due to its biodegradable nature, low cost, non-toxicity, easy availability, proper functionality and film-forming capacity [5-7]. Starch, derived from various sources such as corn, cassava, potatoes and rice, is the most abundant renewable biopolymer on Earth, being cultivated on practically every continent [8-9]. Starch-based biodegradable films have been applied in the food industry worldwide as packaging materials for fruits, vegetables, fish and meat, as well as for the production of disposable bags, and textiles, agricultural films for soil coverage, contributing to sustainability and reducing environmental impact [10-12]. However, due to its high hygroscopicity and the use of plasticizers such as glycerol, which also have a high affinity with water, starch-based films usually have a compromised structure in terms of water vapor barrier properties and mechanical resistance [13-14]. To improve the mechanical properties of film structures, synthetic and natural materials such as fibers and oils had their performance evaluated for developing films [5, 15-16]. Vegetable oils have become a strategy for replacing synthetic materials in biodegradable films because they are accessible, low cost, contain bioactive compounds and, due to their hydrophobic nature, can influence mechanical, morphological and barrier properties [17-19].

For example, coconut oil (CO) is extracted from the coconut copra (internal white part) of coconut fruit (*Cocos nucifera* L.) species [20]. Coconut is widely cultivated in tropical and subtropical regions. Countries such as Indonesia, the Philippines, India, and Brazil are among the largest producers (high global production of 62.41 million tons in 2022), while coconut and its derivatives are distributed and consumed globally [21-22]. In the global market, the fruit of the coconut palm is destined for the production of copra, with coconut oil (62.0%) being its main derivative [23]. CO is composed of around 90% medium-chain fatty acids, and its antioxidant capacity and antibacterial activity have been demonstrated [24]. Due to its hydrophobicity, the addition of coconut oil to the film matrix decreases water vapor permeability (WVP), besides improving the stretch and flexibility of the films [25-26]. According to Carpiné et al. [25], the use of coconut oil with surfactants led to improved performance of soy protein isolate film, such as the reduction of WVP by 35.36%, and the increase of film elongation by 71%.

Therefore, our study investigates starch-derived biodegradable films as a promising avenue for promoting smart packaging alternatives and sustainability in the food industry. Our hypothesis is that it is possible to develop low-cost, non-toxic films with desirable technical properties using readily available food-derived materials. For this, we investigated different formulations of cassava starch-based films produced by casting with the addition of coconut oil as an additive to improve film performance. Initially, coconut oil was obtained by hot extraction and characterized by key physicochemical parameters. Different film processing conditions, such as drying temperature, and CO and glycerol incorporation levels were assessed to determine a prioritized formulation that was further characterized regarding its morphology, mechanical and barrier properties and biodegradability. The outcomes of this study deliver practical, meaningful results to establish efficient protocols to obtain biodegradable films, and necessary environmental-friendly alternatives to synthetic plastics for food packaging applications.

2. Materials and methods

2.1 *Materials*

Ripe coconuts (*Cocos nucifera* L.) and food-grade cassava starch (moisture content: 12.8%, amylose content: 21.9%; Yoki Alimentos, Brazil) were purchased in the local market (Natal, Brazil). Glycerol used in this work was acquired from Sigma-Aldrich (St. Louis, USA). All reagents were of analytical grade and used without further purification.

2.2 *Coconut oil extraction*

Raw coconut oil (CO) was extracted as described by Almeida et al. [20]. Briefly, 1,500 g of coconut endosperm (flesh, edible part) was manually separated and blended with 1,500 mL of distilled water at 25 °C (1 : 1) using an industrial blender for 4 min. The mixture was filtered using a sterile cheesecloth to remove solid residues. The filtered

content was stored in sterile glass bottles and allowed to sit for 24 h at room temperature (24 °C) away from the light, which allowed the separation of the coconut oil from the aqueous portion. After separation, CO was heated in a hot plate to 100 °C to evaporate the remaining water. After water evaporation and cooling, CO was transferred to sterile glass bottles and stored at 4 °C protected from light until further analysis.

2.3 *Coconut oil characterization*

The physicochemical properties of the extracted coconut oil (CO) were analyzed using standard methodologies. The refraction index was determined by direct reading on a digital Abbe refractometer (model WYA-2S, Nova Instruments, Brazil), pH was determined using a digital pH meter (model pH 2,600, Instrutherm, Brazil) previously calibrated with standard buffer solutions (pH 4.00 and 7.00), melting point was determined using a Fisher-Johns apparatus (model MQAPF-302, Microquimica, Brazil), moisture content was assessed gravimetrically by heating approximately 5 g of CO in an oven at 105 °C until constant weight. The percentage of moisture was calculated based on mass loss and ash content was determined by incineration of 5 g of CO in a muffle furnace at 550 °C for 6 h [27]. The iodine value of extracted CO was determined according to ISO 3961 [28] using the Wijs method. Briefly, CO (0.3 g) was dissolved in chloroform, and an excess of iodine monochloride solution was added. After 30 min in the dark, the residual iodine was titrated with sodium thiosulfate solution using starch as an indicator and results were expressed as mg iodine/g CO. The saponification value was determined according to ISO 3657 [29]. A known mass of CO (~2 g) was saponified by refluxing with an ethanolic KOH solution. The unreacted KOH was then titrated with hydrochloric acid, and the results were expressed as mg KOH/g CO. The relative density was determined with a glass pycnometer at 25 °C, as recommended by the American Oil Chemists' Society (AOCS) [30]. The pycnometer was calibrated with distilled water, and the density of CO was calculated based on the mass-to-volume ratio. The acidity value was determined according to ISO 660 [31]. Approximately 2 g of CO was dissolved in a neutralized ethanol-ether solution and titrated with a standard KOH solution, using phenolphthalein as an indicator and results were expressed as mg KOH/g oil.

2.4 *Preparation of cassava starch-based films*

Table 1. Experimental formulations of cassava starch-based films with coconut oil (CO)

Formulation ID	Temperature (°C)	Glycerol (g)	CO emulsion (g)
CSF[1]	55	8.70	-
COF-1	55	8.70	0.5
COF-2	75	8.70	0.5
COF-3	75	4.70	0.5
COF-4	55	4.70	0.5
COF-5	75	8.70	2.35
COF-6	55	8.70	2.35
COF-7	55	4.70	2.35
COF-8	75	4.70	2.35

[1] Film formulation prepared according to previous report [32]

A control cassava starch-based film (CSF) was obtained via casting technique, using a formulation of 92.0 g distilled water, 6.0 g cassava starch and 2.0 g glycerol, and submitted to drying at 55 °C in a forced-convection drying oven (Luca-82/27, Lucadema, Brazil), according to our previously published method [32]. For films prepared with the incorporation of CO (COF), the mass of cassava starch was kept constant while coconut oil (CO) was added in two

different concentrations. Starch/CO (1 : 0.8 w/w) emulsions were prepared at 25 °C, using a high-speed homogenizer operated at 9,500 rpm for 20 min, according to Colivet Briceño [33], and incorporated in the filmogenic solutions at two different addition rates (0.5 g/100 g and 2.35 g/100 g), based on preliminary trials (data not shown). CO-incorporated starch-based films were formulated with two different addition rates of glycerol (8.70 g/100 g and 4.70 g/100 g), for a total of 8 formulations (Table 1). The filmogenic solutions were homogenized by mixing the starch, glycerol, and CO emulsions under constant stirring for 30 min at 65 °C until complete starch gelatinization. Then, solutions were poured onto the acrylic plates (50 cm × 30 cm) and dried in a forced-convection hot air oven (model Luca-82/27, Lucadema, Brazil), under different temperatures (55 and 75 °C) for 6 h.

2.5 *Film characterization*

2.5.1 *Moisture, physical evaluation and morphology*

The moisture content of film samples was assessed gravimetrically at 105 °C by drying in a laboratory oven (Luca-82/27, Lucadema, Brazil) at 105 °C until constant weight. The water solubility was determined according to Hosseini et al. [34]. Measurements were performed in triplicate and expressed as percentage (%). For thickness evaluation, six replicates (100 mm × 100 mm) of film samples were cut at room temperature (25 °C). Thickness was measured at eight different points across the film surface using a digital micrometer (model IP54, Digimess, China, accuracy: 0.001 mm). The surface morphology was evaluated by atomic force microscopy (AFM; model SPM-9700, Shimadzu, Kyoto, Japan), using the intermittent contact method at a scanning frequency of 1 Hz. Samples were prepared by cutting 10 mm × 10 mm film sections and mounting them onto glass slides. The AFM images were acquired under ambient conditions and analyzed. The procedure was according to the method described by Kuutti et al. [35].

2.5.2 *Water solubility and contact angle*

Contact angle was determined according to Nascimento et al. [36] using a digital goniometer Digidrop (GBX Instrumentation Scientifique) coupled to a Nikon PixeLink camera. Aliquots (15 µL) of distilled water were automatically deposited in the center of the films and images were captured by a digital camera, and the contact angle was determined via image analysis software. A white back light-emitting diode (LED) diffuser was used as light source to provide uniform illumination. To minimize heat and keep constant illumination, a diffusion screen was used between the light source and the sample.

2.5.3 *Barrier properties to water vapor*

The water vapor permeability (WVP) was assessed according to standard American Society for Testing and Materials (ASTM) E96 [37]. WVP was evaluated using circular acrylic cells of exposed area of 3.84×10^{-4} m^2. Each film sample was placed and sealed over the opening of the cell containing anhydrous silica gel and placed in a desiccator containing saturated NaCl solution (to create a controlled relative humidity environment of 75%) for 24 h at room temperature (25 °C). Measurements were taken in triplicate and the WVPR was determined according to Equations 1 and 2:

$$WVPR\left(\frac{g}{m^2 \cdot day}\right) = \frac{w}{t \times A} \tag{1}$$

$$WVP\left(\frac{g \cdot mm}{m^2 \cdot day \cdot kPa}\right) = WVPR \times \frac{e}{P_S \times (RH_1 - RH_2)} \tag{2}$$

Where w is the weight gain (g), A is the cell exposed area (m^2), t is the time (days), P_S is the saturation vapour pressure of water (kPa), RH is the relative humidity outside (RH_2) and inside (RH_1) the cell, and e is the film thickness (mm).

2.5.4 *Mechanical properties*

The maximum tensile strength (MTS) and the elongation at break (EB) were evaluated using a universal testing machine (EMIC equipment, model DL3000, Paraná, Brazil) equipped with a 100 N load cell. The tests were conducted at a crosshead speed of 50 mm/min, with an initial grip separation of 50 mm. For each formulation, ten replicates of film samples were tested according to ASTM D882 [38].

2.5.5 *Biodegradability*

Films biodegradability was qualitatively evaluated according to Medina-Jaramillo et al. [39], with minor modifications. Fifteen sample replicates (50 mm × 50 mm) were cut at room temperature, placed on plastic trays (100 mm × 200 mm × 5 mm) and buried in soil mixture (10 mm depth) composed of 50% vegetal soil and 50% bovine manure. Trays were kept under room temperature (24 °C). Water was sprayed four times a day to maintain moisture levels in the compost. The degradation process was monitored by analyzing the trays after 1, 3, 6, 9 and 12 days. At each time point, triplicate samples were carefully removed, visually inspected, and photographed to document the degradation progress.

2.6 *Statistical analysis*

Statistical analysis was performed with GraphPad Prism 10.0. All experiments were run in three independent triplicates and measurements were performed in triplicates, unless otherwise specified. Results are presented as average ± standard deviation (SD) for $n = 3$. One-way analysis of variance (ANOVA) coupled with Tukey's honestly significant difference (HSD) post hoc test ($p < 0.05$) for multiple comparisons was used to determine significantly different groups among the control (CSF) and COF film formulations for each measured attribute.

3. Results and discussion

3.1 *Coconut oil characterization*

Table 2. Physicochemical characterization of coconut oil (CO) used in this study

Parameter	Coconut oil (CO)
Refraction index	1.454 ± 0.001
pH	4.62 ± 0.05
Melting point (°C)	26.87 ± 0.2
Moisture (%wt)	0.03 ± 0.03
Ash content (%wt)	0.05 ± 0.08
Iodine value (cg Iodine/g oil)	4.69 ± 0.34
Saponification value (mg KOH/g oil)	316.13 ± 12.08
Relative density (at 25 °C)	0.916 ± 0.004
Acidity index (mg KOH/g oil)	0.59 ± 0.12

Results are shown as average ± standard deviation

The refraction index depends on the oil's chemical attributes, such as fatty acid chain size, molecular weight, degree of saturation and conjugation. Our findings (Table 2) are similar to previous literature reports [40-41] and within

the range established by the Codex Alimentarius: 1.448-1.450 [42]. The melting point found for CO (26.87 ± 0.2 ℃, Table 2) is higher than demonstrated by Chaleepa et al. [43] melt temperature, cooling rate, agitation speed (24.83 ± 0.15 °C) which indicates a lower number of non-fat solids when compared to the oil obtained in this study.

Moisture and ash contents were lower than reported by Costa et al. [44] for babassu (*Attalea speciosa*) oil (0.369-3.197% w/w and 0.124 to 3.547% w/w, respectively). The iodine value is below the range established by the Codex Alimentarius of 6.3-10.6 [42], but close to values shown by Kumar and Krishna [45]. The iodine value measures the degree of unsaturation of vegetable oils, and CO has a low degree of unsaturation because it contains higher amounts of medium-chain fatty acids [46]. In fact, these parameters are dependent on the protocol used for coconut oil production, which explains the observed differences.

According to Ndiaye et al. [47], the saponification index is an important oil quality index because it determines the degree of deterioration or stability of the oil. The higher the saponification index, the higher the oil quality and stability [48]. Our result is above the values established by the Codex Alimentarius (248-265 mg KOH/g oil) for coconut oil [42]. It is also higher when compared to what Kumar and Krishna [45] (239.9-260.2 ± 0.40 mg KOH/g oil) and Lugo-Méndez et al. [41] (244.19 mg KOH/g oil) reported. Our results for acidity index are within the maximum permitted limit established by Codex Alimentarius of 0.6 mg KOH/g oil for refined oils [42]. The relative density determined at 25 °C was 0.916 ± 0.004 g·mL^{-1}, this is within the values established by Brazilian legislation [49] of 0.908 g·mL^{-1} at 40 °C and 0.921 g·mL^{-1} at 20 °C.

3.2 *Cassava starch-based films characterization*

3.2.1 *Visual aspect, moisture and physical parameters*

Cassava film formulations prepared with lower addition level of glycerol (4.70 g; COF-3, COF-4, COF-7 and COF-8) yielded very brittle and non-homogenous film structures, which demonstrate that CO could not replace the plasticizing effects of glycerol at that addition level. The studies of Gontard et al. [50] and Mali et al. [51] showed that plasticizers as glycerol increase the flexibility and extensibility of biodegradable films because it reduces the intermolecular forces of the films, increasing the molecular space and mobility of the polymer chains. Formulations prepared with higher glycerol addition rate (8.70 g; COF-1, COF-2, COF-5 and COF-6) yielded malleable and flexible films that did not break upon handling, and those were used for further characterization. Similar moisture and thickness were observed among the analyzed samples ($p > 0.05$; Table 3), indicating the robustness of the formulation and the casting technique to produce cassava starch-based films.

Table 3. Characterization of cassava starch-based films incorporated with coconut oil (CO) in moisture, thickness, water solubility and water vapor permeability (WVP)

Formulation ID	Moisture (%)	Thickness, *e* (mm)	Water solubility (%)	WVP (g·mm/m^2·day·kPa)
CSF[1]	20.67 ± 0.01^a	0.122 ± 0.005^b	31.99 ± 0.03^b	3.81 ± 0.55^a
COF-1	22.96 ± 0.57^a	0.153 ± 0.031^a	25.23 ± 0.54^b	4.14 ± 0.57^a
COF-2	20.64 ± 0.17^a	0.165 ± 0.026^a	59.89 ± 5.52^a	6.80 ± 0.51^b
COF-5	18.13 ± 0.69^a	0.203 ± 0.016^a	67.19 ± 3.09^a	8.13 ± 0.19^b
COF-6	20.25 ± 0.76^a	0.197 ± 0.020^a	23.78 ± 7.47^b	7.65 ± 0.10^b

Results are shown as average ± standard deviation. Results in the same column followed by different letters (a, b) are significantly different by Tukey's test ($p < 0.05$). [1] Film formulation prepared according to the report [23]. Legend: CSF-Cassava starch-based film, control formulation. COF-Cassava starch-based film treatments developed with the incorporation of coconut oil. Please refer to Table 1 for detailed description of experimental COF groups

3.2.2 *Water solubility and water vapor permeability (WVP)*

Water solubility is an important parameter for packaging materials developed from starch. Potential applications

may require low water solubility to improve product integrity and water resistance [52]. Anis et al. [53] reported that using polysaccharides (hydrophilic) and essential oils (hydrophobic) for film production significantly alters the water solubility of final products. COF-1 and COF-6, dried at 55 °C, had similar ($p > 0.05$) water solubility (23.78-25.23%, Table 3), significantly lower ($p < 0.05$) than COF-2 and COF-5 (59.89-67.19%, Table 3), dried at 75 °C. This result indicates that the film drying temperature significantly affects the material water solubility.

According to Mali et al. [54], thermoplastic materials such as starch and glycerol undergo more accelerated recrystallization when subjected to temperatures above the corresponding glass transition temperature (*Tg*) and the recrystallization process leads to poorer barrier properties, such as higher rigidity and fragility of materials. Souza et al. [6] showed two different *Tg* results for film-forming solutions of glycerol and cassava starch in ratio similar to our work (0.25 g of glycerol/ g of cassava starch): 38.71 ± 1.92 °C and 62.89 ± 0.04 °C. Xiao et al. [55] studied the incorporation of CO into the polysaccharide matrix (konjac glucomannan/agar/gum Arabic). The higher the CO content (0; 0.1%; 0.3%; 0.4% and 0.6%), the lower the water solubility of the films (45.2 ± 2.6%; 33.5 ± 1.1%; 28.7 ± 1.2%; 23.6 ± 1.1% and 23.1 ± 1.4%) indicating a clear influence of CO on the hydrophobicity of the films.

Water vapor permeability is an important characterization parameter that dictates the use of films for food packaging applications. For example, certain products require packaging with an excellent water vapor barrier to prevent loss or gain of moisture, as well as microbiological and enzymatic reactions during the conditioning period. Others need a less restrictive system where selective gaseous exchanges may occur to minimize possible reactions and sensory changes in food [56]. WVP analysis (Table 3) revealed that all samples had similar WVP values between 4.137 ± 0.565 $g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$ and 8.127 ± 0.191 $g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$. The COF-1 sample showed lower water vapor permeability ($p < 0.05$, WVP = 4.137 ± 0.565 $g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$) compared to COF-2, COF-5 and COF-6 formulations, but similar to CSF ($p < 0.05$). The lower the permeability of the film, the lower the chance of water vapor from the external environment migrating to the inner side of the package. This is an important feature to maintain the sensory aspects of food and minimize powder agglomeration and the development of microorganisms [56-57].

The hydrophilic nature of both starch and glycerol promotes low moisture barrier properties [54, 57]. Xiao et al. [55] studied the incorporation of CO into the polysaccharide matrix (konjac glucomannan/agar/gum Arabic) and observed that the WVP of the control film (0% CO) was 10.49 ± 0.43 $g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$ and the highest concentration (0.6% CO) showed lower permeability to water vapor 6.89 ± 0.32 $g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$. All samples of this work had WVP values close to or lower than Xiao et al. [55]. Jusoh et al. [26] evaluated the behavior of different concentrations of CO incorporated into gelatin films. Although there was no statistically significant difference between treatments, films with higher CO concentration (30% CO) showed WVP = 69.6 ± 17.0 $g \cdot mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$. Our COF-1 results were almost 17 times more effective as a barrier to WVP. Other authors described that the inclusion of vegetable oils originated a hydrophobic phase in the polymeric matrix, limiting the permeation of water vapor in the film, and the hydrophilic-hydrophobic proportion of the components also affected the diffusion of water vapor from the films, promoting a decrease in the rate of water vapor permeation [52, 58]. The results for COF-1 were almost 2-fold lower than the WVP for cellophane films (7.23 ± 0.06 $mm \cdot m^{-2} \cdot day^{-1} \cdot kPa^{-1}$) reported by Phan The et al. [59], which also suggests the potential application of COF-1 for food packaging, since cellophane is an industrially used food packaging material. Therefore, COF-1 sample had the best results for water solubility and WVP and it was chosen for further characterization and comparison to control cassava starch-based film (CSF).

3.3 *COF-1 film characterization*

3.3.1 *Morphology*

AFM images (Figure 1) were collected to evaluate film surface morphology. The scale of the surface roughness for both films (COF-1 versus control CSF) was in the nanometer range. COF-1 film (Figure 1c, d) showed a lower average mean superficial roughness with a maximum height of peaks of 555.28 nm, which was 34% lower than the maximum height for peaks of control CSF sample (Figure 1b). In addition, COF-1 samples had a more homogeneous superficial structure (Figure 1c) when compared to CSF samples (Figure 1a). Our results agree with Fangfang et al. [19]. The authors reported that the surface morphology of films prepared with potato starch and glycerol were smoothened when virgin coconut oil was incorporated to the formulation (14-56% w/w), creating a more uniform surface. On the contrary, Acevedo-Fani et al. [60] showed that alginate-based films with essential oils had rougher microstructure than oil-free

control film. The authors reported that this phenomenon could be attributed to the migration of oil droplets and further aggregation caused by drying during formulation and film production.

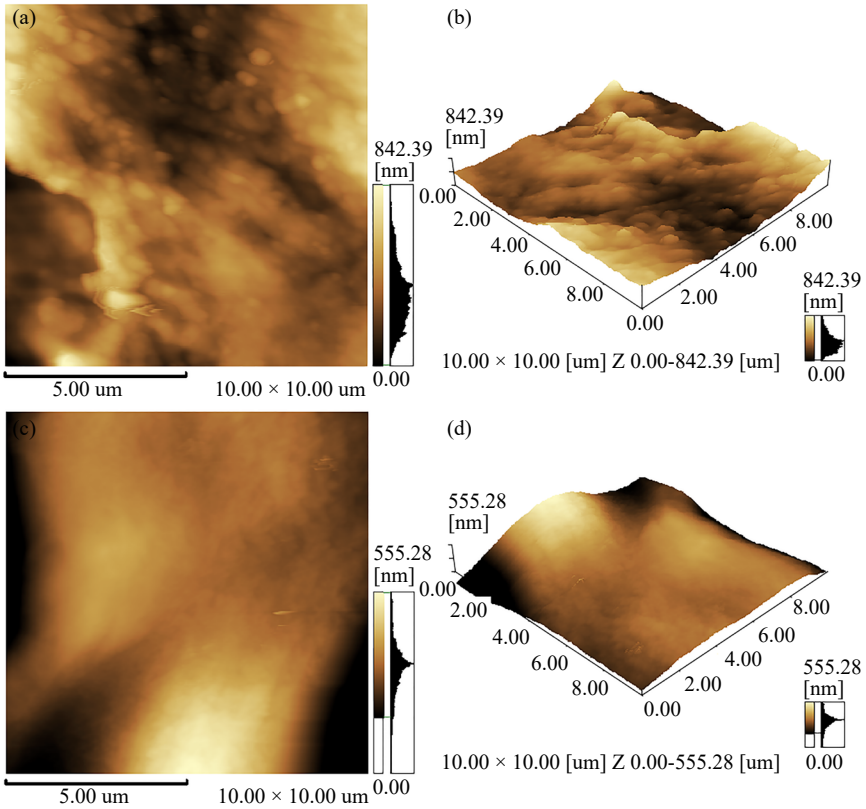

Figure 1. Representative atomic force micrographs (AFM) for cassava starch-based films (CSF; (a) and (b)) and coconut oil-incorporated films (COF-1; (c) and (d))

Gutiérrez et al. [61] showed the incorporation of 25% w/w of coconut oil as an alternative plasticizer to glycerol for the extrusion process of corn starch-based films. The authors observed the presence of starch granules on the extruded matrices, which was attributed to the poor compatibility between corn starch and coconut oil that resulted in moisture evaporation during extrusion. Films with lower concentrations of coconut oil showed a smoother surface, which infers that the excessive use of coconut oil might lead to film structural defects. Overall, our results show that COF-1 formulation (8.70 g of glycerol and 0.5 g of starch/CO emulsion) enabled the production of films with homogeneous surfaces, resulting from good compatibility between the ingredients used in appropriate concentrations.

3.3.2 *Water contact angle*

COF-1 and CSF samples showed water contact angles of 64.9° ± 4.9° and 58.1° ± 2.9°, respectively (Figure 2a), which corroborates to the increase in film hydrophobicity with the addition of CO/starch emulsion due to the hydrophobic nature of CO. Surfaces with contact angles under 90° show hydrophilic characteristics. The hydrophilicity of the material controls the release and disintegration of the film, and in this regard, films with higher hydrophilicity tend to have lower disintegration times [62]. Białopiotrowicz [63] studied the contact angle of water and other liquids in corn and potato-based starch films at different concentrations of starch (2-12% w/w). The author observed that the differences in the contact angle for water in corn and potato starch-based films are not statistically significant and that the contact angle decreases linearly with the increase of starch concentration in films. When 6% w/w of corn or potato starch was used, the contact angle was approximately 35° and 38°, respectively.

Colivet Briceño [33] studied the hydrophilicity of chemically modified cassava starch films compared to

unmodified starch films. A contact angle of 41.1° was observed for unmodified cassava starch, while modified starch films (acetylated starch and cross starch) showed higher contact angles of 67.0° and 81.7°, respectively. Tavares et al. [14] and Luchese et al. [64] considered that higher concentration of amylose may promote greater intermolecular forces with glycerol molecules leading to increased difficulty for water migration into the starch structure. Since cassava starch is usually amylopectin-rich, cassava starch films tend to have higher water affinity [33].

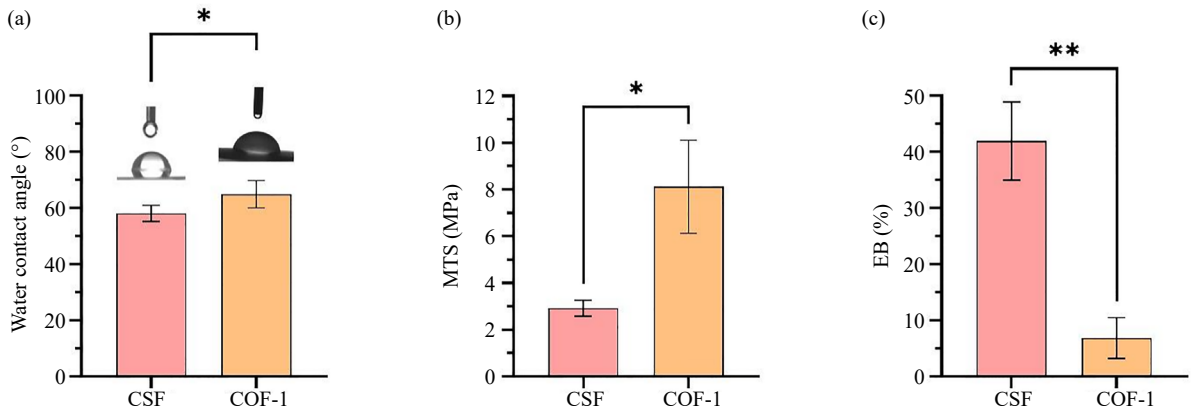

Figure 2. (a) Water contact angle (°), (b) Maximum tensile strength (MTS, MPa) and (c) Elongation at break (EB, %) of cassava starch-based films (CSF) and coconut oil-incorporated films (COF-1)

When Andretta et al. [65] used sorbitol replacing glycerol and less quantity of water in the formulation, the contact angle (59.0 ± 5.0°) was lower than the results reported here for COF-1. Sapper et al. [66] observed that for coating formulations without Tween 85, the contact angle decreased when they contained emulsified essential oil (48.0° at 92.0°), and suggested that emulsified essential oil and liposomes affected the interactions of the coating-forming system with the fruit/air interfaces. Other researchers have described the interaction between amylose with surfactants, which can give rise to low amounts of free surfactant molecules to act at surface level [66-67].

3.3.3 *Mechanical properties: maximum tensile strength and elongation at break*

The evaluation of the maximum tensile strength (MTS) of materials is of great importance to define their applicability in food packaging [68]. The incorporation of 0.5 g of CO/starch emulsion not only promoted higher homogeneity and uniformity on films surface (Figure 1), but also improved its mechanical properties. Results (Figure 2b) obtained for MTS of COF-1 (8.12 ± 1.99 MPa) showed almost a 3-fold increase when compared to control sample CSF (2.92 ± 0.34 MPa). The increased MTS led to an expected lower EB (6.90 ± 3.62% and 41.97 ± 6.98%, for COF-1 and CSF, respectively, Figure 2c). According to Kester and Fennema [69], greater structural cohesion (and consequent higher MTS), leads to lower film flexibility [59]. Souza et al. [6] described the use of glycerol as an efficient plasticizer when used in concentrations up to 1.25% w/w. They also reported that higher concentrations of glycerol led to lower EB and MTS results. Similar relationship was reported by other authors [70]. However, the opposite effect was observed in our study. Lower glycerol content (4.70 g) yielded brittle films, and it was impossible to analyze the mechanical properties of these samples. On the contrary, samples with 8.70 g glycerol showed good interaction between the plasticizer and biopolymer and more flexibility. In this regard, MTS results obtained in this study were higher than previously reported by Souza et al. [6] (3.49 ± 0.55 MPa), Gutierrez et al. [61] (1.16 ± 0.05 MPa) and Andretta et al. [65] (5.70 ± 1.40 MPa), which confirms the positive interaction between the glycerol and CO/starch emulsion in our film formulation.

Mali et al. [54] and Souza et al. [6] reported the importance of the optimization of the drying stage when using thermoplastic materials, such as starch and glycerol. The proximity of drying temperature to the glass transition temperature of cassava starch was considered relevant for improving mechanical properties of cassava starch films. Neves Horta Lima et al. [32] studied how different drying conditions (temperature and time) of casting method would affect the mechanical properties of cassava starch-based films. Drying temperatures between 45 °C at 80 °C were tested

and the best results for maximum tensile strength were found at 55 °C for 8 h and 60 °C for 5.5 h. These temperature/time conditions are close to the glass transition temperature of cassava starch, which resulted in homogeneous surface and cross-section regions for cassava starch-based films [32], similar to the effects observed in our work. Moreover, a drying temperature of 55 °C is within the temperature range used by the cast tape drying (CTD) technique that allows scaling up and preparation of films larger than those produced by classical casting, according to [71].

Basiak et al. [72] and Luchese et al. [64] observed that the physicochemical properties of starch-based films are influenced by the amylose/amylopectin ratio, as well as the molar mass and morphological characteristics. The cassava starch used in this study is composed of 22% linear amylose [73]. Higher molar mass found on cassava starch promotes a more viscous filmogenic solution which affects the re-association of the biopolymer structure, and therefore, impacts film properties. Amylopectin branching reduces water vapor permeability by introducing a tortuous path to vapor molecules inside the cassava starch matrix [64]. Furthermore, Fangfang et al. [19] and Garcia et al. [74] also reported that the amylose content positively correlates with the number of starch/lipid inclusions, which may have led to synergic effects contributing to producing COF-1 film with smooth surface and higher MTS.

Jusoh et al. [26] studied the mechanical properties of chicken skin gelatin films produced with different CO concentrations (0-30% w/w). They observed that control samples (no addition of CO) had 0.70 ± 0.12 MPa and the addition of CO in the gelatin film decreased the film's tensile strength (0.48 ± 0.03 MPa with 10% CO and 0.29 ± 0.19 MPa with 30% CO). CO might act as a plasticizer most likely to disrupt the protein-protein interaction in the film network, promoting discontinuity of polymeric matrix, and affecting the tensile strength of the film [17, 68]. In this study, the CO emulsion increased the interaction of polymeric matrix with starch. Our results represented a 20-fold increase when compared to the tensile strength results presented by Jusoh et al. [26].

3.3.4 *Biodegradability*

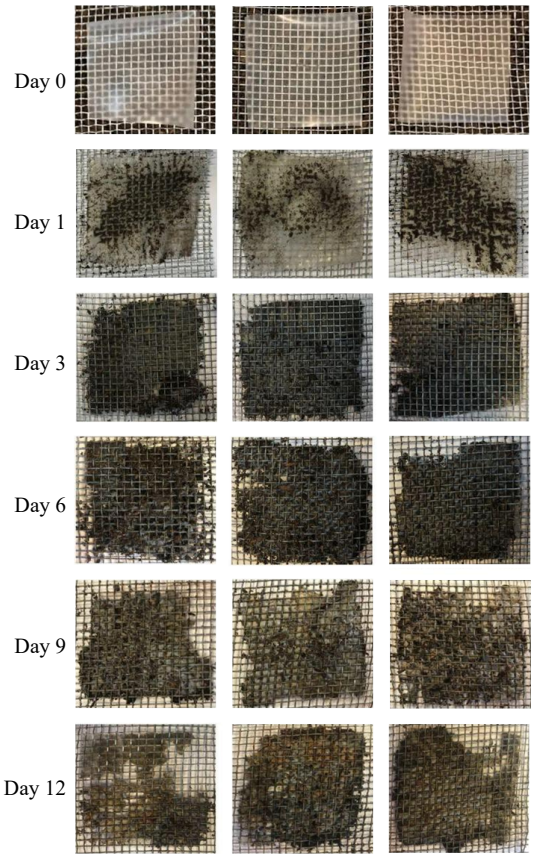

Figure 3. Biodegradability results of cassava starch-based films incorporated with coconut oil (COF-1) during 12 days under soil-buried conditions. Images show triplicate experimental samples

After the first day, COF-1 films showed a wrinkled appearance due to the loss of moisture content to the soil. On the third day, samples were well adhered to the soil and early signs of decomposition were observed. From days 6-9, the film degradation progressed and on day 12, films were visibly degraded (Figure 3). Likewise, Medina-Jaramillio et al. [39] showed similar results after 12 days for biodegradable packaging films produced with starch and natural extracts. Our qualitative results confirmed the biodegradable nature of COF-1, being easily decomposed under simulated natural conditions. Thus, disposal of COF-1 films should have no negative impact on the environment.

4. Conclusions

This study evaluated the formulation of cassava starch-based films with the incorporation of different concentrations of coconut oil and glycerol for the development of biodegradable food packaging. Cassava starch-based film COF-1 produced with 0.5 g of CO/starch emulsion, 8.70 g of glycerol and dried at 55 °C showed greater water solubility and vapor permeability performance among treatments. This prioritized formulation COF-1 produced a more hygroscopic film with greater contact angle and maximum tensile strength when compared to the control film produced without the incorporation of coconut oil. Taken altogether, this study established a protocol to produce cassava starch-based films with coconut oil with enhanced mechanical properties and desirable biodegradability. Notably, COF-1 showed the best barrier and mechanical properties when dried at 55 °C, a temperature compatible with laboratory and commercial scale operations.

Our promising results constitute an innovative effort to improve the performance and cost-effectiveness of starch-based films, making them more competitive compared to traditional plastics, using vegetable oils as active phases. Further investigation regarding possible antimicrobial and bioactive activities of the developed films derived from starch with added coconut oil is warranted in the future.

Conflict of interest

The authors declare no conflict of interest.

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