Biodegradable films of arrowroot starch (*Maranta arundinacea*) incorporated with floral extract of *Tabebuia impetiginosa* and copper sulfate: physical and physicochemical properties, and biodegradability and antibacterial activities

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Received: December 19, 2021 Accepted: January 08, 2022 Published: February 01, 2022

Abstract

Biodegradable films of arrowroot starch were incorporated with floral extract of *Tabebuia impetiginosa* and copper sulfate and determined to the physical, physicochemical, biodegradability and antibacterial properties. The films were produced from arrowroot starch with different concentrations of glycerol, floral extract and copper sulfate solution. Thickness, humidity, solubility, biodegradability, water vapor colorimetry, color density, transparency, FT-IR and antibacterial activity tests were performed for *Escherichia coli*, *Staphylococcus aureus*, *Salmonella serovar* Thyphymurium and *serovar* Enteritidis. The films had a thickness between 0.23-0.33 mm, humidity between 8.53-12.22%, biodegradability between 88.98-100%, water vapor between 255.79-433.57 g m^2 day, L* between 4.77-22.36; a* between -1.20 to 0.30; b* between -1.89 to 0.13; color density between -0.09 to 0.180; maximum transparency of 80%, inhibition activity between 9.13-26.26 mm for *E. coli*, 5.83-24.14 mm for *S. aureus*, 13-42-19.39 mm for *S. serovar* Thyphymurium, and 22.14-26.89 mm for *S. serovar* Enteritidis. The arrowroot biodegradable films incorporated with floral extract of *Tabebuia impetiginosa* and copper sulfate showed good physical, physicochemical, biodegradability and potential antibacterial activity.

Keywords: Active packaging; *Escherichia coli*; *Salmonella* Thyphymurium; Biopolymers.

Resumo

Filmes biodegradáveis de amido de araruta foram incorporados com extrato floral de *Tabebuia impetiginosa* e sulfato de cobre e determinados quanto às propriedades físicas, físico-químicas, biodegradabilidade e antibacteriana. Os filmes foram produzidos a partir de amido de araruta com diferentes concentrações de glicerol, extrato floral e solução de sulfato de cobre. Espessura, umidade, solubilidade, biodegradabilidade, colorimetria de vapor d'água, densidade de cor, transparência, FT-IR e testes de atividade antibacteriana foram realizados para *Escherichia coli, Staphylococcus aureus, Salmonella sorovar* Thyphymurium e *sorovar* Enteritidis. Os filmes tinham espessura entre 0,23-0,33 mm, umidade entre 8,53-12,22%, biodegradabilidade entre 88,98-100%, vapor d'água entre 255,79-433,57 g m2 dia, L* entre 4,77-22,36; a * entre -1,20 a 0,30; b * entre -1,89 a 0,13; densidade de cor entre -0,09 a 0,180; transparência máxima de 80%, atividade de inibição entre 9,13-26,26 mm para *E. coli*, 5,83-24,14 mm para *S. aureus*, 13-42-19,39 mm para *S. serovar* Thyphymurium e 22,14-26,89 mm para *S. serovar* Enteritidis. Os filmes biodegradáveis de araruta incorporados com extrato floral de *Tabebuia impetiginosa* e sulfato de cobre apresentaram boa capacidade física, físico-química, biodegradabilidade e potencial atividade antibacteriana.

Palavras-chave: Embalagem ativa; *Escherichia coli*; *Salmonella* Thyphymurium; Biopolímeros.

Resumen

Se incorporaron películas biodegradables de almidón de arrurruz con extracto floral de *Tabebuia impetiginosa* y sulfato de cobre y se determinaron las propiedades físicas, fisicoquímicas, biodegradables y antibacterianas. Las películas se produjeron a partir de almidón de arrurruz con diferentes concentraciones de glicerol, extracto floral y solución de sulfato de cobre. Se realizaron pruebas de espesor, humedad, solubilidad, biodegradabilidad, colorimetría del vapor de agua, densidad de color, transparencia, FT-IR y actividad antibacteriana para *Escherichia coli*, *Staphylococcus aureus*, *Salmonella serovar* Thyphymurium y *serovar* Enteritidis. Las películas tenían un espesor entre 0.23-0.33 mm, humedad entre 8.53-12.22%, biodegradabilidad entre 88.98-100%, vapor de agua entre 255.79-433.57 g m2 día, L * entre 4.77-22.36; a * entre -1,20 a 0,30; b * entre -1,89 a 0,13; densidad de color entre -0,09 y 0,180; transparencia máxima del 80%, actividad de inhibición entre 9,13-26,26 mm para *E. coli*, 5,83-24,14 mm para *S. aureus*, 13-42-19,39 mm para *S. serovar* Thyphymurium y 22,14-26,89 mm para *S. serovar* Enteritidis. Los films biodegradables de arrurruz incorporados con extracto floral de *Tabebuia impetiginosa* y sulfato de cobre mostraron buena biodegradabilidad física, fisicoquímica y potencial actividad antibacteriana.

Palabras clave: Embalaje activo; *Escherichia coli*; *Salmonella* Thyphymurium; Biopolímeros.

1. Introduction

Packaging is widely used in the modern world, as a form of protection during transport, storage of solid, liquid and pasty products mainly for the food industries, which requires the maintenance and preservation of the quality and integrity of the final product. The first packaging prototype were produced from the processing of oil, with its synthetic polymer matrix, which is highly efficient in a variety of uses, from product coating to electronic components (Shen; Kamdem, 2015; Valadares et al., 2020).

Although this advent was important for the economy, plastic packaging presents serious environmental problems with accumulation, poor compaction, production of microplastics and long stay in the environment, with the main characteristic of the low degradability rate, which can be over 500 years (Piñeros- Hernandez et al., 2017; Olivatto et al., 2018; Kalpana et al., 2019).

Environmental pollution in recent years has been gaining strength with activist movements in favor of the maintenance and balanced use of minerals, fossil and renewable fuels, and yet integrated with this purpose, is the ban on the use of plastic packaging. With this, new studies are being developed aiming to find alternative and more conscious ways in the development of packaging from biopolymers existing on a large scale in the environment. A strong candidate has been gaining prominence among the researches, starch, which is an excellent biopolymer, in addition to being easily obtained in the plant environment. In addition to this biopolymer, there are studies that evaluate chitosan and lipids, where they also present good results, and these efficiencies are substitutes for synthetic polymers (Bonilla et al., 2014; Caetano et al., 2018).

Among starch vegetables, we can mention the arrowroot (*Maranta arundinacea*) belonging to the Marantaceae family, which has rhizomes rich in starch (> 85%), which has specific characteristics that are different from other sources of natural starches and can also be used for therapeutic purposes (Madineni et al., 2012). *M. arundinacea* is a tropical and perennial tuberous plant from America, and is also cultivated for agricultural purposes in São Vicente and the West Indies, being exported to the USA, Canada, Great Britain and Europe. It is still little known about the possible scientific-technological uses, although the number of studies with this plant is already interesting at a scientific level (Madineni et al., 2012; Jayakumar; Suganthi, 2017). It is already known that the starch of this species has high digestibility efficiency, gelatinization capacity and high amylose content, with the characteristic of producing high quality biodegradable films, and good physical, physicochemical, morphological and mechanical characteristics, also having aptitude during the incorporation process with metals, plant extracts, plasticizers and fixed and essential oils, producing a consistent and effective intermolecular rearrangement promoting barriers against water and gas vapors (Nogueira et al., 2018; Santos et al., 2021).

Biodegradable packaging has several positive factors, mainly for food, human and animal use, and also for the environment, as they are non-toxic, having good luminosity, malleability, transparency and using renewable raw material (Chacon et al., 2021). In addition, antioxidant agents, essential oils, fixed oils, oil-resins, metals (copper, aluminum, cobalt) and antimicrobials (bactericidal and fungicidal) can be incorporated during the production of the biopolymer matrix (Balti et al., 2017; Youssef et al., 2019). These agents can migrate from the packaging to the food, increasing the shelf life and decreasing the action of lipid oxidation, degradation of fat-rich products such as meat, inhibiting the growth of phytopathological fungi and pathogenic bacteria of food interest (Nor Adilah et al., 2018; Sharma et al., 2020).

Thus, considering that *M. arundinacea* starch has high potential in the development of natural packaging enriched with plant extracts and metals, both with characteristics of an active and efficient natural product, this study aimed to produce biodegradable packaging incorporated with *Tabebuia impetiginosa* floral extract a species of flora from the Brazilian Cerrado domain and with different concentrations of copper sulfate that have antioxidant and antimicrobial activities, and also evaluate the physical, physicochemical, and biodegradability properties of the resulting biofilms.

2. Materials and Methods

Study location

The study was carried out in the laboratory of technological chemistry in the department of Agrochemistry and in partnership with the Department of Agricultural Sciences in the postgraduate program in Agricultural Sciences of the Goiano Federal Institute, Campus Rio Verde, Goiás, Brazil.

Reagents and digital instruments

Ethanol (LS Chemical), copper sulfate II (Êxodo Científica), glycerol (Dinâmica), chloride sodium (Anidrol), azithromycin disc (15 µg) (DME), cephalexin disc (30 µg) (DME), dextrose tryptone agar (DTA, Kasvi), plant count agar (PCA, Kasvi), arrowroot starch (Torres Alimentos LTDA), bacterial strains (Centerlab), UV-*Vis* spectrometer (Belphotonics, Mod. M-51), analytical balance (Shimadzu, AY 220), digital colorimeter (ColorFlex, Mod. EZ), FT-IR spectrophotometer Frontier (PerkinElmer, Mod. Spectrum Two), magnetic shaker with heating (Solab, Mod. SL 91), air circulation stove (Thoth, Mod. Th-510-480), rotary evaporator (Fisaton, Mod. 802), lyophilizer (Qualicien, Mod. L101), and digital caliper (Digimess, Mod. 100-174BL).

Plant material and extract production

Fresh flowers of *T. impetiginosa* were collected in September 2020 in the morning. The species was identified and deposited at the Herbarium of Goiano Federal Institute. (number HRV 14862) (Menezes Filho et al., 2021). The extract was produced by static maceration with 250 g of flowers in 70% hydroethanolic solution for 7 days in an amber glass bottle. After this period, the extract was filtered and reduced in a rotary evaporator with negative pressure. Then, lyophilized and stored in a freezer at -12 °C until analysis.

Production of biodegradable films

The biodegradable films were obtained by a casting technique, with the use of the methodology described by Issa et al. (2017) modification. In order to produce every packaging, 5 g commercial arrowroot starch was dissolved in 100 mL distilled water. The emulsion was then moderately agitated at room temperature 25 °C. Afterwards, it was heated at 70 °C, at constant mechanical agitation for 30 min. After starch gelatinization, glycerol was added as a plasticizer 30% (p/p) and this emulsion was agitated for ten more minutes.

When the films emulsions reached 30 °C the floral extract of *T. impetiginosa* and copper solution was incorporated according to (Table 1).

Table 1. Emulsion formulations preparation of starch arrowroot in floral extract and copper solution.

Films	**Emulsion formulations**
1 cont	5 g starch + 1.5 g glycerol
2 cont	5 g starch + 2 g glycerol
3	5 g starch + 1.5 g glycerol + 1000 µL floral extract
4	5 g starch + 2 g glycerol + 1000 µL floral extract
5	5 g starch + 1.5 g glycerol + 1000 µL copper solution 1 Mol L^{-1}
6	5 g starch + 1.5 g glycerol + 1000 µL copper solution 0,5 Mol L^{-1}
7	5 g starch + 1.5 g glycerol + 1000 µL copper solution 0,25 Mol L^{-1}

Source: Authors, 2021.

All film emulsion were poured on polystyrene slabs (20 cm^2) and dried in an air circulation oven at 35 °C for about 48 hours.

Physical and physicochemical and biodegradability films characterization

The films was measured by digital caliper, with 0.01 mm accuracy. Ten spots were measured on every biodegradable packaging and thickness mean was calculated. The moisture was determined by loss of packaging mass after had been dried in na oven at 105 °C. The assays were carried out in triplicate as described by Valadares et al. (2020).

The solubility assay in water of the biodegradable films was determined with (2 cm^2 diam.) were dried in na oven at 105 °C for 3 hours and then weighed so that initial mass (***Mi***) could be determined Afterwards, they were immersed in 50 mL distilled water and kept under constant agitation (170 rpm) at 20 °C for 24 hours. Then, samples packaging were filtered through quantitative filter paper. The films were dried at 100 °C for 4 hours and weighed (***Mf***) as described by Kavoosi et al. (2014) with modifications. The packaging water solubility (WS%) was calculated Equation [1].

$$\text{WS\%} = \frac{Mi - Mf}{Mi} * 100 \quad \text{Eq. [1]}$$

The biodegradability was carried out by the methodology described by Martucci and Ruseckaite (2009), with modifications. Films samples (2 x 2 cm^2) were dried up to constant weight (***Mi***) could be determined. Samples were then placed in open polyethylene packaging to enable microorganisms and moisture to gain access to them 40%. After that, they were buried *in natural* soil, at constant moisture and room temperature and natural luminosity. Three, ten, fifteen and thirty days after the experiment installment, the artificial packaging with the samples were removed from the soil, washed with distilled water and dried up to constant weight (***Mf***). The time of biodegradability, was calculated by equation [2].

$$\text{Bio (\%)} = \frac{Mf - Mi}{Mi} * 100 \quad \text{Eq. [2]}$$

Water vapor transmission rate (WVTR) and water vapor permeability (WVP) were evaluated in agreement with Santos et al. (2021). In order to carry it out, 10 mL borosilicate glass bottle were filled with 5.0 g distilled water, sealed with the biodegradable films and placed in a desiccator which held a saturated solution of chloride sodium conc. 14% (NaCl). Masses in the borosilicate bottle were measured every hour for 8 hours and then again at the 24 hours. Both (WVTR and WVP) were calculated by equation [3 and 4]. The assay was performed in quadruplicate.

$$WVTR = \frac{m}{t \; x \, A} \quad \text{Eq. [3]}$$

$$WVP = \frac{WVTR}{(\Delta P)} \; x \; E \quad \text{Eq. [4]}$$

Where: m = mass loss, t = time, A = packaging area, E = packaging thickness, (ΔP) = pressure difference.

The color analysis was carried out by a colorimeter. Parameters under evaluation were L* luminosity and chromaticity a* and b*. The films density was determined from the ratio between the weight and volume described by Kurt and Kahyaoglu (2014). Ultraviolet-visible (UV-*Vis*) light transmission of the films samples were placed in quartz cuvette and transmittance was measured at wavelengths that ranged from 900-200 nm, in agreement with by Santos et al. (2021). Fourier transform infrared (FT-IR) spectra of the biodegradable films in different concentrations of glycerol, floral extract of *T. impetiginosa* and sulfate copper solutions were analyzed using FT-IR spectroscopy operated at a resolution of 4 cm^{-1}. Film sample was placed on the ray exposing stage and the spectrum was recorded between the wave number ranges of 650-4000 cm^{-1}.

Antibacterial activity

Antibacterial assay was evaluated *in vitro* against four bacteria *Escherichia coli* (ATCC), *Staphylococcus aureus* (ATCC), *Salmonella serovar* Thyphymurium and *Salmonella serovar* Enteritidis (ATCC) commercially acquired and maintained from the bacteriological bank of the first author. Briefly, 150 µL of bacterial culture $1x10^4$ cells mL^{-1}, were grown on *Petri* dishes with dextrose tryptone agar (DTA) and 150 µL of spore suspension $1x10^6$ CFU mL^{-1} on *Petri* dishes with plant count agar (PCA). Biodegradable packaging (7 mm diam.) were then placed on the surface agar and incubated at 36 °C for 36 hours. The diameter of the zone of inhibition was measured with a digital caliper. As a positive control, Azithromycin (15 µg disc) and Cephalexin (30 µg disc) discs was used, and negative control biodegradable starch packaging disc without floral extract. The minimum acceptable diameter was 5 mm. The assay was performed in triplicate by described Oliveira Filho et al. (2020).

Statistical analysis

SISVAR (version 2019) were used to analyze the data. The means were compared by Tukey's test at a 5% level of significance using analysis of variance (ANOVA).

3. Results

Table 1 shows the results for different films based on arrowroot starch and 1.5 g of glycerol where it showed thickness = 0.23 mm (1), where was the same base containing 2 g of glycerol showed a greater thickness = 0.24 mm with statistical difference by the Tukey's test ($p < 0.05$). For films (3 and 4), no significant difference was observed by the Tukey's test ($p < 0.05$), although the glycerol concentration was 1.5 and 2.0 g incorporated with *T. impetiginosa* floral extract. The films incorporated with different concentrations of copper sulfate solution, and the same concentration of glycerol, showed to be thickness, especially for the formulated (5 and 6) with higher concentration of copper sulfate = 0.33 and 0.34 mm, respectively, not showing a significant difference by the Tukey's test ($p < 0.05$), although the formulated (7) had a thickness = 0.30 mm, there was a significant difference between the films with a higher concentration of copper.

The moisture content was higher for the formulated (1 and 5) with results of 12.22% and 11.31%, with a significant difference as obtained by the Tukey's test ($p < 0.05$), the others formulated presented humidity below 9.90%. The solubility test showed a superior result only for the formulated (7) = 58.59%, this being the only one with a significant difference between the other formulations that varied between 40.59%-48.58% solubility, with no significant difference by the Tukey's test ($p < 0.05$). The biodegradability test demonstrated that the formulated (1 and 2) showed 100%, and it is not possible to measure the mass of the residual biopolymer, with no statistical difference according to the Tukey's test ($p < 0.05$). The formulation (5) containing 1 Mol L^{-1} of copper sulfate solution showed a degradability rate of 88.98%, showing a statistical difference between the other films by the Tukey's test ($p < 0.05$).

The water vapor permeability test showed no significant difference between the formulated (1, 2 and 7) = 433.57 g m^2; 427.97 g m^2 and 427.96 g m^2 day forming the group (a), the same was observed for the formulated ones (3 and 6) 379.25 g m^2 and 381.94 g m^2 day forming the group (b), already for the formulated ones (4 and 5) 313.96 g m^2 and 255.79 g m^2 day, where a significant difference was observed according to the Tukey's test ($p < 0.05$).

Table 1. Thickness, moisture, solubility, biodegradability and water vapor permeability of arrowroot films incorporating floral extract and copper solution.

F	Thickness (mm)	Moisture (%)	Solubility (%)	Biodegradability (30 days, %)	Water vapor permeability (g m^2 day)
1	0.23±0.01e	12.22±0,04a	48.31±2.35b	100a	433.57±4.83a
2	0.24±0.01d	9.30±0.10f	43.96±2.83b	100a	427.97±3.18a
3	0.26±0.01c	8.53±0.11c	40.59±0.74b	97.06±0.95ba	379.25±18.36b
4	0.27±0.08c	9.90±0.05c	40.78±1.77b	95.02±2.57cba	313.96±12.05d
5	0.33±0.01a	11.31±0.13b	44.55±1.05b	88.98±2.13c	255.79±9.73e
6	0.34±0,01a	9.64±0.05dc	48.58±3.90b	90.15±2.96cb	381.94±12.84b
7	0.30±0.01b	9.56±0.04ed	58.59±4.62a	94.07±3.49cba	427.96±6.84a

Note: F = Films. Different letters in a column show significant difference ($p < 0.05$) by the Tukey's test. Source: Authors, 2021.

Figure 1 shows photographic images of the films produced using standard arrowroot starch (A), varying the concentration of glycerol (B-C), and 1.5 and 2.0 g of glycerol incorporated with floral extract of *T. impetiginosa* (D-E) and 1.5 g of glycerol incorporated with different concentrations of copper sulfate (F-H) solution.

Figure 1. Arrowroot starch films incorporating floral extract of *Tabebuia impetiginosa* and copper solution. A: B: C: D: E: F: G: H: Source: Authors, 2021.

Table 2 shows the color parameters of arrowroot films incorporating floral extract of *T. impetiginosa* and copper solutions. In the measurement of color, the L* value corresponds to brightness (0 black and 100 white) and the a* and b* parameters correspond to the chromaticity coordinates green (-)/red (+) and blue (-)/yellow (+), respectively. The value of L* for the formulated (4) with 2 g of glycerol and floral extract showed greater luminosity = L* 22.36, followed by the formulated (5 and 7) L* = 19.34 and 17.99 with 1.5 g of glycerol and 1 and 0.250 Mol L^{-1} of copper sulfate, showing no significant difference between the other films by Tukey's test ($p < 0.05$). The formulated (4-7) demonstrated chromaticity tending to green (-a*) with significant differences by the Tukey's test ($p < 0.05$), for the formulated (1-3) the chromaticity tending to red (+a*) also with significant difference between them by the Tukey's test ($p < 0.05$).

For chromaticity b*, the films (1, 4, 5, 6 and 7) tended to blue, while the films (2 and 3) tended to yellow. Among the formulated (1, 2 and 3) there was no significant difference by the Tukey's test ($p < 0.05$) even with a negative result, for the other filmogenic formulations, a significant difference is observed. The color density obtained for the films (5-6) showed negative results corroborating the color test due to high concentrations of copper sulfate solution, and the other films showed positive results with statistical difference, except for the formulated ones (1 -3) that showed no statistical difference by the Tukey's test ($p < 0.05$).

Table 2. Measurement parameters of arrowroot starch films incorporating floral extract of *Tabebuia impetiginosa* and copper solution colors, color density and hue tint.

Films	L*	a*	b*	Color density
1	16.65±0.23d	0.17±0.02b	-0.13±0.05a	0,092±0.00b
2	9.33±0.73c	0.30±0.03a	0.08±0.04a	0.049±0.00c
3	7.51±0.10d	0.17±0.02b	0.13±0.00a	0.097±0.00b
4	22.36±0.74a	-0.16±0.03c	-1.30±0.02c	0.180±0.01a
5	19.34±0.60b	-1.07±0.02e	-1.70±0.03d	-0.05±0.00d
6	4.77±0.08e	-0.57±0.05d	-0.40±0.04b	-0.09±0.00e
7	17.99±0.16b	-1.20±0.04f	-1.89±0.01e	0.09±0.00b

Different letters in a column show significant difference ($p < 0.05$) by the Tukey's test; parameters CIELab of color L* (luminosity), a* and b* (chromaticity).

Figure 2 shows light transmission rates (%) of arrowroot films incorporated of glycerol concentrations, and floral extract *T. impetiginosa* and copper sulfate solutions, in the range from 200 to 900 nm. Results found in the UV-*Vis* region 200-380 nm showed that the control films made from arrowroot starch exhibited good barrier property against UV light, since it decreased up to 80% of UV-*Vis* transmission in this range. In the visible region (between 400 and 700 nm), arrowroot films incorporated with copper sulfate solution proved to be least effective as a barrier to light transmission.

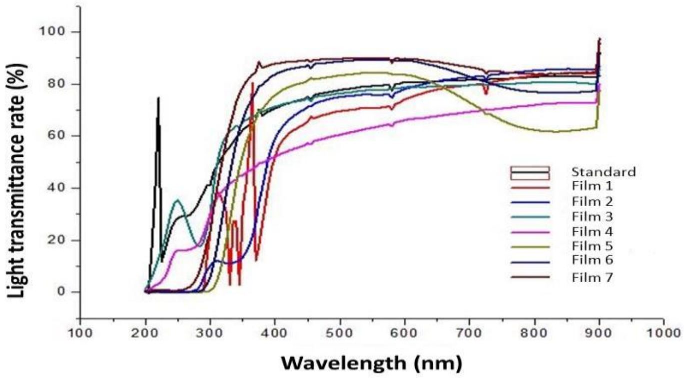

Figure 2. UV-*Vis* light transmittance rate (%) in arrowroot films incorporating floral extract os *Tabebuia impetiginosa* and copper solution. Source: Authors, 2021.

In Figure 3, the band centered at 3291.21 cm^{-1} for all samples, can be attributed to the stretching of the -OH bonds, related to the hydrogen bonds established between the components of the biopolymeric matrix. In 2929.36 cm^{-1} it refers to the CH bonds of sp^3 carbons, the bands in 1646.55 cm^{-1} are associated with the angular flexion of the -OH group in the water molecules, indicating the formation of interaction between starch and glycerol, bands at 1415.45 cm^{-1} can be attributed to the folding of the CH_2 group. Bands at 996.31 cm^{-1} and 925.68 cm^{-1} between the regions 1200 cm^{-1} to 900 cm^{-1} are associated with C-O, C-C connections and C-O-H vibrations. And bands between 858.82 cm^{-1} and 760.06 cm^{-1} are related to the presence of Cu.

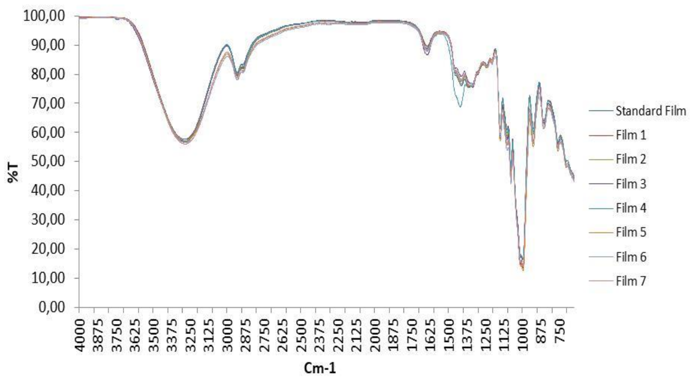

Figure 3. FT-IR spectra of arrowroot starch films incorporating floral extract of *Tabebuia impetiginosa* and copper solution. Source: Authors, 2021.

Antimicrobial activity of the films samples was tested against food borne pathogenic bacteria *E. coli*, *S. aureus*, *S. serovar* Thyphymurium, and *S. serovar* Enteritidis was the results are shown in Table (3). It is observed that the films purity of arrowroot starch do not present antibacterial activity in any of the strains tested. The floral extract of T. impetiginosa showed moderate antibacterial activity for *E. coli* for the films (3 and 4) with inhibition = 9.13 and 9.22 mm respectively, with no significant difference by the Tukey's test ($p < 0.05$). Exciting results were observed in the three formulations containing different concentrations of copper sulfate solution (5, 6 and 7) with the highest rates of bacterial inhibition showing a significant statistical difference between them by the Tukey's test ($p < 0.05$) of 26.26; 24.21 and 21.45 mm respectively.

Similar results were observed in the strain of *S. aureus* for the formulated (3 and 4) with inhibition rate = 6.01 and 5.83 mm respectively, with no significant difference by the Tukey's test ($p < 0.05$). Again, a high antibacterial inhibition effect is observed in formulations incorporated with copper sulfate solutions (5, 6 and 7) = 24.14; 20.59 and 19.05 mm respectively, with significant difference between them, except for the film (6) = according to the Tukey's test ($p < 0.05$). The strains of *Salmonella serovar* Thyphymurium and *S. serovar* Enteritidis showed inhibition only in the formulated (5, 6 and 7) due to the presence of copper sulfate, *S.*

Thyphymurium with less activity between 19.39 to 13.42 mm and high inhibition activity between 26.89 to 22.14 mm for *S.* Enteritidis. The results obtained by the different biodegradable films were simply compared without statistical analysis.

Table 3. Antibacterial activity of arrowroot starch films incorporating floral extract of *Tabebuia impetiginosa* and different copper solution.

Films	**Inhibition zone (mm)**			
	E. coli	***S. aureus***	***S. serovar* Thyphymurium**	***S. serovar* Enteritidis**
1	- e	- d	- d	- d
2	- e	- d	- d	- d
3	9.13±0.05d	6.01±0.03c	- d	- d
4	9.22±0.03d	5.83±0.03c	- d	- d
5	26.26±0.55a	24.14±0.04a	19.39±0.60a	26.89±0.04a
6	24.21±0.16b	20.59±0.02ba	16.80±0.22b	24.18±0.06b
7	21.45±0.03c	19.05±0.04b	13.42±0.02c	22.14±0.05c
Antibacterial references [a/b]	27.29±0.26[b]	24.28±0.23[a]	28.09±0.02[a]	29.11±0.09[a]

Same lowercase letters in the same column do not differ statistically by the Tukey's test ($p < 0.05$); equal capital letters on the same line do not differ statistically by the Tukey's test ($p < 0.05$). [a]Cephalexin and [b]Azithromycin. Source: Authors, 2021.

4. Discussion

Biodegradable films vary in thickness depending on the biopolymeric matrix used (chitosan, starch) and also the numerous substances incorporated in this matrix (glycerol, plant extracts, essential oils, fixed oils and nano or micro particles of metals) that can have an effect antioxidant, photoprotective, prolong the product's life and avoid gas exchange between the product and the environment. Norajit et al. (2010) found thickness between 0.13 to 0.07 mm and moisture content between 29.64 to 23.90% in alginate films incorporated with ginseng extract. Costa et al. (2017) obtained a thickness less than that of this study between 0.09 to 0.10 mm in films based on solid bean starch with different amounts of glycerol and low solubility between 11.43 to 21.51%.

Medina-Jaramillo et al. (2017) evaluated two films of cassava starch using two plant extracts where they found moisture content of 28.6 and 25.3% higher than this study and reports of studies using different sources of starches. Nor Adilah et al. (2018) found solubility between 40.06 to 20.06% for gelatin films incorporated with different concentrations of mango pulp extract. Youssef et al. (2019) found for films based on carboxymethyl cellulose incorporated with zeolite modified with silver and gold, water vapor permeability between 2014.56 to 623.95 g m^2 day. Santos et al. (2021) verified in films produced based on arrowroot starch incorporated with extract of the mature fruit of *Capsicum chinense*, thickness varying between 0.24 to 0.34 mm, humidity between 7.69 to 11.44% and solubility between 20.63 to 41.83% similar to this study.

Different production processes, from the choice of polymeric raw material to dry, are directly involved in the thickness of the film. In addition, the thickness affects the mechanical and physicochemical properties of the biodegradable packaging such as moisture content, solubility, malleability and water vapor permeability (Hosseini et al., 2015; Nor Adilah et al., 2018; Santos et al., 2021). The moisture content is another critical factor of great importance during the biopolymer option process, arrowroot starch has a high concentration of amylose, inducing high water absorption from the environment, negatively interfering in the moisture test.

This is another observed problem that can affect the mechanical properties of biodegradable packaging (Thakur et al., 2019). Although there is no legislation that recommends a humidity range for biopolymers, several studies suggest that its content is equal to or less than 15% (Hosseini et al., 2015; Rambabu et al., 2019). The solubility is also na important parameter that should be analyzed in starch and chitosan films. The ideal percentage of film solubility depends on their final use.

The according Bertuzzi et al. (2007), Kim et al. (2015) and Santos et al. (2021) the starch is a hydrophilic composite thus, when a starch films is exposed to water, its polymeric molecules form hydrogen bonds with water and lead to film dissolution. This study it was observed that the films produced with 1.5 and 2 g of glycerol incorporated with floral extract and 1.5 g of glycerol and copper sulfate with conc. 1 Mol L^{-1} showed lower solubility content.

Biodegradable films must have a rapid biodegradability rate, which is in the natural environment after discarding a nutritional source for the soil's microbial flora that will facilitate the decomposition of these biopolymers. In this study, formulated 1 and 2 (Table 1) demonstrated 100% biodegradability and with plant extracts and different solutions of copper sulfate the biodegradability rate decreased, although they were above 80% in a maximum period of 30 days of experiment. The lower rate of biodegradability for both formulated with copper extract and sulfate is easily explained, possibly the floral extract has some phytochemical group capable of promoting an inhibitory activity of the soil's microbial flora, as well as copper sulfate. We suggest this explanation, although new tests should be performed evaluating both solutions in vitro with the natural microbial flora of the soil.

In the study by Medina-Jaramillo et al. (2017) evaluating two films of cassava starch with incorporated plant extracts, they obtained total degradability with twelve days of *in vitro* assay. Santos et al. (2021) observed that the addition of fruit extract of *C. chinense* did not interfere in the decomposition of films based on arrowroot starch after 30 days of testing. An important comparison between this study, and the Santos et al. (2021), among others, is that extracts from different plants, as well as extraction solvents and phytochemistry itself, with or without groups that have antimicrobial activity, influence the degradation of the biopolymer in the natural environment. Although there is a certain limitation in biodegradability, the objective is to evaluate the active action of the film, mainly for food use, where the antioxidant and antimicrobial biological activity is of greater interest among researchers. However, it does not make the product unfeasible, since it presents slower and continuous biodegradation with less time when compared to synthetic or semi-synthetic packaging.

The permeability to water vapor also presents a high variation when considering a biopolymeric matrix, the control films (1 and 2) demonstrated a greater capacity for water vapor exchange, where as the films incorporated with floral extract and 1 Mol L^{-1} of sulfate copper showed lower permeability, although at lower concentrations of copper the permeability increase.

Optical properties (color and transparency) of seven arrowroot starch films were evaluated on both matte and shiny sides. Films incorporating glycerol (1.5 and 2 g), floral extract of *T. impetiginosa* and different concentrations copper sulfate got more colorful as its doses increased in the assay photographic. In the quantitative colorimetric tests for color and color density (colorimeter and UV-*Vis*) for luminosity (L*), chromaticity (-/+ a* and b*) the results confirm this statement by the qualitative test. The color of the film influences consumer acceptance of the product. Studies have evaluated this claim, in which consumers have opted for brighter and cleared films (Kurt and Kahyaoglu, 2014).

Although this acceptance process undergoes changes, as the consumer is becoming more demanding regarding the maintenance of the planet, thus decreasing the use of synthetic packaging, there is an increasing evolutionary process regarding colored natural packaging. We observed these color effects mainly in plant extracts containing anthocyanins, beta-carotene and lycopene, although they are not restricted only to these groups of substances. Kurt and Kahyaoglu (2014) obtained for glucomannan films L* between 81.61 to 81.82, a* between 5.79 to 5.69 (green) and b* between 1.28 to 0.67 (yellow). Luchese et al. (2018) incorporated starch-based blueberry extract films in which they obtained L* luminosity between 96.7 to 61.1, a* 26.7 to 0.05 and b* between 5.4 to 2.3. Nor Adilah et al. (2018) obtained in gelatin films incorporated with L* mango pulp extract between 89.07 to 85.67, a* between -1.17 to -1.96 and b* between 5.74 to 2.25. Roy and Rhim (2020) found shades in yellow for carboxymethyl cellulose films incorporated with curcumin and zinc oxide. As for the colorimetric test, the group of films studied presented L* between 90.7 to 74.2, a* 10.50 to -0.81 and b* between 86.0 to 5.4 for different concentrations. Santos et al. (2021) obtained films incorporated with extract from the ripe fruit of *C. chinense* L* between 10.57 to 15.51, chroma a* ranging from -0.3 to 4.20, chroma b* between -0.58 to 9.14 (tones between red and yellow).

The transparency assay for the seven films of arrowroot starch incorporated with different concentrations of glycerol, floral extract and copper sulfate solutions demonstrated the potential for barrier properties against ultraviolet light, being close to 80%. The action caused by ultraviolet light negatively interferes in food causing deterioration, often caused by lipid oxidation in foods with high fat content such as meat (Hosseini et al., 2015; Fathi et al., 2018). Roy and Rhim (2020) observed that carboxymethyl cellulose films incorporated with curcumin had low transmittance at wavelengths below 450 nm, while films incorporated with zinc oxide showed superior results with a lower rate of light transmission for both UV and visible light.

FT-IR spectra were analysis to identify possible interactions between functional groups of arrowroot starch with glycerol, floral extract and sulfate copper solutions. According Xu et al. (2005) and Shen et al. (2010) when two or more substances are mixed, physical blends *versus* chemical interactions are reflected by changes with intensed in the spectra peaks (FT-IR).

The FT-IR data supported the physicochemical, mechanical, and permeable properties data of the seven arrowroot starch films incorporated with glycerol, floral extract and different solutions of cupper sulfate. Thus, it is absolutely concluded that the addition of potential antimicrobial agents (vegetable extract and solutions copper sulfate) discreet disturbed the crystalline systems of films starch and the crystallinity of arrowroot starch is the most vital factor to determine the physicochemical, mechanical and permeable properties of biodegradable films.

Equivalent results are also observed using other polymeric matrices such as chitosan, sweet potato, cassava starch, rice and English potato incorporated with plant extracts and metals mainly (copper chloride or sulfate, potassium sorbate) with antioxidant, antifungal, antiviral and antibacterial properties (Shen et al. 2010; Kanmani and Rhim, 2014).

The neat arrowroot starch films did not exhibit any antibacterial activity against bacteria as expected, but starch films incorporated with floral extract and with solution cupper sulfate had distinctive and potential antibacterial activity. The antibacterial activity of the arrowroot films varied on the floral extract and solutions copper sulfate concentration and the type of bacteria. The solutions copper sulfate, exhibited more pronounced antibacterial activity against Gram-negative bacteria *E. coli* than Gram-positive bacteria *S. aureus*. The same was observed for *S. serovar* Enteritidis Gram-negative which proved to be more sensitive to copper sulfate solutions than when compared to *S. serovar* Thyphymurium Gram-negative.

Different extracts and metals have important antibacterial activity as observed by Kanmani and Rhim (2014) where they found antibacterial activity between 5.66 and 5.23 mm (13.3 and 10.0 µg mL^{-1}) in agar films incorporated with grapefruit seed extract. Shankar et al. (2017) observed in films with copper oxide (CuO) nanocomposite important antibacterial activity on *Listeria monocytogenes* and *E. coli*. The strong antimicrobial activity in this study, and the mechanism of the action against the microorganisms could be associated release of copper ions with in this films incorporating with the sulfate copper solutions. Shankar et al. (2017) presents the same idea in this study with packaging incorporated with CuO. In addition to copper, other noble metals exhibit strong antimicrobial and antifungal activity. Films based on carboxymethyl cellulose with modified zeolite incorporating particles of silver (Ag^{+}) and gold (Au^{+3}) exhibited potential bacteriostatic (*S. aureus* and *E. coli*) and fungistatic (*Candida albicans* and *Aspergillus niger*) effects in the study by Youssef et al. (2019). According by Shankar et al. (2017) and Youseef et al. (2019) different concentrations, the cations of Cu, Ag or Au acts directly on nuclear content of the microorganisms (bacteria and fungal) and present the greatest surface contact area due to the size of the metal particles.

5. Conclusions

The packaging from arrowroot starch (*Maranta arundinacea*) with different concentrations of glycerol, and incorporated with the floral extract of *Tabebuia impetiginosa* and solutions with different concentrations of copper sulfate, has great potential to be applied in the large-scale manufacture of new bioactive packaging. Both the floral extract and the copper sulfate solutions demonstrated increase thickness, decreased solubility, less water vapor transmission, greater light transmission, color change at different doses, and potential antibacterial activity.

In short, the results obtained for the seven biodegradable films, reinforce the need to evaluate these new biopolymers in food use and in the development of tubes for agronomic use during the process of plant germination and can also evaluate the possible incorporation of other chemical elements such as nitrogen (N), phosphorus (P) and potassium (K).

6. Acknowledgements

The authors are grateful to Goiano Federal Institute, Campus Rio Verde, Technological Chemistry and Post Harvest laboratory, Multi-User Analytics Center, FAPEG, CAPES, CNPq and FINEP for their financial support.

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