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Incorporation of Oil From *Hymenaea stigonocarpa* and *Hymenaea courbaril* Into Biofilms Made From Arrowroot Starch: Physicochemical, Biodegradability and Antifungal Activity

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Abstract

Incorporating of fixed oils in biodegradable packaging has an important action on the polymer matrix and biological activities on phytopathogens. This study aimed to evaluate the incorporation of fixed oils from the seeds of *Hymenaea stigonocarpa* and *Hymenaea courbaril* in the arrowroot starch biofilm matrix, evaluating the physicochemical parameters of biodegradability and antifungal activity on *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Aspergillus tubingensis*, *Aspergillus fumigatus*, *Aspergillus niger* and *Rhizopus stolonifer*. Both fixed oils from *Hymenaea* seeds showed biological antioxidant activity in reducing DPPH. Biofilms showed increasing variation in thickness ranging from 0.23-0.43 mm and decreasing moisture content and solubility 15.01-5.14% and 51.07-34.10%, respectively, as oil concentrations increased. The oil concentration also reduced the transparency rate, a considerable variation between the color and biodegradability parameters. However, the biofilms presented a mass reduction of more than 90% for this test. Biofilms still demonstrate considerable antifungal activity for the evaluated phytopathogens. The seed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril* played important roles in developing biopolymer matrices and special biological activity on potential phytopathological agents of fruits and grains.

Keywords: *Colletotrichum acutatum*, *Aspergillus niger*, antioxidant activity, *Rhizopus stolonifer*

1. Introduction

Synthetic packaging is widespread in any industrial segment and even in products of "green" origin, these are produced from petroleum processing presents serious and potential environmental problems due to the extended stay in the mainly marine environment (Nielsen et al., 2020). The degradation process of the different types of plastics used for the storage of agricultural, biological and chemical products is long, exceeding 500 years in a favorable environment for this process. It is also worth mentioning that synthetic polymers used as packaging can be associated with food contamination (Kalpana et al., 2019; Santos et al., 2020).

In addition, the packaging industry increases its production by 8% a year, which negatively impacts accumulation, where 90% of all plastic produced is accumulated in the environment, and only 5% is recycled (Nor Adilah et al., 2018). The microplastics produced from the abrasion process can be inserted in both animal and human food, and studies carried out on the absorption of this material by humans generate an accumulation of 1 kg per year, which is toxic (Nor Adilah et al., 2018; Henry et al., 2019).

Observing from several studies that characterize synthetic packaging as a harmful promoter for man and the environment, thus, the need arose to develop biodegradable packaging comparable to synthetic plastics. Green chemistry is called the area that develops products that combine natural bases such as fats, oils, chitosan, starches, among other biopolymers, as raw materials in the development of sustainable packaging (Gandini et al., 2018). According to Tavassoli-Kafran et al. (2016) and Beikzadeh et al. (2020), their growing application is due to the advantages such as being environmentally friendly, preventing losses of moisture, aromas, colors, gas barrier attributes (O_2, CO, CO_2 and C_2H_4), reduction enzymatic of spoilage, microbial contamination; consequently, they could extend the shelf life of main food products with no side effects by inhibiting the dehydration, browning, and oxidative rancidity.

Furthermore, the incorporation of substances such as fixed oil, essential oil, oil-resin, and plant extracts positively aggregate in a resistant polymeric matrix and have bioactive characteristics in the inhibition of fungal and bacterial agents. Several vegetable oils incorporated in biopolymers have potential activity on many pathological and phytopathological fungicide species (Arruda et al., 2019; Aydogdu et al., 2020).

In agricultural production, mainly of fruits and grains, the community's constant pressure on producers to provide products free of fungal agents that present health problems for both humans and animals. Several *Colletotrichum*, *Aspergillus* and *Rhizopus* genus produce adverse effects every year on the economy from natural products such as fruits, vegetables and grains used in food. Several of these microorganisms during the metabolic rate can produce aflatoxins involved in the death of animals fed on contaminated feed. There are reports that these toxins may be associated with an increase in CD4 cells in carriers of the acquired immunodeficiency virus (HIV) (Ikarugi et al., 2008; Souza et al., 2014; Li et al., 2017; Tahir et al., 2018).

The constant need for studies to evaluate natural products from plants as the main renewable source makes it necessary to evaluate species in the most varied biomes and Cerrado domain (Bueno et al., 2018). Thus, there is a need to study native plant species of this domain such as *Hymenaea stigonocarpa* and *Hymenaea courbaril* popularly known as “jatobá-do-Cerrado and jatobá-do-campo”, both belonging to the Fabaceae family that annually have large fruit harvests. Each *Hymenaea* fruit produces around 5-10 seeds where they present in their cotyledons compounds such as fatty acids (fixed oil) (Pereira et al., 2011; Silva, 2018). This oil can be used in the incorporation of polymeric matrices where they can present important characteristics such as increased impermeability, antioxidant activity, average extended shelf life, visible and ultraviolet light transmission, elasticity and water vapor transfer. It is also noteworthy that both species of *Hymenaea* still have a limited number of studies, especially for the use of seeds used in different industrial processes (Menezes Filho et al., 2020).

In addition, eco-friendly bioactive packaging that features natural preservatives may be better options to overcome health concerns, environmental issues and mitigate losses in agriculture. And for that, arrowroot-based high-performance biodegradable packaging materials are considered a relatively easy and suitable method for developing edible emulsion materials and biodegradable packaging with antioxidant, antifungal and antibacterial biological activities.

This study aimed to evaluate the incorporation of fixed oil from the seed of two species of *Hymenaea stigonocarpa* and *Hymenaea courbaril* and evaluate the characteristics of biofilms regarding structural antifungal characteristics on potential agricultural phytopathological agents.

2. Method

Six hundred (600 g) of *H. stigonocarpa* and *H. courbaril* seeds were collected in two Cerrado areas in Rio Verde, Goiás state, Brazil (17°47′17.6″S and 50°57′55.6″W, 17°42′58.6″S and 50°53′28.6″W) respectively. The specimens were authenticated by M.Sc. Antonio Carlos Pereira de Menezes Filho, and are stored was Herbarium in Vegetable Systematic Laboratory, Biology Departament, Goiano Federal Institute. (HRV No. 14097 and 14098).

Seeds were sprayed with a mixer grinder and dried in an oven at 35 °C for 12 h. Fixed oil was obtained using a Soxlhet type system as described by Elagbar et al. (2016) adapted. The extractant solvent used was *n*-Henaxe. Fifteen (15 g) of processed seed was used in the extraction. The system was refluxed for 8 h. Then, the solvent was evaporated using a rotary evaporator. The oil was collected in amber-colored bottles and stored in the refrigerator until analysis at -12 °C.

Physicochemical properties specific gravity (pycnometer 1 mL) and refractive index (digital refractometer) were determined according to Adegbe et al. (2016). The methodology used for Thin Layer Chromatography (TLC) of *Hymenaea* seed oils followed as proposed by Ferreira et al. (2021). The analyzed samples and standards were

placed on a silica chromatographic plate (Xtra SIL G/UV_{254}). The standards used were oleic acid (Sigma-Aldrich), linoleic acid (Vetec), myristic acid and palmitic acid (Sigma-Aldrich). The distance for the chromatographic run was 10 cm. A binary mixture of *n*-hexane and ethyl acetate (8:2) was used as the mobile phase. The mobile phase and the chromatographic plate were placed inside a glass vat and kept at rest for 20 mL for the chromatographic run. After this period, the chromatoplates were developed with solid iodine vapor (I_2) and ferric chloride, and the R*fs* compared between oil samples and standards.

Oil samples were evaluated using DPPH radical (2,2-Diphenyl-1-pikryl-hydrazyl) for free radical scavenging activity using the method described by Elagbar et al. (2016). Solutions were freshly prepared. DPPH (0.006 g%), different concentration of oil conc. (10-2000 µg mL^{-1}) prepared in *n*-hexane, and Ascorbic acid (10-2000 µg mL^{-1}) were prepared in ethanolic solution (70%) (*v/v*). One mL of DPPH was mixed with either oil (1 mL) or Ascorbic acid (1 mL). These solutions were homogenized at 25 °C for 30 s and kept aside for 30 min in dark room. Using *n*-hexane as blank at Abs 517 nm the instrument UV-*Vis* spectrophotometer was set at zero. The free radical scavenging activity of residual DPPH against the blank was determined at Abs 517 nm using the following Equation 1.

$$DPPH\ scavenging\ activity\ (\%) = (1 - AbsC/AbsS) \times 100 \quad (1)$$

Where, *AbsC* = Absorbance control; *AbsS* = Absorbance sample.

All the experiments were conducted in quadruplicate. The values of the calculated inhibition concentration (IC_{50}) µg mL^{-1}.

Biodegradable films were obtained by the "casting" method, agreeing with the methodology proposed by Valadares et al. (2020) adapted. In order to obtain biofilms, 5 g commercial arrowroot starch *M. arundinacea* was dissolved in 100 mL distilled water. The mixture was moderately agitated at room temperature (25 °C) for 5 minutes. Afterwards, this solution was heated at 70 °C under constant agitation for 30 minutes. After starch gelatinization, glycerol was added conc. 30% (*w/v*). This dispersion was then agitated for 5 minutes. When the filmogenic solution reached 30 °C, a previously prepared suspension of fixed oil from *H. stigonocarpa* or *H. courbaril* in Tween 40 conc. 0.25 (g/g fixed oil) was incorporated into it under constant agitation for 15 minutes. Final concentrations of seed fixed oil were 0.25%, 0.50%, 0.75% and 1% (*v/v*), besides a control treatment with no fixed oil. Filmogenic solutions made from arrowroot starch into which fixed oil was incorporated were poured on a polyethylene plate and dried in an oven with air circulation at 35 °C for about 48 hours.

A digital caliper measured biofilm thickness. Measurements were carried out in ten spots on every biofilm, and the thickness mean was calculated by according Santos et al. (2021). The moisture content was obtained in an oven at 105 °C for 4 h. Four replicates per film treatment were used, in agreement with the methodology described by Rambabu et al. (2019) adapted. Measurement of water solubility was performed as described by Santos et al. (2020) and proposed by Jahed et al. (2017). Biofilms which measured about 2 cm^2 were dried in an oven at 105 °C for 4 hours and then weighed so that initial mass (*Mi*) could be determined. They were immersed in 50 mL distilled water and kept under constant agitation at 25 °C for 24 hours. Afterwards, solutions with the films were filtered through filter paper which had been previously weighed. Sheets of filter papers with films were dried at 105 °C for 24 hours and weighed so that final mass (*Mf*). The analysis was analyzed in triplicate. Biofilm solubility (%) was calculated by Equation 2.

$$Water\ solubility\ assay\ (\%) = (Mi - Mf/Mi) \times 100 \quad (2)$$

Ultraviolet and visible light transmittance of biofilms was conducted by UV-*Vis* spectrophotometer. Biofilm samples were cut and placed in cuvettes so that transmittance could be measured over a wavelength range between 850 and 200 nm (Hosseini et al., 2015) adapted. The FT-IR data were obtained in the range of 600-4000 cm-1, with 60 scans and a resolution of 4 cm-1, equipped with a diamond attenuated total reflectance (ATR) accessory. Data were evaluated using Microsoft Excel. Analysis of biofilm color was carried out by a colorimeter. Parameters under evaluation were L* (luminosity) and chromaticity parameters [(+60/-60) a* and (+60/-60) b*]. Measurements were conducted on five randomly selected film spots (Valadares et al., 2020).

The analysis of biodegradability was carried out by according Martucci and Ruseckaite (2009) adapted. Biofilm samples (2 × 2 cm^2) were dried up to constant weight so that initial mass (*Mi*). Samples were then placed in open high density polyethylene packages to enable microorganisms and moisture to gain access to them. After that, they were buried *in natural* soil, which had been previously prepared, at constant moisture and room temperature (70% R.U and 25 °C). Five, ten, fifteen, twenty, twenty-five and thirty days after the experiment installment, the packages with the samples were removed from the soil, washed with distilled water and dried up to constant weight (*Mf*). The percentage biodegradability was calculated by Equation 3.

$$Biodegradability\ (\%) = (Mf - Mi/Mi) \times 100 \quad (3)$$

The morphology of the biofilms was evaluated under high-resolution optical microscopy. A (2 × 2 cm^2) film sample was adhered to a microscope slide and analyzed at different magnifications of 4, 10, 40 and 100 X in an optical microscope with an attached camera. Micrographs of the biofilm surface area were analyzed in ImageJ software in 3D pixel stacking analysis.

The antifungal activity of biofilms was analyzed against the phytopathogenic fungi *Colletotrichum acutatum* (BW-101), *Colletotrichum gloeosporioides* (BW-102), *Aspergillus tubingensis* (N2A), *Aspergillus fumigatus* (V1G), *Aspergillus niger* (VIF) and *Rhizopus stolonifer* (BW-116) belonging to the mycological bank of the Technological Chemistry laboratory of the Goiano Federal Institute, Goiás State, Brazil, by a diffusion test on disk described by Ma et al. (2016). *Petri* dishes, half full with medium potato dextrose agar (PDA), were inoculated with 100 µL suspension with 1 × 10^8 CFU mL^{-1} with 0.5 McFarland scale in UV-*Vis* spectrophotometer. Then, three samples of biofilms which had been cut in circles with about 7 mm in diameter were placed on every dish. Dishes were incubated at 26 °C for 10 days (Weir et al., 2012; Damm et al., 2012). Finally, diameters of the zone of inhibition (mm) were measured with digital caliper. As standard fungicide Frowncide 500 SC concentration 10 µL mL^{-1} was used (*C. gloeosporioides* and *C. acutatum*), Anphotericin B 100 MCG (*A. tubingensis*, *A. fumigatus* and *A. niger*) and Botector® 50 µL mL^{-1} Westbridge.

Analyses were carried out in quadruplicate±SD were calculated. The data was statistically analysed by ANOVA and means were compared by the Duncan multiple range test significance with the use of the IBM SPSS Statistics 26 software program. The *P* level of < 5% was supposed to be significant in determining the variations among mean values of biofilm aspects.

3. Results and Discussion

The physicochemical and antioxidant properties of the analyzed *Hymenaea* oils presented for specific gravity g mL^{-1} (20 °C) 0.9274±0.04a and 0.9280±0.06a, refractive index (25 °C) 1.44718±0.01a and 1.46237±0.03b, *H. stigonocarpa* and *H. courbaril*, respectively. According by Dias et al. (2013) the refractive index is mainly related to the saturation degreeand the ratio of fatty acids *Cis* and *Trans* double bonds, besides in isinfluenced by oxidative processes. In the analyzed oils of *H. courbaril* by Dias et al. (2013), the refractive indices at 40 ºC were 1.4653 for the pulp and 1.4655 for the seeds.

In both oil samples it was verified the presence of R*fs* close to the standards for oleic, linoleic and palmitic acid (*H. stigonocarpa* and *H. courbaril*), and myristic (*H. courbaril*). The TLC method showed good separation results for the evaluated fatty acids. Several retention spots were observed on chromatoplates containing *Hymenaea* oil on I_2 vapor and UV_{365} nm light, suggesting the presence of other fatty acid groups. Ferric chloride developer revealed a blue spot at 33 mm which suggests the presence of the hydrolyzable or gallic tannin group in *H. courbaril*. This result corroborates the study by Dias et al. (2013) where they evaluated total phenolic compounds in *H. courbaril* seed oil with a result of 3.43 mg GAE 100 g^{-1}.

Antioxidant activity IC_{50} = 2.19±0.06a µg mL^{-1} (Ascorbic acid), IC_{50} = 398.17±1.08c and IC_{50} = 211.30±1.96b µg mL^{-1}, *H. stigonocarpa* and *H. Courbaril*, respectively. Observed that there is no significant difference by the Duncan (p < 5%) for specific gravity, however, there is a statistical difference for the refractive index assay and in the DPPH free radical reduction for both samples and the standard antioxidant. Fixed oils extracted not only from seeds, they have potential antioxidant agents. Dias et al. (2013) found for the pulp and seeds of *H. courbaril* moderate DPPH free radical reduction efficiency of 22.19% (IC_{50} = 49.04 g/g) and 83.49% (IC_{50} = 48.56 g/g) respectively. This anti-free radical activity has potential activity incorporated in polymeric matrices capable of reducing the deleterious effects on food (Santos et al., 2020).

The incorporation of fixed oils of *H. stigonocarpa* and *H. courbaril* into the arrowroot biopolymer matrix showed significant effects when compared to the control on thickness, moisture and solubility (Table 1). These effects are due to the high concentration of amylose found in arrowroot starch (Thakur et al., 2019; Santos et al., 2020). Thickness analysis showed that the biofilms varied in terms of thickness according to Duncan's test (p < 5%) noting that the biofilm incorporated with 1% of *H. stigonocarpa* oil had a higher thickness than the others. Aydogdu et al. (2020) found thinner thicknesses between 0.11-0.18 mm for guar gum as a polymer matrix incorporated with 1% and 2% of orange oil, emphasizing that the polymer and the oil concentration considerably influence this characteristic.

According by Husseini et al. (2015) the biofilms is a crucial parameter on mechanical properties and water vapor permeability values. These results indicated that the addition of *Hymenaea* oils altered the thickness and microstructure of the films.

In the study of Kadzińska et al. (2020) the moisture content of the researched biofilms ranked from 15.49-19.18% for the biofilms with coconut oil and rapeseed oil, respectively, with sodium alginate polymer matrix. In the study by Niknam et al. (2019) the researchers obtained a thickness of biodegradable films inferior to that of this study, ranging from 0.12-0.19 mm incorporated in different oilseed oils (olive, canola and maize). It is noteworthy that the polymer matrix differs from this study, where each biopolymer has a unique behavior. According by Peres-Mateos et al. (2009) and Niknam et al. (2019) addition of fixed oil to the biofilm matrix may lead to the replacement of strong polymer-polymer interactions with weak polymer-oil interactions and thus increasing the biofilm volume which in turn cause to increasing of the biofilm thickness.

As for the moisture content, biofilms are directly dependent on the oil concentration in both *Hymenaea* species. Although there are no standards for moisture content in biofilms in organs regulatory bodies of food products, the packages obtained in this study had similar moisture percentages observed in other studies (Niknam et al., 2019) and were considered low. Biodegradable packaging with starch-based polymers, gelatine, gum and chitosan have different moisture content, this is observed in the study by Niknam et al. (2019) where researchers found moisture content ranging from 22.46-15.05%. Galus et al. (2016) obtained higher moisture content than in this study evaluating whey protein biofilms incorporated with rapeseed oil between 16.8-17.9%.

Solubility is an important property of edible biofilms as they are used as protective layers on food. In its various uses, potential applications may require water insolubility for harmonic interactions between product integrity and water resistance (Hosseini et al., 2015), with this it is observed that, a variation between solubility results between the control biofilm and other concentrations of both oils. Biofilms incorporated with higher concentration of 1% oil did not show significant difference according to statistical analysis, although it is observed that FOHs and FOHc 1% had the lowest solubility. Similar results were obtained in the study by Galus et al. (2016) evaluating whey protein biofilms incorporated with rapeseed oil between 37.4-42.4%.

Table 1. Thickness, moisture and solubility of arrowroot biofilms incorporating fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*

Biofilm	Thickness (mm)	Moisture (%)	Solubility (%)
Control	0.23±0.01f	15.01±0.59a	51.07±2.26a
FOHs 0.25%	0.25±0.01ef	12.35±0.92c	49,35±1.57ab
FOHs 0.50%	0.28±0,01d	10.55±0.56d	46.88±1.29b
FOHs 0.75%	0.40 ±0.02b	8.21±0.91e	39.34±1.84c
FOHs 1%	0.43±0.01a	5.14±0.60f	35.35±2.10d
FOHc 0.25%	0.24±0.01f	13.73±0.50b	49.93±1.92a
FOHc 0.50%	0.26±0.01e	11.65±0.49c	46.86±1.76b
FOHc 0.75%	0.29±0.02d	8.84±0.32e	40.74±1.40c
FOHc 1%	0.32±0.03c	5.69±0.43f	34.10±2.18d

Note. Different letters in a column show significant difference ($p < 5\%$) Duncan's test. FOHs: Fixed oil *Hymenaea stigonocarpa*. FOHc: Fixed oil *Hymenaea courbaril*.

Regarding the colors of developing biofilms (Figure 1, A-B), there is a decrease in the light transmittance rates of biofilms that incorporate different doses of fixed oil of *H. stigonocarpa* and *H. courbaril* in the visible region (850 at 250nm). Light transmission rates were higher at lower concentrations when compared to the control. Concentrations between 0.75-1% showed low transparency rates, especially for biofilms incorporated with oil of *H. stigonocarpa* due to the opacity promoted by the emulsion incorporated with large volumes of oil. The according Pereda et al. (2012) and Galus and Kadzińska (2016) the transparency of emulsion-based biofilms is related to their internal structure, which is affected by the fixed and essential oil and droplet size distribution in biofilm-forming emulsions and its rearrangement during drying. Galos and Kadzińska (2016) also emphasizes the solvent termical evaporation during drying induces changes in the emulsion structure by destabilization phenomena such as creaming and aggregation, which have important role in the visual and optical properties of emulsion-based biofilms.

According to Santos et al. (2020), Rambabu et al. (2019) and Romani et al. (2018), the color is an important parameter to be evaluated, as it directly influences the acceptance of the product by consumers. Biofilms used as packaging generally have a high transparency rate so that the product can be seen by the consumer as fruits, vegetables and legumes. However, opaque or colored biofilms offer potential protection, especially to foods

exposed to visible and UV light, especially foods with high fat content, such as meat products, thus preventing them from suffering oxidative degradation of fats and proteins.

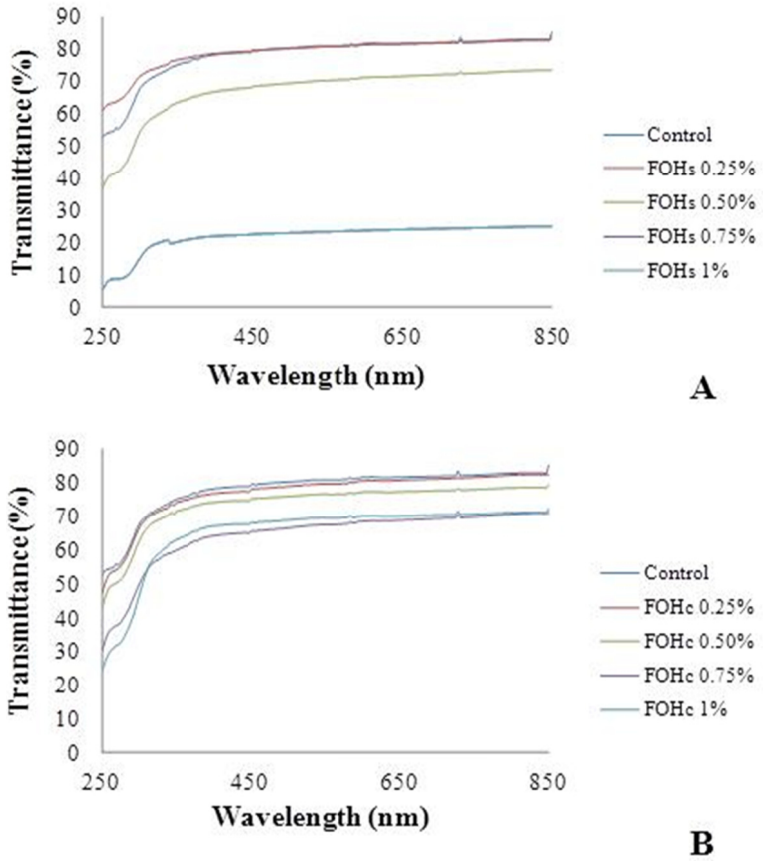

Figure 1. UV-*Vis* light transmittance rate in arrowroot biofilms incorporating different doses of fixed oil *Hymenaea stigonocarpa* (A) and *Hymenaea courbaril* (B)

Biofilms were analyzed in agreement with color parameters L*, a* and b*, were L* is the biofilm luminosity (100 light/0 dark), a* is the red/green coordinate (+/-); and b* is the yellow/blue coordinate (+/-) (Table 2).

The values of L* in this study showed statistical difference in all samples, demonstrating that the arrowroot starch-based polymer has low luminosity. This was also verified by Santos et al. (2020) where they developed arrowroot starch-based biofilms obtaining maximum for L* = 15.51. Comparing with other natural polymers, Galos and Kadzińska (2016) found L* between 89.5-90.8 for whey protein biofilms incorporated with rapeseed oil. In the study by Kadzińska et al. (2020) describe for pure sodium alginate biofilm high transparency with a high L* value of 93.69.

For a* chromaticity, all packages showed a tendency towards red and for b* a tendency towards blue was verified, except for FOHs 1% and FOHc 1% with a tendency to yellow. As discussed, polymers have different colorations, although what is incorporated into the matrix influences this color characteristic considerably. Thus, this ability to incorporate substances in different matrices is observed, as in the study by Galos and Kadzińska (2016) where the researchers found values of a* with a tendency to green (0.81 to 0.95) and b* to blue (-0.1 to -0.7).

Table 2. Measurements of biofilm colors incorporating fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*

Biofilm	L*	a*	b*
Control	14.59±0.24h	-0.71±0.09e	-0.91±0.05e
FOHs 0.25%	16.43±0.07g	-0.70±0.05e	-0.93±0.02e
FOHs 0.50%	18.21±0.48e	-0.71±0.05e	-1,26±0.08f
FOHs 0.75%	17.65±0.16f	-0.57±0.03d	-0.96±0.04e
FOHs 1%	30.29±0.12b	-0.54±0.01d	0.40±0.01a
FOHc 0.25%	26.13±0.55c	-0.24±0.01a	-0.50±0.04d
FOHc 0.50%	31.35±0.03a	-0.32±0.01b	-0.23±0.10c
FOHc 0.75%	22.89±0.01d	-0.39±0.04b	-0.56±0.05d
FOHc 1%	30.24±0.01b	-0.47±0.02c	0.23±0.06b

Note. Different letters in a column show significant difference ($p < 5\%$) Duncan's test. Parameters CIELab of color L* (luminosity), a* and b* (chromaticity). (±) mean standard deviation. FOHs: Fixed oil of *Hymenaea stigonocarpa*. FOHc: Fixed oil of *Hymenaea courbaril*.

Biodegradation can be designated as degradation occurring in a in nature biological environment, where microorganisms (fungi and bacterial), moisture (RU%) and enzymes are responsible by degrading biopolymers (Fernandes et al., 2020). The biodegradability test (Figure 2), evaluates how much in percentage the biopolymers incorporated with varying concentrations of *H. stigonocarpa* and *H. courbaril* oil influence over time in the soil. It is observed that the lowest oil concentrations (0.50%) for both species statistically presented biodegradability results close to the standard. Concentrations between 0.75 and 1% for *H. stigonocarpa* showed statistically similar degradation effects. All concentrations except 1% had a biodegradability rate greater than 90% for this study.

After the 25-day analysis, the hollow polyethylene packages that contained arrowroot starch films were removed from the soil. Thus, the conclusion may be the fact that incorporation of fixed oil into films does not decrease biodegradability of arrowroot starch, which may be considered a promising material for biodegradable packaging (Santos et al., 2020). Several studies show biodegradability time longer than 30 days (Seligra et al., 2016), Fernandes et al. (2020) obtained a time greater than 40 days in the soil biodegradation test for chia mucilage biofilm incorporated with chia oil. Arancibia et al. (2014) evaluated the biodegradability in topsoil for biofilms of a mixture of soy protein lignin with citronella essential oil, where they obtained a rate of reduction in the mass of the biopolymer over six months. It is suggested that the degradation period is influenced by the type of polymer, incorporation, moisture and degree of crosslinking, this is also suggested by Arancibia et al. (2014) and González et al. (2011). Where a low degree of crosslinking makes biopolymers more degradable in a shorter period.

As reported by Pantini and Sorrentino (2013) denser and more crystalline structures are expected to have a slower degradation rate compared to amorphous structures once it affects the water diffusion into de biofilm structure. The rapid degradation of the films in this study demonstrates this thesis proposed by the researchers, suggesting that the crystalline structure of arrowroot starch biofilms are less dense and easier to degrade in an environment with uncontrolled climatic effects.

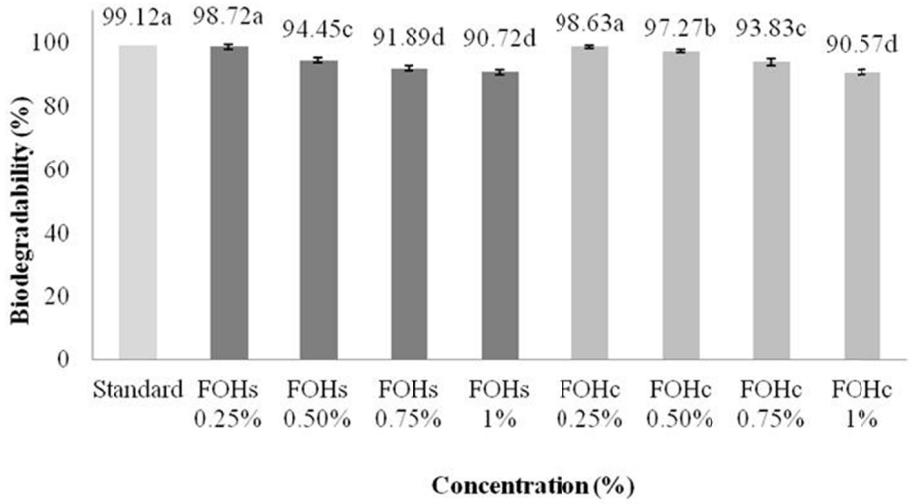

Figure 2. Biodegradability assay of arrowroot biofilms incorporated with fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*. Different letters in the bars show significant difference ($p < 5\%$) Duncan's test

Arrowroot starch biofilms incorporated with oils of *H. stigonocarpa* and *H. courbaril* at concentrations 0.25-0.50% showed homogeneous surface area (Figure 3, B-C, K-L) 3D imaging through pixel stacking provided a color image over the surface area and its relief, where the red color represents a homogeneous plateau (Figure 3, G-H, P-Q). And green color presents a shapeless sinuous plateau. It is suggested that this characteristic may be involved with the drying process, emulsion and molecular conjugation between the biopolymer and fixed oil.

Embedded biofilms with concentrations of 0.75-1% showed bubbles and small cracks for both fixed lobes of *Hymenaea* (Figure 3, D-E, M-N). As for the 3D imaging, a deep heterogeneous plateau can be seen in (Figure 3, I), the same is observed in (Figure 3, R). Arrowroot polymer showed homogeneity in all samples when compared to concentrations (Figure 3, A-F). 3D imaging presents a satisfactory attribute in the morphological analysis of biofilms, as also stated by Menezes Filho et al. (2019) where they evaluated this imaging technique in watermelon biofilm.

This means that most formulations were successful in forming biofilms that were not too sticky or brittle (Figure 3). Guar gum biofilms incorporated with orange oil and glycerol as plasticizer also showed similar characteristics to the treatments in this study, although Aydogdu et al. (2020) have not reported the formation of bubbles. Guar gum biofilms incorporated with orange oil and glycerol as plasticizer also showed similar characteristics to the treatments in this study, although Aydogdu et al. (2020) have not reported the formation of bubbles. This study suggests that mechanical stirring by Vortex at the time of oil addition may be the cause of bubbles, however, future studies should be carried out evaluating an alternative to substitute for concentrations above 0.75%.

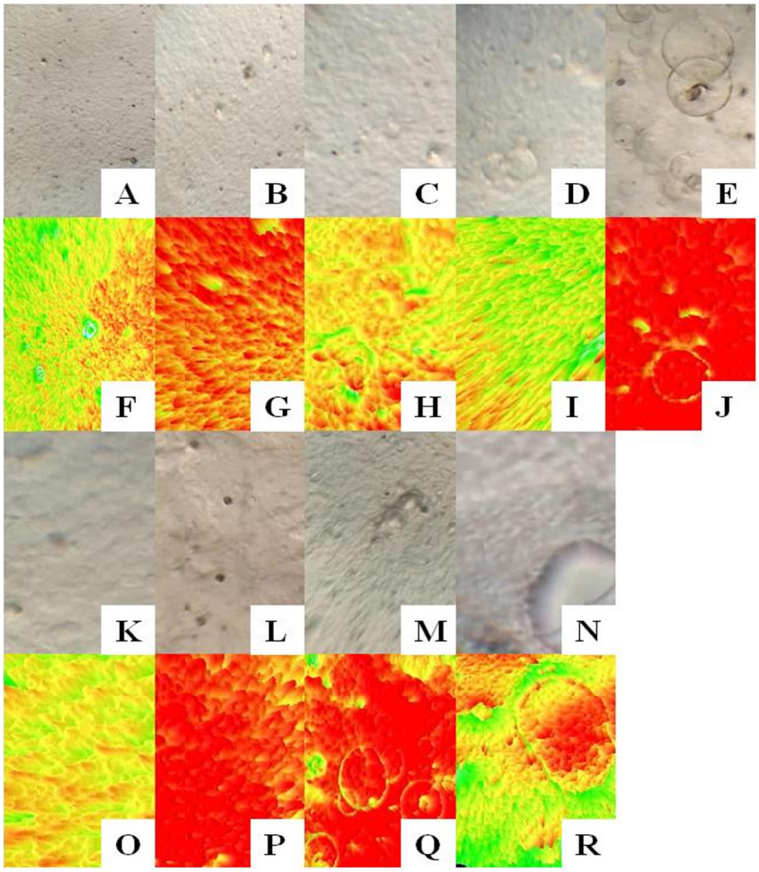

Figure 3. Micrographs and 3-D surface area imaging analysis of arrowroot starch biofilms incorporated with fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*. (A-F) Control biofilm. (B-G) FOHs 0.25%. (C-H) FOHs 0.50%. (D-I) FOHs 0.75%. (E-J) FOHs 1%. (K-O) FOHc 0.25%. (L-P) FOHc 0.50%. (M-Q) FOHc 0.75%. (N-R) FOHc 1%

According Aydogdu et al. (2020) FTIR analyses permit the investigation of particular interactions between functional groups of the chains and can be recognized as shifts in the chain's and vibration IR bands. FTIR spectra of the films are given in (Figure 4).

The spectra of the control and biofilms fixed oil concentration, showed similar behavior with elongation bands of OH and N–H flexion at 3329 and 3315 cm^{-1}, C=O stretching at 1662 and 1650 cm^{-1} (amide I), C–N and N–H vibration at 1377 and 1369 cm^{-1} (amide III), and C–H stretching band at 2925 and 2924 cm^{-1}. The bands located between the 800 and 1150 cm^{-1} region are related to C–C bonds (864 and 858, and 998 and 996 cm^{-1}) and C–O (at 1050 and 1151 cm^{-1} corresponds to the C–O bond at C_1 and C_3 and at 1111 and 1114 cm^{-1} is the C–O bond in C_2) of glycerol, respectively of biofilms incorporated FOHs and FOHc. At 2362 cm^{-1}, in Figure 3 (A), a strong and narrow band is observed, suggesting the presence of (alkyne) C≡C for the biofilm with 1% FOHs (Aydogdu et al., 2020; Khah et al., 2021; Hejazi et al., 2021). There were no significant changes in the characteristic peaks of oils in the biofilm matrix. In addition, the reduction in the intensity of the characteristic bands of glycerol and a slight displacement of the bands at 1050 and 1110 cm^{-1} suggest interaction between glycerol and biopolymer (Aydogdu et al., 2020).

Gelatin-pectin biofilms incorporated with virgin olive oil and grape seed oil analyzed by Khah et al. (2021) presented similar results for most functional groups characteristic of natural polymers incorporated with fatty acids obtained from vegetables. Aydogdu et al. (2020) also did not observe changes in functional groups in guar gum biofilms incorporated with orange oil, similar to this study. The researchers add that the low concentration of oil used may be related to this characteristic, where no extra band is due to the lack of covalent bonds between the oil of *Hymenaea*, with the matrix and the plasticizer glycerol.

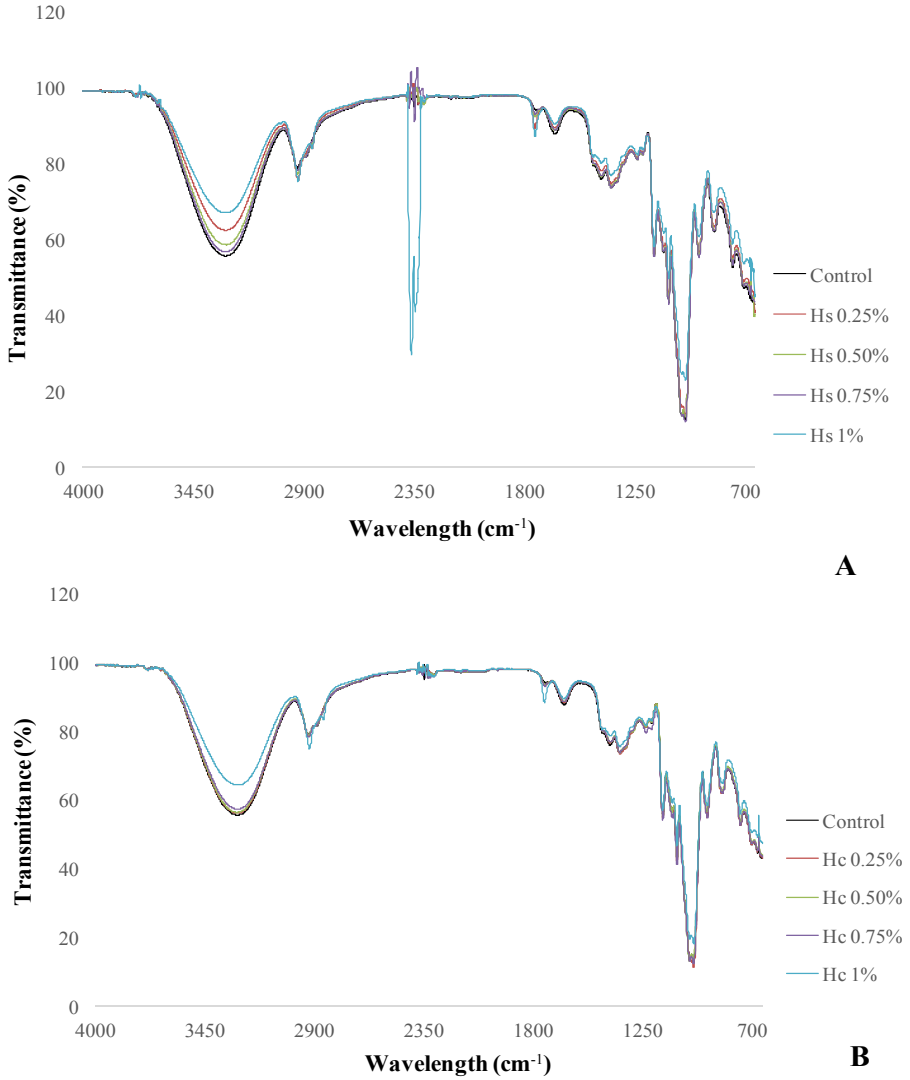

Figure 4. FTIR-ATR spectra of the arrowroot biolfims incorporated with fixed oil *Hymenaea stigonocarpa* (A) and *Hymenaea courbaril* (B)

Biofilms incorporated with concentrations above 0.50% showed antifungal activity on all evaluated phytopathogens. However, the 1% concentration proved to be more effective inhibiting the development of fungi, especially for *C. acutatum*, *C. gloeosporioides*, *A. niger* and *R. stolonifer* (Table 3) for FOHs and FOHc. When compared to standard antifungals, the Duncan test ($p < 5\%$) demonstrates a statistical difference between concentrations and standards. It is noteworthy that the antifungals Frowncide 500 SC and Amphotericin B are synthetic molecules and the Botector is a conjugation of microorganisms, which is a biological antifungal.

Control biofilm exhibited no antimicrobial activity, as expected. It is suggested that the different inhibition activities on the evaluated fungi are involved in the structural characteristics of the cell wall. Arruda et al. (2019) also suggests such a statement because the complex structure of this wall is constituted by polysaccharides, linked or not to proteins or lipids, polyphosphates and inorganic ions. Although the results of this study demonstrate that the use of both oils from *Hymenaea* species are promising for the alternative control of these phytopathogens when used in biodegradable packaging.

The study with fixed oils incorporated in biopolymeric matrices is scarce in the literature. Although there are studies evaluating the lipid fraction extracted from numerous vegetables on different forms of pathogenic and phytopathogenic fungi. Aydogdu et al. (2020) found potential antibacterial activity against *Escherichia coli* and lower inhibition activity against *Bacillus subtilis* in conc. 1%, and higher activity in conc. 2%.

Arruda et al. (2019) did not find antifungal activity on *Rhizoctonia solani* and *Sclerotium rolfsii* evaluating the fixed oil of *Jatropha curcas* seed. Ali et al. (2017) developed nanoemulsions incorporated with neem and

citronella oils where they found important phytopathogenic activity on *R. solani* and *S. rolfsii*. Passos et al. (2002) report in a study using *Caryocar brasiliense* seed and almond oil high antifungal action on *Cryptococcus neoformans* in conc. 15.6 μg mL^{-1}, where the seed oil showed 21.1% inhibition, and 62.5 μg mL^{-1} with 10.5% for the almond oil.

Table 3. Antifungal activity of biofilms incorporated with fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril* at different concentrations on phytopathological microorganisms

Microorganisms	Inhibition Zone (mm) Concentrations (%) FOHs					Standard antifungal
	Control	0.25%	0.50%	0.75%	1%	
C. acutatum	0.00±0.00d	0.00±0.00d	0.00±0.00d	6.34±0.09c	8.17±0.08b	100±0.00a
C. gloeosporioides	0.00±0.00d	0.00±0.00d	0.00±0.00d	8.53±0.04c	10.85±0.09b	100±0.00a
A. tubingensis	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	6.57±0.43b	100±0.00a
A. fumigatus	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	6.22±0.29b	100±0.00a
A. niger	0.00±0.00d	0.00±0.00d	0.00±0.00d	7.89±0.98c	13.28±0.99b	100±0.00a
R. stolonifer	0.00±0.00e	0.00±0.00e	7.62±0.52d	9.39±0.68c	14.42±0.86b	100±0.00a
Microorganisms	Inhibition Zone (mm) Concentration (%) FOHc					Standard antifungal
	Control	0.25%	0.50%	0.75%	1%	
C. acutatum	0.00±0.00d	0.00±0.00d	0.00±0.00d	6.15±0.08c	10.10±0.07b	100±0.00a
C. gloeosporioides	0.00±0.00c	0.00±0.00c	0.00±0.00c	4.63±0.09b	5.47±0.07b	100±0.00a
A. tubingensis	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	100±0.00a
A. fumigatus	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	7.41±0.08b	100±0.00a
A. niger	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	9.77±0.04b	100±0.00a
R. stolonifer	0.00±0.00d	0.00±0.00d	6.15±0.07c	7.42±0.09c	11.07±0.09b	100±0.00a

Note. Different letters in a line show significant difference ($p < 5\%$) Duncan's test.

4. Conclusion

This study revealed that fixed oil from the seed of *Hymenaea stigonocarpa* and *Hymenaea courbaril* have great potential for application in the manufacture of bioactive packaging. Both oils have antioxidant activity. The presence of oleic, linoleic, palmitic and myristic acids was observed in the samples. Physicochemical observations related to the incorporation of fixed oil of *H. stigonocarpa* and *H. courbaril* into arrowroot biofilms led to increase in thickness, decrease in moisture, solubility em transparency, and change in color as its concentrations increased.

Biofilms also showed variation according to the increase in oil concentration over the percentage of biodegradability, in the structural morphology of the surface area with bubbles at concentrations above 0.75%, functional groups are in accordance with expectations, and antifungal activity was also observed on *Colletotrichum*, *Aspergillus* and *Rhizopus*.

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