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AMAZONIAN OILS AS SUSTAINABLE AGENTS IN GREEN HYDROGELS FOR BIOMEDICAL APPLICATIONS

ÓLEOS AMAZÔNICOS COMO AGENTES SUSTENTÁVEIS EM HIDROGÉIS VERDES PARA APLICAÇÕES BIOMÉDICAS

ACEITES AMAZÓNICOS COMO AGENTES SOSTENIBLES EN HIDROGELES VERDES PARA APLICACIONES BIOMÉDICAS

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Dener Ferreira Horta Fialho[1], Guilherme Lucas Silva Pereira[2], Morgana Louise de Souza[3], Isadora Moreira Borges[4], Sariny Baeta Santini[5], Renata Carolina Zanetti Lofrano[6]

ABSTRACT

The demand for sustainable and biofunctional materials has driven the development of green hydrogels based on biopolymers and Amazonian bioactives. This study explores the incorporation of four Amazonian oils — Copaiba (Copaifera spp.), Murumuru (Astrocaryum murumuru), Brazil nut (Bertholletia excelsa), and Buriti (Mauritia flexuosa) — into alginate-based hydrogels for biomedical applications. Films were prepared using sodium alginate (2 %), glycerin (2 %), and sodium dodecyl sulfate (0.1 %), with calcium chloride ($CaCl_2$) crosslinking to enhance mechanical resistance and stability. Oils were added as emulsified phases. Resulting films were analyzed for visual appearance, thickness, solubility, and swelling. All oil-based films were homogeneous, transparent, and flexible. The control film showed 0.286 mm thickness, 45.71 % solubility, and 1601.13 % swelling. Murumuru oil produced the thinnest film (0.153 mm), increased solubility (62.72 %), and maintained high swelling (1531.10 %). Brazil nut oil yielded the thickest film (0.311 mm), with 54.05 % solubility and reduced swelling (901.10 %). Copaiba oil showed the lowest swelling (644.45 %), moderate thickness (0.230 mm), and solubility (57.86 %). Buriti oil had the lowest solubility (41.42 %), moderate thickness (0.230 mm),

[1] Undergraduate in Chemical Engineering.Universidade Federal de São João del-Rei.
E-mail: denerhorta@gmail.com Orcid: 0009-0005-6462-7018
Lattes: http://lattes.cnpq.br/0034147808276729
[2] Undergraduate in Chemical Engineering.Universidade Federal de São João del-Rei.
E-mail: guilsp55@gmail.com Orcid: 0009-0006-8646-1036
Lattes: http://lattes.cnpq.br/4886548650022299
[3] Undergraduate in Chemical Engineering.Universidade Federal de São João del-Rei.
E-mail: momorgana01@gmail.com Orcid: 0009-0005-8926-6108
Lattes: http://lattes.cnpq.br/8906739362518090
[4] Undergraduate in Chemical Engineering.Universidade Federal de São João del-Rei.
E-mail: isadora.moreira98@gmail.com Orcid: 0009-0002-1328-8800
Lattes: http://lattes.cnpq.br/7229773389130430
[5] Undergraduate in Chemical Engineering.Universidade Federal de São João del-Rei.
E-mail: sarinybaetasatini@gmail.com Orcid: 0009-0000-9668-2939
Lattes: https://lattes.cnpq.br/0201630029320581
[6] Professor. Universidade Federal de São João del-Rei.
E-mail: renataczlofrano@ufsj.edu.br Orcid: 0000-0002-5688-3936
Lattes: http://lattes.cnpq.br/5561482457720983

and swelling of 1204.53 %. Each oil induced distinct effects on film properties. Copaiba and buriti oils reduced swelling and solubility, suggesting suitability for moist environments like wound sites. Murumuru oil enhanced plasticity, favoring applications needing thinner, softer films. The results highlight the potential of Amazonian oils as natural functional agents for tailoring hydrogel properties and advancing sustainable, high-value biomedical materials.

Keywords: Biopolymers. Copaiba Oil (*Copaifera Spp*.). Murumuru Oil (*Astrocaryum Murumuru*). Brazil Nut Oil (*Bertholletia Excelsa*). Buriti Oil (*Mauritia Flexuosa*).

RESUMO

A demanda por materiais sustentáveis e biofuncionais tem impulsionado o desenvolvimento de hidrogéis verdes baseados em biopolímeros e bioativos amazônicos. Este estudo explora a incorporação de quatro óleos amazônicos — copaíba (Copaifera spp.), murumuru (Astrocaryum murumuru), castanha-do-Brasil (Bertholletia excelsa) e buriti (Mauritia flexuosa) — em hidrogéis à base de alginato para aplicações biomédicas. Os filmes foram preparados utilizando alginato de sódio (2%), glicerina (2%) e dodecil sulfato de sódio (0,1%), com reticulação por cloreto de cálcio ($CaCl_2$) para aumentar a resistência mecânica e a estabilidade. Os óleos foram adicionados como fases emulsificadas. Os filmes resultantes foram analisados quanto ao aspecto visual, espessura, solubilidade e intumescimento. Todos os filmes contendo óleos apresentaram-se homogêneos, transparentes e flexíveis. O filme controle apresentou espessura de 0,286 mm, solubilidade de 45,71% e intumescimento de 1601,13%. O óleo de murumuru produziu o filme mais fino (0,153 mm), aumentou a solubilidade (62,72%) e manteve elevado intumescimento (1531,10%). O óleo de castanha-do-Brasil resultou no filme mais espesso (0,311 mm), com solubilidade de 54,05% e redução do intumescimento (901,10%). O óleo de copaíba apresentou o menor intumescimento (644,45%), espessura moderada (0,230 mm) e solubilidade de 57,86%. O óleo de buriti apresentou a menor solubilidade (41,42%), espessura moderada (0,230 mm) e intumescimento de 1204,53%. Cada óleo induziu efeitos distintos nas propriedades dos filmes. Os óleos de copaíba e buriti reduziram o intumescimento e a solubilidade, sugerindo adequação para ambientes úmidos, como sítios de feridas. O óleo de murumuru aumentou a plasticidade, favorecendo aplicações que requerem filmes mais finos e macios. Os resultados destacam o potencial dos óleos amazônicos como agentes funcionais naturais para o ajuste das propriedades de hidrogéis e o avanço de materiais biomédicos sustentáveis e de alto valor agregado.

Palavras-chave: Biopolímeros. Óleo de Copaíba (Copaifera spp.). Óleo de Murumuru (Astrocaryum murumuru). Óleo de Castanha-do-Brasil (Bertholletia excelsa). Óleo de Buriti (Mauritia flexuosa).

RESUMEN

La demanda de materiales sostenibles y biofuncionales ha impulsado el desarrollo de hidrogeles verdes basados en biopolímeros y bioactivos amazónicos. Este estudio explora la incorporación de cuatro aceites amazónicos — copaíba (Copaifera spp.), murumuru (Astrocaryum murumuru), nuez de Brasil (Bertholletia excelsa) y buritÍ (Mauritia flexuosa) — en hidrogeles a base de alginato para aplicaciones biomédicas. Las películas se prepararon utilizando alginato de sodio (2%), glicerina (2%) y dodecil sulfato de sodio (0,1%), con reticulación mediante cloruro de calcio ($CaCl_2$) para mejorar la resistencia mecánica y la estabilidad. Los aceites se añadieron como fases

emulsificadas. Las películas resultantes se analizaron en cuanto a apariencia visual, espesor, solubilidad y hinchamiento. Todas las películas con aceites fueron homogéneas, transparentes y flexibles. La película control presentó un espesor de 0,286 mm, solubilidad del 45,71% e hinchamiento del 1601,13%. El aceite de murumuru produjo la película más delgada (0,153 mm), incrementó la solubilidad (62,72%) y mantuvo un alto hinchamiento (1531,10%). El aceite de nuez de Brasil generó la película más gruesa (0,311 mm), con solubilidad del 54,05% y reducción del hinchamiento (901,10%). El aceite de copaíba mostró el menor hinchamiento (644,45%), espesor moderado (0,230 mm) y solubilidad del 57,86%. El aceite de buritĺ presentó la menor solubilidad (41,42%), espesor moderado (0,230 mm) e hinchamiento de 1204,53%. Cada aceite indujo efectos distintos en las propiedades de las películas. Los aceites de copaíba y buritĺ redujeron el hinchamiento y la solubilidad, lo que sugiere su idoneidad para ambientes húmedos, como los sitios de heridas. El aceite de murumuru mejoró la plasticidad, favoreciendo aplicaciones que requieren películas más delgadas y suaves. Los resultados destacan el potencial de los aceites amazónicos como agentes funcionales naturales para ajustar las propiedades de los hidrogeles y avanzar en materiales biomédicos sostenibles y de alto valor añadido.

Palabras clave: Biopolímeros. Aceite de Copaíba (Copaifera spp.). Aceite de Murumuru (Astrocaryum murumuru). Aceite de Nuez de Brasil (Bertholletia excelsa). Aceite de Buritĺ (Mauritia flexuosa).

1 INTRODUCTION

The search for sustainable biomedical materials has driven the development of green hydrogels based on biopolymers integrated with Amazonian oils. These innovative systems use natural bioactive compounds to offer practical and environmentally responsible therapeutic solutions [1]. Hydrogels of biopolymeric origin — such as alginate, chitosan, and gelatin — stand out for their biocompatibility and biodegradability, with proven application in controlled drug release and tissue regeneration [2-4]. Among the Amazonian oils studied, Copaiba oil (*Copaifera spp.*) has received special attention for its potent antimicrobial and anti-inflammatory activity. For example, a hydrogel containing copaiba oil nanoemulsions showed significant reduction in edema in animal models, with up to 72% inhibition in the rat paw edema test [5]. Furthermore, its bactericidal action has been proven both in vitro and in vivo, especially against Streptococcus agalactiae and gram-positive biofilms [6, 7]. These results support its potential as a therapeutic agent in hydrogel formulations aimed at topical and systemic applications. Lesser-explored oils, but with high potential, such as Murumuru (*Astrocaryum murumuru*), Brazil nut (*Bertholletia excelsa*), and Buriti (*Mauritia flexuosa*), have also been considered in biomedical formulations. Recently, a literature review pointed out advances in using essential oils in hydrogels with anti-inflammatory and antioxidant activity, standing out especially in healing applications and release of natural active ingredients [8]. These oils, rich in fatty acids, carotenoids, and tocopherols, are promising for improving the biocompatibility of polymeric systems and enhancing their bioactive effects. Therefore, exploring the synergistic use of these oils in green hydrogels is relevant, combining the functional structure of biopolymers with the therapeutic efficacy of compounds from Amazonian biodiversity. This article presents the results obtained by combining Amazonian vegetable oils such as Copaiba, Murumuru, Brazil nut, and Buriti with sustainable alginate hydrogels and their visual characteristics, water solubility, thickness, and degree of swelling, aiming at biomedical applications.

2 MATERIALS AND METHODS

2.1 MATERIAL

Reagents: Glycerin (ÊXODO CIENTÍFICA®), Sodium Alginate (ÊXODO CIENTÍFICA®), Brazil Nut Oil (BIG ESSÊNCIAS), Absolute Ethyl Alcohol 99.5% (TWA®), Calcium Chloride Dihydrate (NEON®), Sodium Dodecyl Sulfate (SDS) (LABSYNTH®).

Equipment: Analytical balance (Sartorius), hot plate with magnetic stirring (IKA C-MAG HS10), and orbital shaker (NOVATÉCNICA).

2.2 METHODS

2.2.1 Preparation of biofilms

The film-forming mixture was prepared using the Casting technique, following the methodology developed by [9]. In a 250 mL beaker, 1 mL of glycerin was dissolved in 50 mL of distilled water and stirred for 20 min at room temperature (~25 ºC). Then, 1 g of sodium alginate was added, constantly stirring for another 20 min at 70 ºC. The vegetal oil mixture was prepared in a 100 mL beaker to which 10 mL of distilled water, 0.1 g of Brazil nut oil, and 0.01 g of sodium dodecyl sulfate (SDS) were added. Once ready, the mixture was poured into a 25 mL volumetric flask for later storage. This mixture was then added dropwise to the previously prepared film-forming mixture, which was stirred constantly for 20 minutes at 70 ºC. After this process, the film-forming mixture with Brazil nut oil was poured into a previously sterilized Petri dish in an oven. The plate containing the material was then placed in an oven to dry at 50 °C for 48 hours. For comparison purposes, control biofilms, i.e., without oil, were also produced and prepared using the methodology already mentioned. After preparing the additive and control biofilms, they were subjected to a rectification process of adding these dry biofilms to 10 mL of a 2.0 % (m/v) calcium chloride solution for 30 s [10]. All tests were performed in triplicate. This methodology was used for all oils studied (Copaiba, Buriti, Brazil nut, and Murumuru).

2.2.2 Characterization of the biofilms

Visual analysis

Visual analyses of the biofilms were performed after crosslinking, observing the uniformity, transparency, malleability, and flexibility of the additive and control biofilms. The process was performed in triplicate.

Thickness

Using a digital caliper, measurements were taken at seven points, randomly chosen for both prepared films. This determination was triplicate [11, 9].

Water Solubility

The solubility (*S*) of the biofilms in water was determined for both biofilms. These were randomly cut into 2 cm × 2 cm pieces and weighed on an analytical balance. They

were then placed in a 100 mL Erlenmeyer flask containing 25 mL of distilled water and then subjected to orbital agitation (shaker) at 150 rpm for 10 min, as per the experiments by [9]. Then, the samples of both biofilms were transferred to Petri dishes and kept in a desiccator for 3 days to complete drying and subsequent weighing. The process was performed in triplicate. The solubility percentage was calculated by Equation 1:

$$S\% = ((M_i - M_f)/M_i) \text{ x } 100 \quad (1)$$

S% is the amount of soluble matter (%); *Mi* is the initial mass of the sample (g); and *Mf* is the final mass of the sample (g).

Degree of swelling (GI%)

Each film's swelling degree was measured for random samples of 2 cm x 2 cm and weighed on an analytical balance [9]. Each film was then submerged in 5.0 mL of distilled water for 40 min by immersion. The process was performed in triplicate. With the data obtained, the degree of swelling could be calculated using Equation 2.

$$GI\% = ((M_u - M_i)/M_i) \text{ x } 100 \quad (2)$$

GI% is the degree of swelling of the film (%), *Mi* is the initial mass of the sample (g), and *Mu* is the mass of the sample removed from the solution (g).

3 RESULTS AND DISCUSSION

Determining the optimized condition for the preparation of biofilms supplemented with the different oils studied resulted from several preliminary tests in which the concentrations of alginate, glycerin, and sodium dodecyl sulfate (SDS) were varied. Because of such tests, it was defined that the concentration of alginate (1 g/50 mL = 2 % w/v) is consistent with the results presented by [12], in which the use of 2 % (w/v) of alginate allowed the formation of stable emulsions with high oil load. The choice of 2 % balances adequate viscosity for the incorporation and stabilization of oil droplets and good formation of the polymeric matrix for drying via casting. It avoids excessive rigidity in the dry films, ensuring flexibility, which is important for application in packaging or cosmetics. Furthermore, this concentration allows a more homogeneous distribution of oils in the matrix, reducing coalescence and phase separation during drying as observed by [12].

The addition of glycerin as a plasticizer is common in the production of biofilms. The concentration of 2 % v/v is a balanced choice to reduce the brittleness of the final biofilm, maintain good structural integrity without making it excessively sticky or elastic, and prevent oil migration to the film surface, helping in the overall stability of the matrix [13]. Although the article by [11, 12] focuses more on emulsifiers, plasticity is crucial for the mechanical integrity of films during drying and after application.

Adding SDS (sodium dodecyl sulfate) at a low concentration of 0.1 % w/v acts as an anionic emulsifier, facilitating the incorporation of hydrophobic oils into the aqueous alginate solution. As discussed in [13], using surfactants allows the formation of stable emulsions that remain homogeneous during the time required for drying and biofilm formation. Although [12] used nonionic surfactants (Tween 80; Span 20), the choice of SDS is justified by its low cost and high efficiency in small concentrations, if the critical micelle concentration (CMC) is not exceeded. The low concentration of SDS also minimizes possible irritant or cytotoxic effects, which is important if the films are applied in contact with the skin. This formulation was considered ideal because it avoids phase separation during the drying process and maintains the stability of vegetable oil emulsions without damaging the polymeric structure of the film. The resulting biofilms have good integrity, flexibility, and uniform appearance, as required in biomedical applications. The oils used (Murumuru, Copaiba, Buriti, and Brazil Nut) are rich in lipophilic bioactive compounds, whose release and protection are favored by this matrix. In addition, the tests with control biofilms (without oil) allow us to evaluate the real contribution of emulsification and the presence of oil in the structural and functional properties of the films, such as elasticity, permeability, opacity and antioxidant or antibacterial potential, which will be evaluated in the continuation of this study.

The cross-linking of alginate-based biofilms using $CaCl_2$ is a crucial step in modifying their structural and functional properties. This provides the final material with characteristics that favor its use in various applications, such as dressings, controlled release systems, and food packaging.

Crosslinking is a process in which alginate polymer chains are interconnected by calcium ions (Ca^{2+}), forming a three-dimensional network. This process is based on the interaction between calcium ions and the carboxylate groups present in the guluronic acid units of alginate, resulting in the "egg-box" network model [14, 15]. Crosslinking affects the formation of ionic bonds between the chains, which generates a denser and more

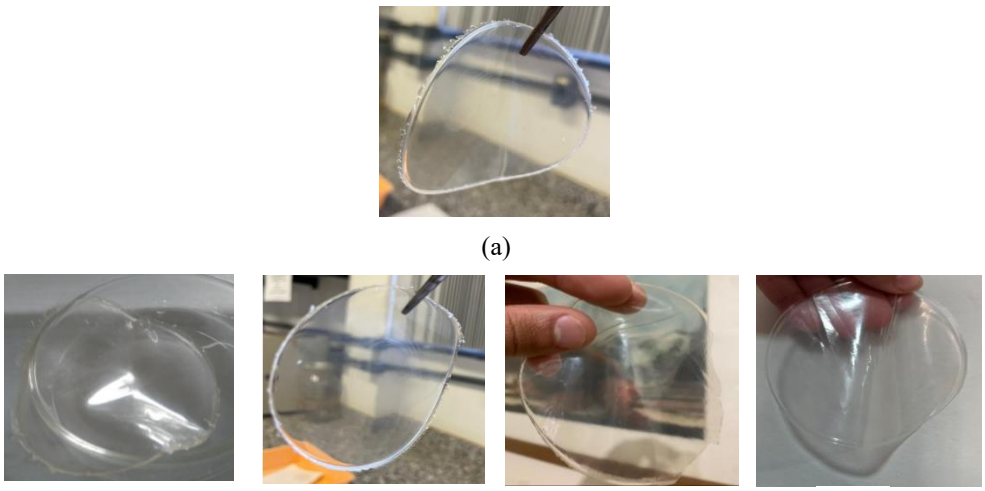
stable structure, essential for conferring desirable properties to biofilms. In practical terms, the $CaCl_2$ solution is added to the alginate film, and the calcium ions interact with the carboxylate groups, promoting crosslinking. The degree of rigidity and thickness of the biofilm can be adjusted according to the $CaCl_2$ concentration and the time the film is exposed to the solution, allowing customization of the mechanical and structural properties of the material [16]. This process has the advantage of increasing mechanical strength, which promotes the formation of ionic bonds between the alginate chains and significantly increases the resistance of the biofilm, making it stronger and more resistant to handling, which is crucial for applications involving packaging or dressings. In addition, reducing the brittleness of the alginate film is an important improvement for its durability and performance [17, 18]. Reducing water solubility, in this case, crosslinking with $CaCl_2$, also reduces the solubility of the biofilm in water, which is essential for using the material in humid environments. This ensures that the biofilm maintains its integrity even when exposed to moisture, such as in dressings or controlled release systems [17, 19]. The versatility and customization obtained through control over the $CaCl_2$ concentration and the exposure time to the crosslinking solution allow the customization of the biofilm properties. This makes it possible to create films with different degrees of rigidity, thickness, and flexibility, adapting them to different applications. The flexibility of adjusting properties is one of the main advantages of this technique, as highlighted by [14] and reinforced by [20].

Other researchers, such as [21], investigated the influence of crosslinking with calcium ions on alginate films' structural and thermomechanical properties. The results indicated that crosslinking with calcium improves the rigidity and thermal stability of the films, an important characteristic for applications in which resistance to temperature variations is essential. In addition, the improvement in structural integrity facilitates the use of these films as coating materials and in encapsulation technologies. [22] focused on comparing the effects of $CaCl_2$ and KCl as crosslinking agents for chitosan-carrageenan composite films. The study observed that $CaCl_2$ provides better crosslinking and mechanical strength and reduces the film's solubility in aqueous environments. Although the study focused on other polymers, the results reinforce the effectiveness of $CaCl_2$ as a crosslinker to improve the functionality of polymeric biofilms. [23] explored the ionic crosslinking process of chitosan and alginate to form biodegradable films. Although the focus is on chitosan, crosslinking with Ca^{2+} has also been applied to modify the

properties of biofilms. The study highlighted how crosslinking with $CaCl_2$ can improve mechanical properties and increase the material's stability and strength in controlled release systems. [15] analyzed the influence of different crosslinking ions on the morphology and performance of alginate films. The study concluded that $CaCl_2$ promotes greater structural compaction and strength than other metallic salts [16] demonstrated how the concentration of $CaCl_2$ directly impacts the barrier and mechanical properties of alginate-based films. They propose an optimization model to maximize physicochemical performance. Crosslinking with $CaCl_2$ promotes reduced water solubility and increased mechanical resistance, making these biofilms ideal for applications in dressings, especially in humid environments.

Figure 1

Images of biofilms supplemented with: (a) Control, (b) Murumuru Oil, (c) Brazil Nut Oil, (d) Copaiba Oil, (e) Buriti Oil



(a)

(b) (c) (d) (e)

Figure 1 shows representative images of biofilms developed with and without the addition of Amazonian vegetable oils: (a) control, (b) Murumuru oil, (c) Brazil nut oil, (d) Copaiba oil, and (e) Buriti oil. In all cases with lipid addition, transparency, homogeneity, and absence of lumps are maintained, indicating good dispersion of the oils in the polymer matrices and efficient structural compatibility. This performance can be partially explained considering the data from [24], who report the functional efficacy of lipophilic compounds in biopolymeric matrices, which may contribute to the visual and mechanical stability observed in the images. Furthermore, [25] analyzed the structural and functional

characteristics of biofilms produced with zein and vegetable oils, observing that the incorporation of lipids promotes improvements in malleability, flexibility and moisture barrier, without compromising the integrity of the film — characteristics that align with the results obtained in the formulations with murumuru, Brazil nut, copaiba and buriti. Although the base matrix Almeida uses is zein, the behavior described can be partially extrapolated to alginate-oil systems, given the hydrophobic nature of the incorporated additives.

The fact that all biofilms presented homogeneity and flexibility suggests that vegetable oils acted not only as structural modifiers but also as natural plasticizing agents. They partially replaced water as an agent of molecular mobility, conferring greater elasticity to the material—an effect also observed by [25] in mixtures with oleic acid.

Table 1

Results were obtained by determining the thickness, solubility (S%), and degree of swelling (GI%) of the oils studied

Oil	Thickness (mm)	S%	GI%
Control	0,286 ± 0,035	45,71	1601,13
Murumuru	0,153 ± 0,015	62,72	1531,10
Brazil Nut	0,311 ± 0,053	54,05	901,10
Copaiba	0,230 ± 0,080	57,86	644,45
Buriti	0,230 ± 0,042	41.42	1204,53

The results obtained in Table 1 demonstrate that adding Amazonian vegetable oils significantly influenced the physicochemical properties of the biofilms, especially in terms of thickness, solubility, and degree of swelling. The biofilm incorporated with murumuru oil presented the smallest thickness (0.153 mm), which may indicate greater compatibility and compaction of the polymer matrix, probably due to the lipophilic nature and reduced viscosity of this oil [26]. On the other hand, the film with Brazil nut oil presented the most significant thickness (0.311 mm), suggesting a less compact structure, possibly due to the interference of unsaturated fatty acids in its dispersion in the polymer matrix.

Regarding solubility (S%), the film with buriti oil demonstrated the lowest value (41.42 %), surpassing the control. This result can be attributed to the high concentration of hydrophobic compounds such as carotenoids and tocopherols, which contribute to

greater moisture resistance [15]. In contrast, the biofilm with murumuru oil showed the highest solubility (62.72 %), suggesting a lower water barrier capacity, which may be related to the more saturated composition of the oil, which reduces the density of the polymer network formed.

The degree of swelling (GI%) highlights an important functional distinction. The control showed the highest value (1601.13 %), followed by buriti (1204.53 %), which indicates high water absorption in both cases. Copaiba oil, on the other hand, gave the film the lowest degree of swelling (644.45 %), which is in line with its ability to form denser and more hydrophobic matrices, favoring the dimensional stability of the material in humid conditions [24]. This characteristic is especially desirable in biomedical applications and food packaging, where the structural integrity of the biofilm is essential.

These differences demonstrate that each oil exerts specific effects on the structure and properties of the biofilm, allowing the development of customized materials according to the purpose. Oils such as copaiba and buriti stand out for their hydrophobic performance and stability in the face of humidity. At the same time, murumuru favors greater malleability and less thickness, which are functional characteristics in formulations where flexibility and lightness are priorities [25].

4 CONCLUSION

The choice of alginate (2 %), glycerin (2 %), and SDS (0.1 %) concentrations for the preparation of biofilms was based on preliminary experiments that indicated good emulsion formation, stability, and mechanical properties, and compatibility with the physicochemical properties of the incorporated vegetable oils. These conditions ensured the production of an efficient film-forming system for functional and sustainable applications.

The crosslinking of alginate biofilms with $CaCl_2$ is a well-established strategy that provides several benefits, such as increased mechanical strength, reduced water solubility, and film stiffness and thickness adjustment. Studies published by different authors reinforce the advantages of this approach and indicate the versatility of biofilms crosslinked with $CaCl_2$ for applications in areas such as food packaging, dressings, and controlled release of substances.

Based on the results obtained, it can be concluded that adding Amazonian vegetable oils to biofilms contributes significantly to the formation of homogeneous,

transparent, flexible, and visually stable structures. The absence of lumps and the sound dispersion of the oils indicate efficient compatibility with the polymer matrix, favoring the structural integrity of the films. In addition to acting as natural plasticizing agents, the oils provide greater malleability and resistance to the material, desirable characteristics in applications such as dressings, coatings, and functional packaging. These results reinforce the potential of these compounds in the formulation of biomaterials with improved properties and versatile applications.

The results demonstrate that incorporating Amazonian vegetable oils into biofilms significantly modified their structural and functional properties. The control film presented a thickness of 0.286 mm, solubility of 45.71%, and swelling degree (GI%) of 1601.13, serving as a comparative basis. Murumuru oil promoted the smallest thickness (0.153 mm), although it increased the solubility to 62.72 %, maintaining the GI% at 1531.10. Brazil nut oil resulted in the most significant thickness (0.311 mm), with intermediate solubility (54.05 %) and a reduced GI% (901.10). Copaiba oil significantly reduced the swelling degree to 644.45 %, with a thickness of 0.230 mm and solubility of 57.86 %. Finally, buriti oil showed good performance, low solubility (41.42 %), GI% of 1204.53, and moderate thickness (0.230 mm). These data reinforce that the choice of oil directly influences the functional performance of biofilms, allowing their applications to be targeted according to need, whether for greater resistance to moisture, flexibility, or dimensional stability.

In summary, this study demonstrated that adding Amazonian vegetable oils influences the physicochemical properties of biofilms in distinct ways, allowing the modulation of characteristics such as thickness, solubility, and swelling. The variation in the results highlights these oils' potential as functional agents for developing customized bioactive materials, with promising applications in sustainable packaging, dressings, and controlled release systems. The valorization of these natural resources reinforces technological innovation and the responsible use of Amazonian biodiversity.

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