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PRODUCCIÓN DE BIOFILM A PARTIR DE CULTIVO SIMBIÓTICO DE BACTERIAS Y LEVADURAS (SCOBY) EN Euterpe oleracea Mart. (AÇAÍ)

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ABSTRACT

Biofilms are complexes of microbial aggregates surrounded by a self-produced matrix of extracellular polymeric substances (EPS), usually adhered to the surface. This research investigated biofilm production from a symbiotic culture of bacteria and yeast (SCOBY), using Euterpe oleracea Mart. (1824) [açaí] extract as a substrate. The studies combined with the essays produced in the Microbiology and Biotechnology Laboratory - MicroBioTec, IFRO, Calama campus in Porto Velho, RO in these conditions: room temperature (16 and 35 °C). Fermentation was conducted with an industrial starter culture in a medium containing 42 g of sucrose, 4.2 mL of açaí pulp, 2.7 mL of acetic acid, and 166 mL of mineral water, incubated at a room temperature for 14 (fourteen) days with subsequent renewal of the medium until biofilm formation. The tests were triplicated and evaluated after 233 (two hundred and thirty-three) days, the results showed us the synthesis of gelatinous and translucent biofilms, with an average initial mass of 22.88 g and a final mass of 3.32 g after drying. Microscopic evaluations revealed the presence of polymeric fibers and microorganisms consistent with yeasts, fungal filaments immersed in EPS, which became consistent and opaque after drying. Sensory analyses identified substrates with the following profiles: reddish-brown coloration,

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vinegary aroma and initially gelatinous and translucent textures that became opaque, leafy, and earthy after drying. The data that results from the tests and the studies are relevant and can contribute to studies related to the production of biofilms from plant extracts in the Brazilian Amazon. However, we suggest additional analyses associated with physicochemical, structural, and functional profiles that could improve, evaluate, and enhance biofilm production techniques using E. oleracea extract.

Keywords: Biofilm. *Euterpe oleracea.* Bioproducts. Amazonian Flora.

RESUMO

Os biofilmes são complexos de agregados microbianos envolvidos por uma matriz autoproduzida de substâncias poliméricas extracelulares (EPS) normalmente, aderidas à superfície. Esta pesquisa investigou a produção de biofilme a partir de uma cultura simbiótica de bactérias e leveduras (SCOBY), utilizando extrato de Euterpe oleracea Mart. (1824) [açaí] como substrato. Os estudos combinados com os ensaios de produção, realizados em temperatura ambiente (16 e 35 ºC), foram realizados em etapas no Laboratório de Microbiologia e Biotecnologia – MicroBioTec, do IFRO, campus Calama em Porto Velho – RO. A fermentação foi conduzida com cultura starter industrial em meio contendo 42 g de sacarose, 4,2 mL de polpa de açaí, 2,7 mL de ácido acético e 166 mL de água mineral, incubados à temperatura ambiente por 14 (catorze) dias com posterior renovação do meio até a formação dos biofilmes. Os ensaios, realizados em triplicata e avalizados após 233 (duzentos e trinta e três) dias, proporcionaram a síntese de biofilmes gelatinosos e translúcidos, com massa inicial média de 22,88g e final de 3,32 g após secagem. As avaliações microscópicas revelaram a presença de fibras poliméricas e micro-organismos condizentes com leveduras, filamentos fúngicos imersos em EPS, que se tornaram consistentes e opacos após secagem. As análises sensoriais identificaram substratos com os seguintes perfis: coloração marrom avermelhada, aroma avinagrado e texturas inicialmente gelatinosas e translúcidas que se tornaram opacas, foliáceas e terrosas após secagem. Os dados dos ensaios e dos estudos são relevantes e podem colaborar com estudos relacionados com a produção de biofilmes oriundos de extratos vegetais da Amazônia legal. Entretanto, sugere-se análises complementares associadas aos perfis físico-químicos, estruturais e funcionais que possam aperfeiçoar, avaliar e potencializar as técnicas de produção dos biofilmes a partir do extrato de E. oleracea.

Palavras-chave: Biofilme. *Euterpe oleracea*. Bioprodutos. Flora Amazônica.

RESUMEN

Las biopelículas son complejos de agregados microbianos rodeados por una matriz autoproducida de sustancias poliméricas extracelulares (EPS), generalmente adheridas a la superficie. Esta investigación investigó la producción de biopelículas a partir de un cultivo simbiótico de bacterias y levaduras (SCOBY), utilizando extracto de Euterpe oleracea Mart. (1824) [açaí] como sustrato. Los estudios combinados con ensayos de producción, realizados a temperatura ambiente (16 y 35 °C), se llevaron a cabo en etapas en el Laboratorio de Microbiología y Biotecnología - MicroBioTec, IFRO, campus Calama en Porto Velho, RO. La fermentación se realizó con un cultivo iniciador industrial en un medio que contenía 42 g de sacarosa, 4,2 mL de pulpa de açaí, 2,7 mL de ácido acético y 166 mL de agua mineral, incubado a temperatura ambiente durante 14 (catorce) días con posterior renovación del medio hasta la formación de la biopelícula. Las pruebas, realizadas por triplicado y evaluadas después de 233 (doscientos treinta y tres) días, dieron como resultado la síntesis de biopelículas gelatinosas y translúcidas, con una masa inicial promedio de 22,88

g y una masa final de 3,32 g después del secado. Las evaluaciones microscópicas revelaron la presencia de fibras poliméricas y microorganismos consistentes con levaduras, filamentos fúngicos inmersos en EPS, que se volvieron consistentes y opacos después del secado. Los análisis sensoriales identificaron sustratos con los siguientes perfiles: coloración marrón rojiza, aroma avinagrado y texturas inicialmente gelatinosas y translúcidas que se volvieron opacas, frondosas y terrosas después del secado. Los datos de las pruebas y estudios son relevantes y pueden contribuir a los estudios relacionados con la producción de biopelículas a partir de extractos de plantas en la Amazonia brasileña. Sin embargo, se sugieren análisis complementarios asociados con los perfiles físico-químicos, estructurales y funcionales para mejorar, evaluar y optimizar las técnicas de producción de biopelículas utilizando extracto de E. oleracea.

Palabras clave*:* Biopelícula*.* *Euterpe oleracea.* Bioproductos*.* Flora Amazónica*.*

1 INTRODUCTION

Studies seek to improve and innovate processes for the production of cellulose biopolymers generated through microbial metabolic activities. Of these products, those obtained from matrices that result from kombucha fermentation processes are mentioned, a Scoby – *Symbiotic Culture of Bacteria and Yeasts*, in free translation into Portuguese: Symbiotic Culture of Bacteria and Yeasts (Silva *et al*., 2021; De Souza *et al*., 2023). Kombucha is a Germanized concept for the Japanese etymological name, "kobu-cha". As for the origin, the regions of northeastern China, Manchuria, where it became popular for its detoxifying properties (JAYABALAN *et al*., 2014) are mentioned.

Considering the rich biodiversity of the Amazon, the microbiota and plants represent a range of possibilities for innovations. In this context, cellulose has been considered the most abundant natural polymer on Earth and has been widely used as a sustainable solution, since it is a biodegradable and biocompatible material (Nascimento, 2022), data show that cellulose can be an option to replace conventional polymers derived from petroleum, which, in turn, have a great industrial application (KARGARZADEH *et al*., 2017).

With regard to the microbiota, especially the microorganisms involved in fermentation processes, acetic bacteria of the genus *Acetobacter*, *Gluconobacter* and *Gluconacetobacter* that are present in fermented beverages such as kombucha, have been reported to be responsible for the formation of the cellulosic film (Santos, 2016). However, some genera usually prevail in the initial cultures such as *Komagataeibacter* and *Acetobacter* - acetic acid bacteria (AAB), and yeasts of the genera *Saccharomyces*, *Zygosaccharomyces*, *Brettanomyces* and *Candida* for yeasts (Jayabalan *et al*., 2014; Becchi *et al*., 2023). On the other hand, despite the importance of lactic acid bacteria (LAB) for the probiotic properties of kombucha, they do not always appear in drinks (LAUREYS; BRITTON; CLIPPELEER, 2020; BECCHI *et al*., 2023).

Of the different cellulosic films, one of those that has been gaining visibility in the most diverse researches, not only for its biodegradability and non-toxicity, but also for its potential biotechnological applications is Scoby (Chawla *et al*., 2009; Keshk, 2014a; Davey & O'toole, 2000). With regard to kombucha, it is characterized in two phases: one is made up of a layer of cellulosic membrane produced by bacteria, Scoby, and the tea itself, which has probiotic properties, a characteristic fermented flavor and is slightly bittersweet, based on traditional black or green tea leaves (RODRIGUES, 2018; SILVA *et al*., 2021).

As for Scoby, it represents a product with biotechnological potential. Thus, for its production, applications of new substrates are sought. The final product, consequently, will have biological properties different from those traditionally produced (Maia *et al*., 2020). In

this context, it was proposed, for this study, the use of *Euterpe oleracea* (Açaí) as a base substance for the cultivation of kombucha for new trials for the production of biofilm, from Scoby.

The fruit of *E. oleracea* plays an important role in the Amazon region, due to its abundance, high productivity and socioeconomic importance (Anderson *et al*., 1985; Bezerra; Nery; Lobato, 2001; Marques *et al*., 2024). Among the properties present in the açaí fruit extract, in addition to the pleasant flavor, the high nutritional value and lipids, carbohydrates, proteins, bioactive compounds present in polyphenols, the class of flavonoids and anthocyanins, characteristics explored by the food and pharmaceutical industries (Cedrim; Baby; Nascimento, 2018; Vargas *et al*., 2024), important aspects for further analyses.

For bioprospective processes, the production of biopolymers from cellulose fermented by bacteria due to their properties of biodegradability, purity, and biocompatibility are relevant (Keshk, 2014b; Silva *et al*., 2021). In addition, the applicability in the textile industry, paper industry, food industry, mining and refinery, waste treatment, sewage purification, communications, cosmetics, medicine, laboratories, electronics, energy, materials engineering, in the arts and more recently in the *design* of sustainable products is mentioned (Costa, 2018). In this context, this research aimed at the study and production of biofilm from symbiotic culture of bacteria and yeasts - Scoby - in enriched extract of *Euterpe oleracea* Mart. (Açaí).

2 METHODOLOGY

The studies and production of the biofilms were carried out at the Laboratory of Microbiology and Biotechnology - MicroBioTec - of the Federal Institute of Education, Science and Technology of Rondônia - IFRO, *Porto* Velho Calama Campus, between the years 2024 and 2025. The *Scoby starter* culture was commercially acquired and maintained under technical conditions recommended by the company "Vida bióticos" - Joinville - SC, Brazil.

2.1 EXPERIMENTAL DESIGN

The experimental design was structured based on the methodologies described by De Souza *et al*. (2023), with adaptations. Initially, the starter culture was kept in a solution containing 100 mL of mineral water and 10 g of sucrose, incubated in a beaker sealed with sterile and elastic gauze, at room temperature (25 °C), for seven days. During this period, a reduction in the volume of the solution was observed, keeping the SCOBY hydrated and active. Periodic supplementation was performed to replace nutrients.

For the production trials, aliquots of ≅12.5 g of SCOBY were transferred to three independent containers (triplicate). Each fermentation system was prepared with 15 mL of *Euterpe oleracea* Mart. extract (açaí), 10 g of sucrose ($C_{12}H_{22}O_{11}$) and 100 mL of mineral water. The physicochemical characteristics of the water were: pH 4.9; temperature of 25 °C; electrical conductivity of 63.8 µS/cm. The ionic composition included: sulfate (0.18 mg/L), chloride (10.23 mg/L), sodium (9.02 mg/L), nitrate (6.12 mg/L), bicarbonate (1.42 mg/L), potassium (0.68 mg/L), calcium (0.10 mg/L), bromide (0.05 mg/L) and magnesium (0.01 mg/L).

The cultures were kept in static fermentation for 56 days at room temperature (16 and 35 ºC). In this interval, after 30 days, the medium was renewed, using a solution with the same initial composition. At the end of the process, the biofilms produced were carefully removed and submitted to physical (mass and moisture), sensory (color, texture, aroma), microscopic (optical microscopy and Gram staining) and descriptive microbiological analyses.

2.2 PHYSICAL ANALYSIS

For the physical analyses of mass (g) and percentage of moisture content (Tu%), carried out with adaptations and based on the guidelines of Cunha, Santos and Schneider (2021) and Cruz (2023). The initial evaluations were using a 0.0001g precision scale, model Mark M214A. For the moisture content (Tu%) it was carried out by the following queetion:

$$Tu\ \% = \frac{Pi-Pf}{Pi} x\ 100 \qquad (1)$$

In the equation above, the moisture content (Tu) is given by dividing the variation of the masses, given in weight (P) of the biofilm, that is, [initial weight (Pi) of the subtracted by the final weight (Pf), divided by its initial weight (Pi) and multiplied by 100]. The evaluations were carried out after assisted dehydration and with the use of a complete 160 mm desiccator with a lid and with a 55/38 sleeve accompanied by a porcelain plate. At the end of the experimental phase, the collected data were tabulated and analyzed according to mean notation (±) with standard deviation, using Microsoft 365 Excel programs and GraphPad *Prism 8.0*® software.

2.3 MICROSCOPIC AND MICROBIOLOGICAL EVALUATIONS

The microscopic and microbiological descriptive evaluations performed were through the use of the common optical microscope, biocular and with the use of the Gram staining

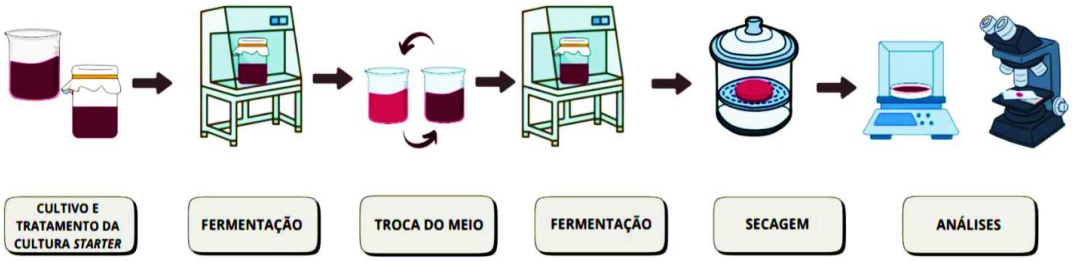
technique, modified from Brazil (1997) and Brazil (2013), with magnification for resolution of 1000x and 2500x for observation and microbial and descriptive structural identification.

2.4 SENSORY EVALUATIONS

The sensory evaluations were adapted from the *Flash Profile* methodology, proposed by Dairou and Siefferman (2002), and which are based on a combination of the Free Profile method with the ordering technique, as well as on the guidelines of Stone *et al.* (2020); Sartor *et al.* (2021) and ABNT (2017; 2018), considering the description observed by the sight of smell, touch – texture for the biofilm. The illustration below shows the steps of the experimental protocol summarized in the flowchart (Figure 1).

Figure 1

Flowchart showing the stages of biofilm/Scoby production from *E. oleracea.*



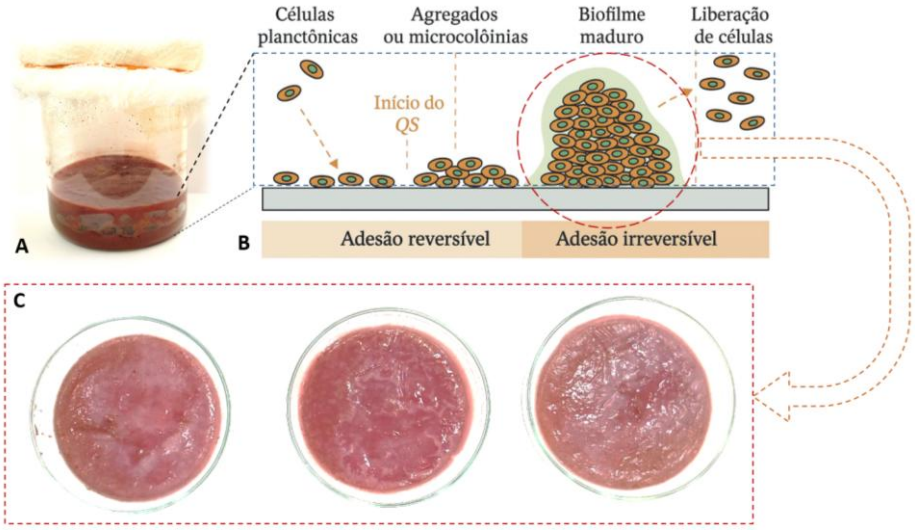
Source: Authors' image bank (2025).

3 RESULTS AND DISCUSSION

The trials of the Scoby cultures, with the addition of *E. oleracea extract,* resulted in the sample production of biofilm films with measurable physical and sensory characteristics that differed between the records of the initial - hydrated, and final - dehydrated (dry) samples (Figure 2 A - C). The development of the biofilm is characterized by the metabolic action of the microorganisms present in the aerobic substrate composed of mineral water, *E. oleracea extract* and sucrose, which provided a favorable environment for the production of extracellular polymeric substances (EPS). As described by Caixeta (2008) and Dalla Costa *et al*. (2016), the formation of biofilm results from sequential events where the initial adhesion of planktonic bacteria to the surface (reversible process) is followed by subsequent proliferation and accumulation of layers or aggregates of cells and, finally, by the formation of the microbial community (irreversible process), involved in an EPS matrix produced by itself (Figure 2 – A-C).

Figure 2

*Images A, B, and C show events and characteristics associated with biofilm production in this and other studies. In **A** – the beaker with enriched substrate for biofilm growth. Figure **B**- The illustration shows the stages of formation and development of the biofilm from planktonic cells, the beginning of QS (Quorum Sensing) - mechanisms of cellular communication between bacteria for the coordination of group activities - in the reversible phase and, later, the mature biofilm with the release of cells for the formation of new microcolonics - irreversible phase. In figure **C**, the biofilm, in the petri dishes, produced in triplicate. A reddish-brown tone refers to the presence of the anthocyanin molecule adhered to the EPS compound in the extract of E. oleracea (acai)*



Source: Images A and C - authors' database; D - Created with the help of ChatGPT-4º's Artificial Intelligence (AI) tool in DALL.And 3 (2025).

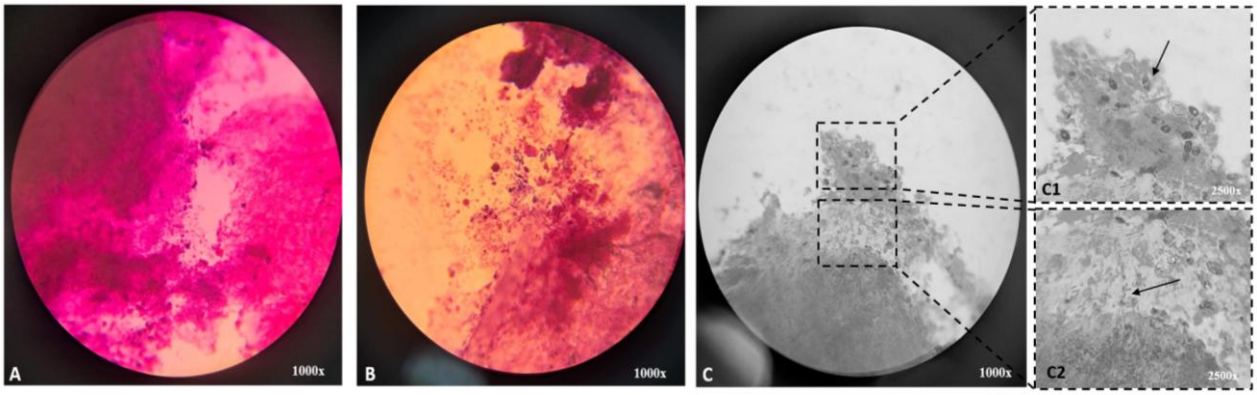
In the biofilms (Figure 2C), the reddish morrom tone is derived from the violet color of the açaí and is the result of the presence of a type of flavonoid - anthocyanin (C15H11O6+), present in different types of plants, which are solubilized in water. Rogez (2000) reports that the characteristic color of açaí is related to the high concentration of anthocyanins in the fruits. Additionally, Bobbio *et al*. (2000) highlight that anthocyanins are pigments responsible for various attractive colors in fruits, flowers and leaves, ranging from red to blue. Heredia *et al.* (1998) and De Souza *et al*. (2023) show that, in aqueous solution, anthocyanins show a balance between different structural forms, such as flavil cation (red), quinoidal anhydrous base (blue), carbitol pseudo-base (colorless) and chalcone base (yellow), and the hydration of the flavil cation favors the carbitol and chalcone forms, resulting in red and yellow colorations (Figure 3 A and B)..

The process of biofilm polymerization, although not well elucidated, is described in the papers and Chawla *et al*. (2009), Donini *et al.* (2010), Lee *et al.* (2014) and De Souza *et al*.

(2023) as a biosynthesis mechanism that begins with the polymerization of glucose residues – joined by glycosidic bonds of the β type (1 → 4) glucan, followed by the excretion of linear chains and ends with the organization and crystallization of glucan chains, resulting in a resistant three-dimensional structure called microfibril (Figure 3C2).

Figure 3

Images of the fields of view used from microscopic evaluations. In ***A*** *and* ***B****, smears containing EPC granules in the fixed colors of the Gram stain are evidenced, as well as the characteristic tones of the presence of anthocyanin in the EPS. In* ***C****, image analyzed in contrast - gray and white color - and the* ***presence of microorganisms consistent with yeasts and microfiber aggregates were observed (C1 and C2 - indicated in the arrows)*** *- structural part of the monosaccharide composition of exopolymer complex (EPC)*



The polymerization process occurred in a uniform and homogeneous manner. In the analyses, it was found that, in addition to the anthocyanin evidenced in the substrate and in the EPS (Figures 2C; 3A, B and C), the extracellular polymeric matrix that was formed is made up of water, different classes of exopolysaccharides, lipids, nucleic acids, proteins, lipopolysaccharides, minerals and bacterial appendages, as reported by Hall-Stoodley, Costerton and Stoodley (2004); Hall-Stoodley and Stoodley (2005) and Shree *et al*. (2023).

In addition, polymers establish functional and structural properties of biofilms, determining their physicochemical properties (Flemming *et al*., 2000), offering support and protection to microorganisms in hostile environments (Fulaz *et al.,* 2019) and resistance that, in the case of biofilms synthesized by microorganisms of medical interest, are responsible for persistent bacterial infections (COSTERTON; STEWART; GREENBERG, 1999; LEWIS, 2005; HOUDT; MICHIELS, 2010).

In the physical analyses, the hydrated (Figure 2C) and deitradate samples showed mean mass and standard deviation of 13.82 ± 4.39 g and 1.72 ± 0.48 g, respectively. The moisture content (Tu%) of the hydrated biofilms was approximately 87.85%, a value close to

that described by Mariano Cunha, Pereira dos Santos, and Schneider (2021), who reported about 80%, diverging by 7.85%. Studies indicate that the moisture content can vary according to the physicochemical conditions of the medium and the microbial composition, but it is generally close to 90% (CZAJA *et al*., 2006; JAYABALAN *et al*., 2014; ULLAH; SAINTS; KHAN, 2016).

The sensory analyses of the biofilm films revealed the following characteristics: In the hydrated state, hydroscopic profile, viscous, translucent, shiny, predominantly reddish brown color and with color intensity reducing to the dehydrated state, being, when dry, opaque, foliaceous and earthy. Mechanically, in the hydrated state, Scoby presented, considering the sensory evaluation, gelatinous texture, "moderate" malleability and, after dehydration, moderate tensile strength.

As for the aroma, it presented perceptible characteristics of acetic acid, data that can be associated with fermentation by microorganisms in the substrates that make up the environment and by the extract of *E. oleracea* (açaí). For the presence of acetic acid, Villarreal-Soto *et al.* (2018) and Bokulich and Bamforth (2013) corroborate by citing that, depending on the desired product for Scoby, there are different species of bacteria and yeasts that can be used, such as acetic acid-producing bacteria - AAB, aerobic, gram-negative, such as those belonging to the *Acetobacter genera; Gluconobacter* and *Komagataeibacter*, the aerobic, gram-positive lactic acid - BAL - producing bacteria, such as those belonging to the genus *Lactobbacillus*, as well as different yeasts of the genera *Sccharomyces* and *Zygosaccharomyces*, among others also identified in the studies of Starter cultures of Scoby used in the Kombucha trade, which are the yeasts of the genus *Brettanomyces* and bacteria of the genus Starmerella, identified in the works of Harrisson and Curtin (2021).

The relevance of sensory evaluations consists of their frequent use in determining the differences between samples, as well as helping in the applications in the analysis of stability, quality, formulations and correlations of sensory and instrumental measurements, data already described by Civille and Oftedal, (2012); Silvia *et al*. (2012) and Scolforo (2014). However, the data of this study preliminarily characterize biofilm as a potential product for industrial applications.

Microscopic evaluations of dry biofilm samples revealed the presence of EPS with predominantly reddish-brown tones, microfibers and glycosidic aggregates, as described in the works of Heredia *et al*. (1998) and De Souza *et al*. (2023). Additionally, the microbiological analyses performed with the Gram staining technique revealed the presence of microbial structures consistent with yeasts and others with characteristics of microfiber structures (Figures 3C1 and C2). The presence of microbial groups has already been described by De

Souza *et al*. (2023) and Zhao, Sun and Liu (2023). In these, they report that the characteristics of bacterial biofilms are generally defined as fixed microbial communities, encapsulated in EPS. However, these aggregates can be composed of a single microorganism or a mixture of bacteria, fungi, archaea, protozoa, and yeasts, with a channel structure that controls the release of gases, nutrients, and antimicrobial compounds (ZHAO; SUN; LIU, 2023).

Regarding the microscopic aspects observed, the samples showed characteristics of a polymeric complex (EPC) with translucent texture and opacity with tones characteristic of the presence of anthocyanins (Figure 3A, B and C). The data are relevant and consistent with those observed in studies with biofilm production from other plant extracts, as well as corroborate different studies carried out on the production of biofilms.

4 CONCLUSION

The *in vitro* studies enabled the homogeneous production of three samples of biofilms in biotechnological production assays from the addition of an extract of *E. oleracea* (Açaí) in a symbiotic culture matrix (*starter)* composed mostly of bacteria and yeasts (Scoby). The results of the overlapping production of the biofilm films showed characteristics similar to those present in the scientific literature, as well as with shades of coloration of the presence of anthocyanins, the presence of microbial strains (yeasts), microfibers and aggregates of EPCs with aggregate of the researched extract.

The data presented collaborate with research and reinforce the promising results previously obtained. However, it is suggested a broad structural and functional characterization of biofilms, studies for new applications, as well as the realization of new research that can improve and direct production on an industrial scale.

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