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Research Article

Development of biofilms derived from bacterial cellulose

Desenvolvimento de biofilmes oriundos de celulose bacteriana

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ABSTRACT

Symbiotic Culture of Bacteria and Yeasts (SCOBY) is one of the main constituents of kombucha, consisting of a bacterial film that forms on the surface of the tea. This is a material that has a favorable fiber composition and biodegradable capacity. Thus, the present study aimed to analyze the factors that influenced the development of SCOBY in order to use it as a raw material to produce new biodegradable materials. The symbiotic culture of bacteria and yeasts showed the best yield (66.8 g) in the experiment prepared with green tea, stored at 30ºC and in a container with a surface area equal to 361 cm^2. The addition of bacterial cellulose to the films resulted in a material with better mechanical and thermal resistance. It was concluded that sample B1, with bacterial cellulose and a smaller amount of glycerin in its composition, would be the biofilm with the best characteristics for the production of biopackaging, considering that it has a lower moisture content, greater weight, better mechanical properties, such as greater tensile strength and a higher percentage of elongation, in addition to being a material resistant to high temperatures.

KEYWORDS: Biofilm; bacterial cellulose; kombucha; SCOBY.

RESUMO

A Symbiotic Culture of Bacteria and Yeasts (SCOBY) é um dos principais constituintes da kombucha, consistindo em uma película bacteriana que se forma na superfície do chá. Este é um material que apresenta composição de fibras favoráveis e capacidade biodegradável. Desta forma, o presente estudo teve como objetivo analisar os fatores que influenciavam o desenvolvimento de SCOBY, a fim de utilizá-lo como matéria-prima para produzir novos materiais biodegradáveis. A cultura simbiótica de bactérias e leveduras apresentou melhor rendimento (66,8 g) no experimento preparado com chá verde, armazenado a 30ºC e em recipiente de área superficial igual a 361 cm2. A adição da celulose bacteriana nos filmes, resultou em um material com melhor resistência mecânica e térmica. Concluindo-se que a amostra B1, com celulose bacteriana e menor quantidade de glicerina em sua composição, seria o biofilme com melhores características para a confecção de bioembalagens, tendo em vista que possui menor teor de umidade, maior gramatura, melhores propriedades mecânicas, como maior resistência a tração e maior percentual de elongamento, além de ser um material resistente a elevadas temperaturas.

PALAVRAS-CHAVE: Biofilme; celulose bacteriana; kombucha; SCOBY.

INTRODUCTION

In recent years, the demand for materials with lower pollution levels has become increasingly necessary. The world faces a major problem with plastic pollution, which will consequently increase with the rise in global plastic production and consumption (NAYAK et al. 2024 & SHEN et al. 2020).

Synthetic plastics are one of the most widely used materials for packaging different

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types of products. However, in addition to polluting oceans because they are difficult to degrade, when discarded in the environment they also generate large amounts of waste and consequently increase the amount of waste in landfills (FRANCHETTI & MARCONATO 2006).

As a result, alternatives have gradually been sought to minimize these environmental impacts. In this sense, SCOBY (Symbiotic Culture of Bacteria and Yeasts), which is a bacterial film that forms on the surface of kombucha, is seen as an alternative for the environment, given that it has a fiber composition that is favorable for the production of resistant packaging (DOMENEGHETTI et al. 2019) and biodegradable capacity (MARTINS et al. 2021).

Bacterial cellulose is one of the main constituents of this symbiotic culture of bacteria and yeast, which is considered a high value-added material with many potential uses. However, it has a high production cost, which limits its use. Therefore, the possibility of using the by-product of kombucha from its fermentation process, SCOBY, becomes an increasingly promising alternative (PALUDO 2021).

In addition, increased awareness of environmental sustainability has highlighted the search for high-performance, more affordable bio-based raw materials (LAHIRI et al. 2021).

Although the symbiosis of microorganisms present in kombucha is fundamental for the generation of SCOBY, it is important to note other variables that influence this development, such as: surface area, given that the productivity of the symbiotic colony of bacteria and yeast is related to the surface area ratio, since the metabolic processes of cellulose synthesis depend on oxygen for fermentation to occur (PALUDO 2021); temperature, since at high temperatures (60°C - 80°C) the bacterial film does not form (AL-KALIFAWI & HASSAN 2014); and type of tea, which, added to other process variables, such as sucrose concentration and fermentation parameters, becomes fundamental for cellulose development (LEAL et al. 2018).

Based on the information provided above, the objective of this study was to analyze the factors that influence the development of SCOBY, in order to use it as a raw material for producing new biodegradable materials.

MATERIALS AND METHODS

Study of variables that may influence the development of SCOBY

Three process variables were selected to study and verify the best way in which SCOBY would develop. These are: surface area, temperature, and type of tea. Therefore, based on the study conducted by AL-KALIFAWI & HASSAN (2014) and SANTOS et al. (2019) with adaptations, temperatures of 20°, 30°, and 40°C were selected, containers with surface areas of 144, 289, and 361 cm^2, and three types of tea (5g/L), namely: black tea, green tea, and hibiscus tea.

Statistical experimental design

Statistical experimental planning was performed using Statistica 7.0 software, employing a fractional 3^3-1 factorial design, totaling 9, with the aim of reducing the number of experiments and optimizing resources while maintaining the quality of the information. The variables were defined as: temperature (°C); type of tea (5 g/L); and surface area (cm^2). Table 1 shows the variables and value ranges, and Table 2 shows the factorial design. Treatment in the study was randomized.

Table 1. Process variables and value range.

Parameters	Levels		
	-1	0	+1
Types of tea	Green tea	Black tea	Hibiscus tea
Temperature (°C)	20	30	40
Surface Area ($cm^{2)}$	144	289	361

Table 2. Factorial design 3^3-1 fractional – 9 treatments.

Treatments	Tea (5g/L)	Temperature (°C)	Surface Area (cm^2)
1	Black tea	40	144
2	Black tea	30	289
3	Hibiscus tea	20	289
4	Black tea	20	361
5	Hibiscus tea	40	361
6	Green tea	20	144
7	Green tea	30	361
8	Hibiscus tea	30	144
9	Green tea	40	289

Kombucha production

The kombucha production steps were adapted from the methodologies described in studies by JAYBALAN et al. (2014), AL-KALIFAWI & HASSAN (2014), and MARTINS et al. (2021). 50 g of white granulated sugar (sucrose) and 5 g of herbs were added to 1 liter of filtered water. After that, the tea was left to infuse for 30 minutes, and then strained. Once the tea had cooled (to a temperature of approximately 25°C), it was transferred to a clean, sterilized container, with 900 ml of the contents and 100 ml of carrier tea being transferred.

Finally, the containers were covered with material ideal for gas flow, preventing insects and dirt from entering, but allowing contact with oxygen, necessary for fermentation. They were stored in a Biochemical Oxygen Demand (BOD) incubator at controlled temperatures of 20°, 30°, and 40° for 18 days.

Characterization of SCOBY (Symbiotic Culture of Bacteria and Yeasts)

Centesimal analysis

The determinations of ash, moisture, protein, and lipids were performed following the methodology of the ADOLFO LUTZ INSTITUTE (2008), and the amount of total carbohydrates present was determined by the difference between the values found for moisture, proteins, lipids, and ash.

Thermal Stability

The thermal stability of SCOBY was performed in a thermogravimetric analyzer (model TGA-50H, Shimadzu) under nitrogen flow, with a heating rate of 10 °C/min from room temperature to 900 °C. A sample of SCOBY with a mass of 12.503 mg was used for analysis.

Production of biofilms

The films were prepared using the casting method, with glycerin as a plasticizer and cassava starch as a base. The methodology of PALUDO (2021) and JACOBS et al. (2020) was followed to prepare their composition. First, control films were prepared without adding SCOBY, in which 4 g of cassava starch were diluted in 100 ml of distilled water. The mixture was then mechanically stirred in a water bath for 30 minutes at 80°C. After that, glycerol was added, and two formulations were made, one with 0.4 g (FC1) and the other with 1.5 g of the plasticizer (FC2). From the control solutions, films were prepared with SCOBY.

Previously, a SCOBY solution was prepared with 50 g of cellulose on a wet basis, homogenized in a mixer with 20 ml of distilled water to obtain more uniform samples. After that, to prepare the biofilm derived from bacterial cellulose, 30 g of this solution were added to 100 ml of the control film-forming solution (30% (w/v)).

The samples were poured into Petri dishes, placing 50 g of solution in each dish, then taken to dry in an oven at 45°C until the solvent evaporated.

Figure 1. Samples ready for drying.

Characterization of biofilms

Mechanical properties

The mechanical properties were tested according to the methods of the American Society for Testing and Materials (ASTM D-882-00, 2001) using a universal testing machine (EMIC-DL-2000) at a test speed of 20 mm/min. Tensile strength (TS) and maximum elongation (%E) were analyzed. For analysis, samples of the film were cut into strips 7 cm long. After that, the ends of the films were glued onto small pieces of EVA foam for analysis.

Thickness Analysis

The thickness of the films was measured using a digital caliper (CARBOGRAFITE), taking measurements at four random points on the films, and calculating the mean and standard deviation of the results (CARVALHO et al. 2015).

Moisture content analysis

The moisture content was determined according to the methodology described in the study by COSTA et al. (2017), in which samples of the films were weighed and then placed in an oven to dry at 105°C for 24 hours. After drying, the material was placed in a desiccator for 30 minutes. Finally, the samples were weighed, and the percentage was calculated using equation 1. Where U (%) is moisture content, mi equals initial mass, and mf equals final mass.

$$U(\%) = \frac{(mi-mf)}{mi} \times 100 \qquad \text{(Eq. 1)}$$

Weight analysis

The weight analysis of the films was performed according to the method of SARANTÓPOULOS et al. (2002) and COSTA et al. (2017) with adaptations, where films cut into 4 cm^2 pieces were weighed on an analytical balance. The results were expressed in g/cm^2.

Biodegradability analysis

The procedure was performed following the methodology of MARTINS et al. (2021) and BIELER (2023) with modifications. A small amount of biofilm fragments was placed in a container containing soil samples, and their degradation was observed after 5 days.

RESULTS AND DISCUSSION

Study of SCOBY development

The samples were prepared in accordance with the experimental design in random order. At the end of fermentation (18 days), each SCOBY was weighed to verify the mass obtained. Table 3 shows the results obtained.

Table 3. Sample mass.

Tea (g)	Temperature (°C)	Surface Area (cm^2)	SCOBY mass (g)
Hibiscus tea	20	289	50
Black tea	20	361	37
Green tea	20	144	49.12
Hibiscus tea	30	144	25.21
Black tea	30	289	18
Green tea	30	361	66.8
Hibiscus tea	40	361	10.81
Black tea	40	289	34.76
Green tea	40	144	22.01

The symbiotic culture of bacteria and yeast showed the best yield, equivalent to 66.8 g, in the experiment prepared with green tea (GT), stored at 30°C and in a container with a larger surface area (361 cm^2). The lowest yield was obtained from the sample of hibiscus tea (HT) fermented at 40°C in a container with a surface area of 361 cm^2, resulting in 10.81 g.

In the present study, it was observed that both the experiment that produced the highest mass (treatment 7) and the one with the lowest mass (treatment 5) were obtained in containers with the same surface area, but with different types of tea and temperatures. AL-KALIFAWI & HASSAN (2014) showed that the ideal fermentation temperature for kombucha to obtain good bacterial cellulose production varies between 20 and 50°C, and a container with a larger surface area (227 cm^2) is capable of producing more bacterial cellulose (104.80 g/L) than another container with a smaller surface area (28.26 cm^2), which produces 20.5 g/L.

It was also noted that experiments prepared with green tea and hibiscus tea, fermented at temperatures of 20 and 30°C, produced larger masses of SCOBY compared to those fermented at 40°C. In their study, RODRIGUES et al. (2021) obtained a SCOBY with a mass of 136.14 g after 12 days of fermentation of kombucha prepared with green tea at 30°C.

Characterization of SCOBY

Centesimal analysis

The centesimal characterization was performed on a SCOBY from kombucha prepared with green tea (5 g), white granulated sugar (50 g), carrier tea (100 ml), and drinking water (900 ml), fermented at room temperature.

The centesimal characterization of the SCOBY prepared with green tea was performed, since according to Table 3, this was the treatment in which the bacterial film obtained the highest mass. Table 4 shows the results obtained for the centesimal characterization of the sample.

Table 4. Centesimal characterization of SCOBY.

Parameters	**SCOBY (%)**
Humidity	92,76 ± 0,06
Ashes	0,26 ± 0,02
Proteins	1,21 ± 0,12
Lipids	0,44 ± 0,15
Total fiber	5,30 ± 0,14
Total carbohydrates	5,33

*Average ± standard deviation of results.

There are limited studies in the literature on the centesimal composition of SCOBY, given that most characterizations related to this material are in relation to its microbial composition, as its potential in other areas, such as food, has only recently been observed (PALUDO 2021).

It was observed that this bacterial film contains high moisture content (92.76%), a result close to that found by SHARMA & BHARDWAJ (2020), who obtained a moisture content of 90.04% in their study. For ash, the sample presented 0.26%, below that found by JAYABALAN et al. (2010), who obtained 3.9% after 21 days of fermentation, 2.9% after 14 days, and 0.85% after 7 days.

For the results of proteins, lipids, and carbohydrates, values lower than those obtained by PALUDO (2021) were found, which were 5.07%, 3.36%, and 19.56%, respectively. This fact may be related to the fermentation time and types of substrates used to prepare the tea.

In relation to total fiber, a result of 5.30% was obtained, which is classified as a source of dietary fiber according to Brazilian legislation, RDC No. 54, dated November 12, 2012, and can therefore contribute to the resistance of materials. For the same parameter, JAYBALAN et al. (2010) obtained 6.3% in 7 days of fermentation.

The production of cellulose from bacteria will offer advantages in terms of fiber composition, as it has high purity and does not contain lignin and hemicellulose (CHAN et al. 2018, BIELECKI et al. 2005). Bacterial cellulose has a unique nanofibril network structure, which resembles, to a certain extent, the characteristics of the extracellular matrix (PETERSEN & GATENHOLM 2011).

According to DOMINI et al. (2010), plant cellulose and bacterial cellulose have the same chemical configuration, but the nanometric proportions of the fibers in bacterial cellulose give it different properties, notably high mechanical and tensile strength, as well as the possibility of adding other materials to obtain composites.

Characterization of biofilms

After drying and removal of the plate, the films were characterized. All analyses were performed in triplicate to obtain the best results.

Evaluation of the biofilms produced

The FC1 samples (control film with 0.4 g of glycerin) detached completely from the plate during drying, resulting in brittle films. This result may be related to the small amount of glycerin used, given that the lack of action of this plasticizer results in a rigid and brittle material (COSTA et al. 2017). For this reason, analyses of thickness, moisture content, and biodegradability were performed, but it was not possible to perform the other analyses, as a complete sample of the film was required.

The FC2 films (control film with 1.5 g of glycerin) did not detach from the plate, so it was not possible to perform analyses on them. Figure 2 shows how the control films appeared after drying.

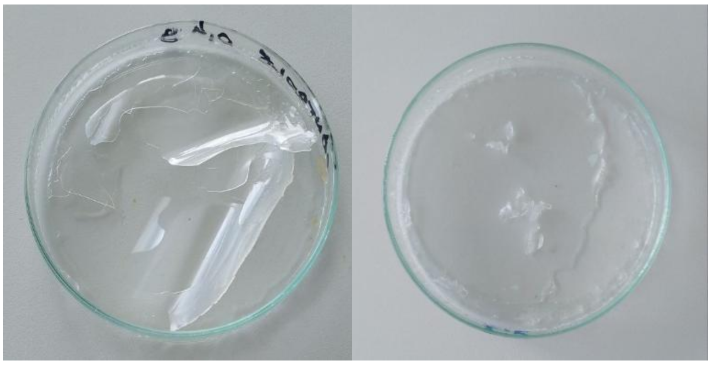

Figure 2. Samples FC1 and FC2.

Sample B1 (0.4 g glycerin + SCOBY) was difficult to remove from the plate. It was firmer than sample B2, but less malleable, resembling a more resistant plastic. In terms of appearance, they had a slightly yellowish color due to the color of the SCOBY, but were transparent and uniform. It can be analyzed in Figure 3.

Figure 3. Sample B1.

Sample B2 (1.5 g glycerin + SCOBY) was easier to remove from the plate than sample B1, being malleable but sticky, easily adhering to other materials. It also showed yellowish discoloration, as can be seen in Figure 4.

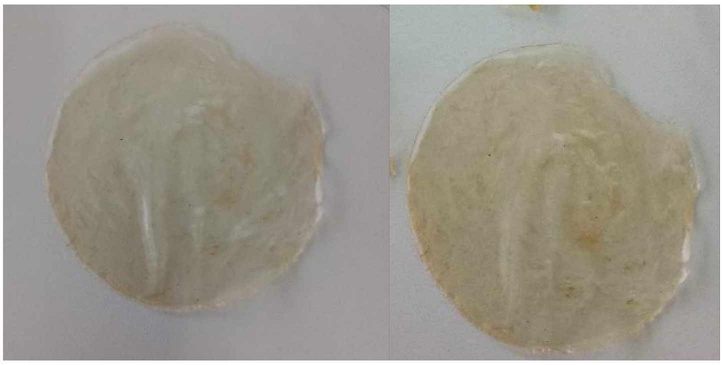

Figure 4. Sample B2.

Given that the control samples became brittle (FC1) or did not detach from the plate (FC2), it was observed that the addition of bacterial cellulose provided resistance to the material, and this may be related to the fact that SCOBY contains fibers such as alpha-cellulose and holocellulose in its composition, which can aid in the manufacture of resistant packaging (DOMENEGHETTI et al. 2019).

It should also be noted that, while a smaller amount of glycerin (0.4 g) produced a film with a drier and less malleable appearance, increasing the amount (1.5 g) produced a more flexible material. The same result was observed by TARIQUE et al. (2021) in their study, in which arrowroot films with 45% glycerol were more flexible than those containing 15 and 30% of the plasticizer, i.e., the increase in glycerol concentration led to increased film malleability.

Thickness

Analyzing Table 5, it can be observed that the addition of bacterial cellulose contributed to the increase in biofilm thickness and that the increase in glycerin resulted in a thinner film (B2). The same result was observed by ALMEIDA et al. (2013) in their study, which developed films formed by blends of bacterial cellulose and potato starch.

The results of the present study were also similar to those found by JACOBS et al. (2020), who obtained a thickness of 0.30 mm for a control film and a thickness equivalent to 0.34 mm for a film with 30% acerola pulp added.

Table 5. Film thickness.

Samples	Thickness (mm)
FC1	$0{,}24 \pm 0{,}01$
FC2	—
B1	$0{,}32 \pm 0{,}01$
B2	$0{,}31 \pm 0{,}02$

*Average $\pm$ standard deviation of results.

It was noted that the sample with the highest amount of glycerin (B2) was thinner than the sample with the lowest amount of plasticizers (B1). This result differs from that found by SHIMAZU et al. (2007), who, when preparing cassava starch films with different glycerin concentrations (0, 5, 10, 15, 30, and 40 g), observed that the thickness of the films varied from 0.07 to 0.10 mm, where films without the plasticizer had a thickness of 0.07 mm and, as the plasticizer content increased, the thickness of the films reached 0.10 mm.

This fact may be related to the increase in the amount of plasticizer used, since the increase in glycerin causes a decrease in the rigidity of the film by increasing the mobility between the polymer chains (ALMEIDA et al. 2013).

Moisture content

Analyzing Table 6, it can be observed that the addition of bacterial cellulose caused an increase in the moisture content of the biofilms, as sample B1 presented

14.4% and its control (FC1) presented 11.57% moisture. This result is consistent, given that the material used to make the biofilm, SCOBY, is composed mainly of water. The present study, for example, obtained a result of 92.76% moisture content for SCOBY, PALUDO (2021) obtained 97.92%, and SHARMA & BHARDWAJ (2020) obtained 90.04%.

Table 6 - Moisture content values.

Samples	Humidity (%)
FC1	$11{,}57 \pm 0{,}01$
FC2	—
B1	$14{,}40 \pm 0{,}01$
B2	$21{,}00 \pm 0{,}00$

*Average $\pm$ standard deviation of results.

It can also be noted that the biofilm with more glycerin (B2) had a higher moisture content than the biofilm with less glycerin (B1). According to DAVOODI et al. (2021), increasing the concentration of the plasticizer causes an increase in the moisture content of the films, as glycerol has hygroscopic properties. Due to the small size of glycerol molecules, water is attracted to the polymer matrix and hydrophilicity increases.

COSTA et al. (2017) prepared films from cowpea starch (Vigna unguiculata (L.) Wap) (3 g) and glycerol (20 and 30%), where the sample with 20% plasticizer had a lower moisture content (14.45%) than the sample with 30%, which had a moisture content of 26.50%. In other words, the increase in glycerin caused an increase in the moisture content of the films.

Weight

Weight, measured as mass per unit area, is of great importance in film production, as it is directly linked to mechanical strength: the higher the weight, the greater the mechanical strength of the material (SARANTÓPOULOS et al. 2002).

Table 7 shows the data obtained in the analysis. The results differed when compared to other studies. ROSSINI et al. (2025), for example, in films made with pine nut starch (24 g), glycerol (12 g), and distilled water (360 ml) obtained results for this same parameter of 0.030, 0.046, and 0.037 g/cm^2. BIELER (2023) obtained average values equivalent to 0.33 and 0.32 g/cm^2, respectively, for the weight of films made from starch (5% w/v), pulp (30%), and apricot peel (30%).

Table 7. Biofilm weight.

Samples	Weight (g/cm^2)
FC1	—
FC2	—
B1	$0{,}18 \pm 0{,}01$
B2	$0{,}16 \pm 0{,}00$

*Average $\pm$ standard deviation of results.

Mechanical properties

Tensile strength is the maximum stress that the film can withstand before failing, i.e., until the moment of rupture (GARCIA et al. 2000). Elongation (E%) refers to the malleability of the material, defining its capacity for deformation before failure (MACLEOD et al. 1997).

These properties of SCOBY composites are shown in Table 8.

Table 8. Tensile strength (TR) and percentage elongation (E) of the biofilms.

Samples	Tensile strength at break (kPa)	E (%)
B1	$0,86 \pm 0,13$	$17,71 \pm 0,01$
B2	$0,71 \pm 0,16$	$11,77 \pm 0,02$

*Average $\pm$ standard deviation of results.

It was observed that sample B1 exhibited greater tensile strength than sample B2. The result may be linked to the fact that sample B1 has a higher weight (0.18 g/cm^2) than B2 (0.16 g/cm^2), since the mechanical strength of a material is directly related to its weight: the higher the weight, the greater the mechanical strength of the material (SARANTÓPOULOS et al. 2002). Thickness is another factor that can also affect sample tension, due to the effect of inter- and intramolecular bonds in the membrane and the orientation of the fibrils (BORRO et al. 2023).

In their study, ANTUNES et al. (2019) observed that increasing the amount of glycerol reduces the breaking tension of the film. The study produced chitosan-based films with different concentrations of glycerol (0.5, 0.75, and 1.0%), noting that the film with the lowest concentration (0.5%) withstood a higher tension than the others.

The plasticizer causes a decrease in mechanical strength and an increase in deformation, since its presence reduces the density of interactions, thus causing an increase in the mobility of polymer chains, resulting in a more elastic and less resistant material (TARIQUE et al. 2021).

For elongation, sample B1 obtained a better result (17.71%) than sample B2 (11.77%), considering that low elongation values result in films with greater fragility (MACLEOD et al. 1997). FARIAS et al. (2012) in films prepared with cassava starch, acerola pulp, and different concentrations of glycerol, noted that the increase in the amount of glycerin was one of the factors that led to increased deformation in the tensile strength of the films, ranging from 23.11 to 44.63%.

MONTERREY-QUINTERO & SOBRAL (2000) noted that the force required to break the films produced in their study ranged from 2.94 N for the film with 70% glycerol to 6.67 N for the film with 30% glycerol. On the other hand, the deformation at break was 2.71% in the biofilm with 30% glycerol and increased to 7.50% in the biofilm with 70% glycerol, indicating that the plasticizer has a major influence on the mechanical properties of these materials.

The mechanical properties of a biofilm depend on the formulation, the origin of the film-forming material, and the method used to make it. The amount of plasticizer used is a factor of paramount importance, as it has the ability to modify the mechanical properties profile of starch films (MALI et al. 2010).

Thermal stability of SCOBY and films

The thermal stability of the materials was evaluated using thermogravimetric analysis (TGA), see Figure 5, a method used to verify mass variation as a function of temperature in a controlled atmosphere under a heating program (OLIVEIRA et al. 2011). It is a thermoanalytical technique that can be used for both raw materials and finished products (SILVA et al. 2007).

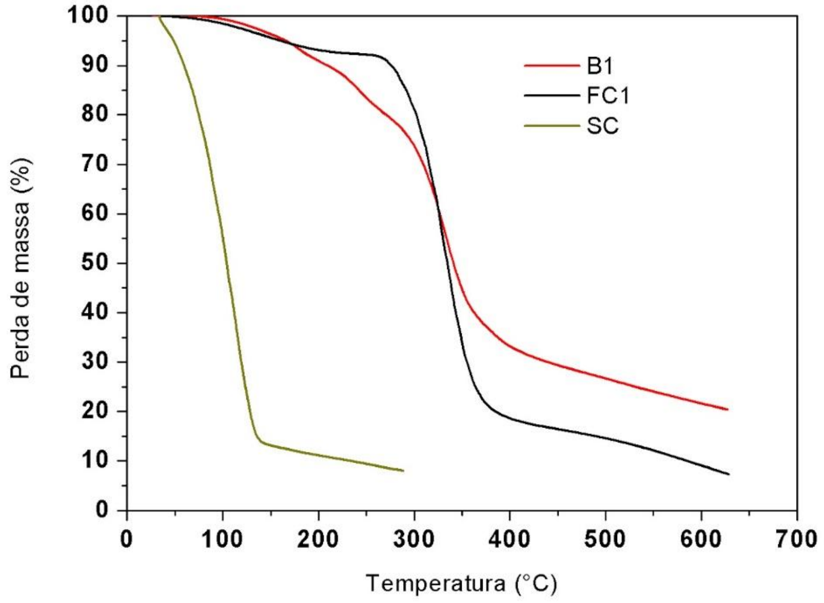

Figure 5. Thermal stability of samples.
*B1: film with 0.4 g of glycerin plus SCOBY; FC1: control film with 0.4 g of glycerin; SC: SCOBY.

Analyzing Figure 5, it was noted that sample B1 began its degradation at approximately 100°C, ending at around 627°C. From 100 to 200°C, a 10% loss of mass was observed, and from approximately 280 to 350°C, a significant loss of mass was observed, approximately 35%, in this range alone. For the same sample, a peak of greater mass loss was also observed at 332°C, beginning at 277°C and ending at 428.09°C. It was also noted that from the moment the sample began its mass loss process at 100°C until the end of the peak (428.09°C), there was a total loss of 68.58%.

Sample FC1 began its degradation process at 126°C, with a mass loss of 80% between this temperature and 432°C. While SCOBY began its degradation process at 34.6°C, from this value up to 163°C it suffered a loss equal to 86.6%.

Comparing the results of the three samples, it was noted that the raw material used for biofilm production, SCOBY, was the material that showed the lowest resistance to temperature, given that it began to degrade at a much lower temperature than the others (34.6°C), which began at 100°C. Based on this result, it was inferred that the addition of glycerin contributed to improved film resistance at high temperatures.

In their research, CELSO et al. (2008) found that the formulation of films is a factor that significantly influences the thermal degradation behavior of the material. The same study, when preparing films from sulfonated poly (ether ether ketone) (SPEEK), derivatives of benzoimidazole and phosphotungstic acid (HPW), obtained results similar to those found for samples B1 and FC1, as the films also began to degrade after 100°C, at 140°C.

PALUDO (2021) found that heat treatment of intact SCOBY resulted in mass loss starting at 150°C, a value much higher than that found in the present study. According to BARROS (2021), fermentation time may be related to the thermal stability of bacterial cellulose, as longer fermentation times make the three-dimensional fiber network of the sample more resistant, requiring more energy to break the bonds.

MARTINS et al. (2021), when analyzing the thermal resistance of this desiccated bacterial cellulose in a more practical way, heating it in a microwave oven for 2 minutes at 100°C, observed that the material underwent significant physical deformation and thermal degradation, presenting cracks and burned areas.

In general, SCOBY had lower thermal resistance and the control sample (glycerin only) had higher thermal resistance. Thermal analysis of the composite composed of glycerin and SCOBY proved that the addition of this matrix increased the thermal resistance of the biofilm made with bacterial cellulose.

Biodegradability

After the 5-day period, fragments of the films were observed in all soil samples, with no significant visual differences between them. The samples were smaller than at the start of the analysis and were stuck together with small pieces of soil, indicating that their biodegradation had begun but had not been completed in 5 days. Figure 6 shows the procedure performed.

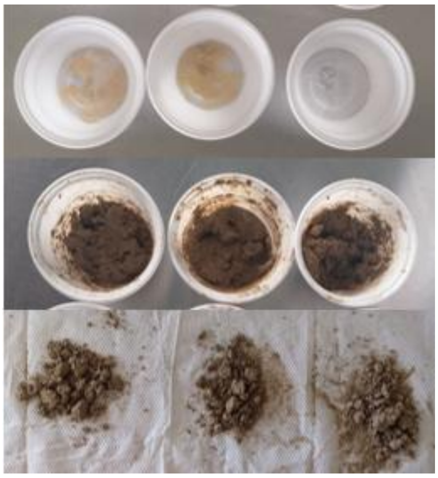

Figure 6. Samples before, during and after biodegradability analysis.

The result was the same as that obtained by BIELER (2023) when testing the biodegradability of films made from starch and apricot pulp over the same period. In their study, MARTINS et al. (2021) also performed the biodegradability test, but on samples of desiccated SCOBY, observing that after a period of one month, the samples had degraded.

It was concluded that although films containing SCOBY appear to be a potentially biodegradable material, they need more time in contact with the soil for complete degradation to occur.

CONCLUSION

From the preparation and subsequent analysis of biofilms made with SCOBY, it was observed that this bacterial cellulose from kombucha fermentation proved to be a raw material with relevant characteristics for the possible development of biopackaging. It is a material with good yield (66.8 g) when made with green tea, stored at 30°C in a container with a surface area of 361 cm^2.

It was observed that the addition of bacterial cellulose to the films provided greater mechanical and thermal resistance to the material. It should be noted that biofilm sample B1, with bacterial cellulose and a lower amount of glycerin in its composition, would be the biofilm with the best characteristics for the possible manufacture of biopackaging, given that it has lower moisture content, higher weight, better mechanical properties, such as greater tensile strength and higher elongation percentage, in addition to being a material resistant to high temperatures.

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AUTHOR CONTRIBUTIONS

Thaís Cristina Honorato Caresto: Conceptualization, methodology and formal analysis, research, writing—preparation of the original draft, writing—revision and editing, visualization. Rodolfo Pessoa de Melo Moura: Methodology and formal analysis, visualization, supervision. Brendo Pablo Ribeiro da Silva: Methodology and formal analysis. Wenderson Gomes dos Santos: Conceptualization, methodology and formal analysis, research, writing - revision and editing, visualization, supervision. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

INFORMED CONSENT STATEMENT

I declare that this research did not involve experiments with humans or animals, therefore, approval by a Research Ethics Committee and the use of a Free and Informed Consent Form or similar document were not necessary.

DATA AVAILABILITY STATEMENT

Data can be made available upon request.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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