

Production and reuse of kombucha waste for cellulose production from different plants*

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Abstract

In this work, kombucha residue (bacterial cellulose) was obtained after fermentation of six herbal infusions. Culture medium was prepared with 5g/L of plants infusion and sweetened with 50g/L of sucrose. Higher film production was 32,33 g.L⁻¹ after 21 days from culture medium formed by 50% of green tea and 50% of *Pereskia aculeate*. All pellicles showed antimicrobial activity against *S.aureus* and SEM of pellicles showed a heterogeneous and dense surface. XDR analysis evidenced cellulose type I properties in pellicles and degree of crystallinity decreased in the pellicles obtained from culture medium with 100% herbal infusions. Thermal properties revealed better thermal stability than kombucha pellicle standard. This research is innovative because it used plants from northeast of Brazil for production of an alternative, low cost and biodegradable material to replace traditional plastics using residues and active agents in addition to reducing pollution.

Keywords: bacterial cellulose; kombucha; herbal infusions.

Data Availability: Research data is available upon request from the corresponding author.

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1. Introduction

Kombucha, an ancient tea of Asian origin, is currently consumed for its beneficial health properties, produced from green tea, traditionally from the *Camellia sinensis* species, sweetened and fermented by a symbiotic culture of bacteria and yeasts^[1]. As a result of fermentation, in addition to the production of a sweet, slightly acidic and carbonated beverage, a by-product known as SCOBY (symbiotic culture of bacteria and yeasts) is formed, which is a bacterial cellulose (BC) film synthesized from the metabolism of bacteria mainly of the genus *Komagataeibacter*^[2,3]. This biofilm, which consists of pure cellulose nanofibrils, high water retention capacity, thermostability and high crystallinity, can be used, after the purification stage, as dressings, biodegradable packaging, clothing, bags, etc^[4,5]. In Brazil, a drink to be recognized as kombucha, according to Normative Instruction N° 41 of 17 September 2019 of the Ministry of Agriculture, Livestock and Supply (MAPA), it must contain drinking water, *Camellia sinensis* infusion, sugars and SCOBY. Other plant extracts can be used, but these are optional^[6].

The SCOBY produced is considered waste and is discarded and disposed of in the environment after the

kombucha production process. In recent decades, bacterial cellulose has been widely researched and proposed as an alternative to replace polluting materials that are difficult to degrade in the environment, due to its various properties such as biodegradability, biocompatibility, low toxicity, high water absorption capacity, among others^[7-9]. It is mainly a component of plants cell wall, but can be synthesized by other organisms such as fungi, algae and especially by the bacteria *Komagataeibacter hansenii*^[10,11]. Some natural plants extracts can be added to the polymer matrix to improve certain desirable properties such as antimicrobial and antioxidant activity due to the presence of active compounds^[12].

In this sense, northeast Brazil is a region with a great biodiversity of plants and vegetables that have biological activity due to the presence of secondary metabolites in their composition. These plants, as well as their waste, can be used to obtain bacterial cellulose after kombucha fermentation, boost the polymeric matrix with antioxidant and antimicrobial properties and reduce the cost process of production^[13]. Some active compounds such as alkaloids, terpenoids and saponins are present in *Centella asiatica*^[14],

caffeine, theobromine, phenolic compounds, flavonoids in *Ilex guayusa*^[15], phenolic acids in *Pereskia aculeata*^[16], flavonoids, steroids, alkaloids in *Duguetia lanceolata*^[17,18] and steroids, flavonoids, catechins, tannins and terpenes in *Pterodon emarginatus*^[19].

Several works develop BC pellicles from different culture medium, using HS or alternative medium, but none of them use kombucha and beverage- like kombucha to produce BC with Brazilian regional herbs. This work aimed to obtain and characterize BC from plant extracts from northeastern Brazilian plants such as ora-pro-nóbis (*Pereskia aculeata*), coronha (*Dioclea violacea*), misteriosa (*Duguetia lanceolata*), sucupira (*Pterodon emarginatus*) to enhance regional sources and compared with other plants such as centelha (*Centella asiatica*) and guayusa (*Ilex guayusa*). Their properties and antimicrobial activity against Gram positive and Gram-negative bacteria were evaluated in order to propose a unique material to apply as biodegradable and antimicrobial packaging.

2. Materials and Methods

2.1 Materials

Leaves of *Camelia sinensis*, *Centella asiatica* (CA), *Ilex guayusa* (IG), *Pereskia aculeata* (PA), *Dioclea violacea* (DV), *Duguetia lanceolata* (DL) e *Pterodon emarginatus* (PE) were purchased in a local market. Sucrose brand olho d'água batch 03A4881 and kombucha from Real kombucha used as starter.

2.2 Pellicle production

Infusions were prepared with 5g.L⁻¹ of corresponding plant (nitrogen source) and sweetened with 50g.L⁻¹ sucrose (carbon source). After the infusion reached room temperature, 24g of SCOBY and 10% of kombucha tea were introduced and remained for 21 days for fermentation at static condition and 25°C. Two culture media of each plant were prepared as follows: 1) 50% of *Camelia sinensis* infusion and 50% of herbal infusion and 2) 100% of herbal infusion as described on Table 1.

2.3 Purification of pellicles

Pellicles were washed with distilled water and then autoclaved for 15 minutes to remove residual microorganisms. They were dried at 50°C in an oven for 3 days and kept in a desiccator with silica gel at room temperature.

2.4 Pellicle characterization

2.4.1 Mid-infrared absorption spectroscopy

The samples were scanned with SPECTRUM 400 spectrometer (Perkin Elmer) with a attenuated total reflectance on following conditions: scanning range was 4000 to 400 cm⁻¹, resolution of 4 cm⁻¹ and 16 scans.

2.4.2 Antimicrobial activity analysis of the pellicles

The disc diffusion method was used to verify the antimicrobial activities against *E. coli* and *S. aureus*, according to Bauer et al.^[20]. Microorganisms were added to sterile water until turbidity was set to 0.5 (McFarland scale), corresponding to 108 CFU/mL to prepare inoculum. Petri dishes with agar

(Müeller-Hinton) were inoculated with 0.1 mL of inoculum and spread on a drigalski spatula. Pellicles samples were added over the agar. So, Petri dishes were incubated in an oven at 35 °C for 24 h. Finally, the diameters of the halos were read with a micrometer.

2.4.3 Thermogravimetric analysis (TGA)

Approximately 4,0 mg of samples were scanned using analyzer Mettler Toledo brand, model TGA/DSC 2 STAR in the following conditions: temperature from 30°C to 600°C, heating rate 10°C.min⁻¹ under a nitrogen atmosphere of flow rate 50mL/min.

2.4.4 X-ray diffractometry (XRD)

XRD pattern was measured with a Rigaku diffractometer model SmartLab with a copper tube, voltage of 40kVe and current of 20mA, on the 2θ scale, with a range of 10° to 70° and scanning speed of 0,5 min⁻¹. Crystallinity index (CI) was calculated according to Segal et al.^[21]:

$$CI = \left[\frac{(cp - ap)}{cp} \right] \times 100\% \quad (1)$$

where cp is crystalline phase and ap amorphous phase.

2.4.5 Scanning electron microscopy (SEM)

SEM was performed with TESCAN MIRA microscopy model MIRA 3 operating at 5kV and magnification of 5kx. Samples were coated with a carbon layer

3. Results and Discussions

3.1 Produced pellicles

The pellicles began to form from day 4 of fermentation as a thin layer at the air-liquid interface and the thickness increased with fermentation time and are shown in Figure 1 after 21 days of fermentation.

Table 1. Description of culture medium.

Medium Code	Description	pH
BC	100% <i>Camellia sinensis</i> tea	2.97
CA100	100% <i>Centella asiatica</i> tea	3.02
CA50	50% <i>Centella asiatica</i> tea	3.01
	50% <i>Camelia sinensis</i> tea	
IG100	100% <i>Ilex guayusa</i> tea	3.10
IG50	50% <i>Ilex guayusa</i> tea	3.05
	50% <i>Camelia sinensis</i> tea	
PA100	100% <i>Pereskia aculeata</i>	3.16
PA50	50% <i>Pereskia aculeata</i>	3.10
	50% <i>Camelia sinensis</i> tea	
DV100	100% <i>Dioclea violácea</i>	3.22
DV50	50% <i>Dioclea violácea</i>	3.11
	50% <i>Camelia sinensis</i> tea	
DL100	100% <i>Duguetia lanceolata</i>	3.06
DL50	50% <i>Duguetia lanceolata</i>	3.03
	50% <i>Camelia sinensis</i> tea	
PE100	100% <i>Pterodon emarginatus</i>	3.11
PE50	50% <i>Pterodon emarginatus</i>	3.04
	50% <i>Camelia sinensis</i> tea	

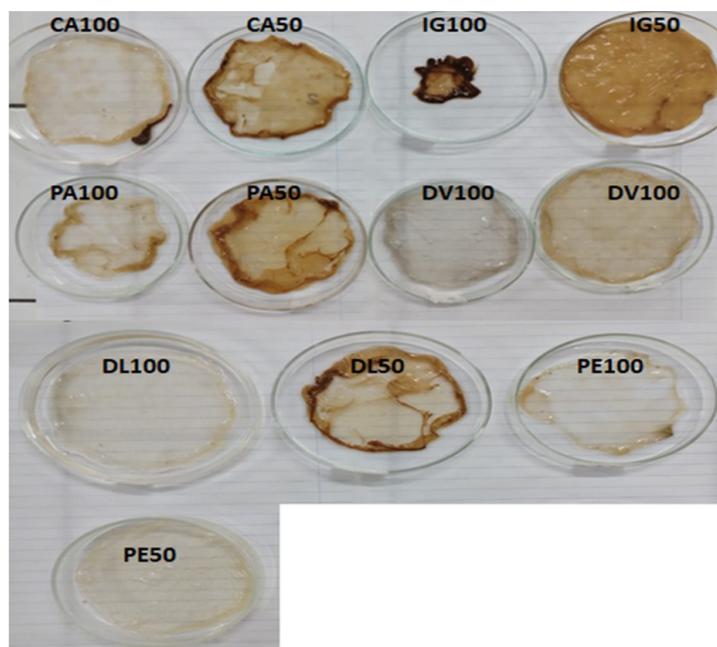


Figure 1. Visual appearance of pellicles.

The pellicles, after fermentation, were homogeneous in appearance, with a gelatinous aspect and translucent and some with a brownish color due to the culture medium in which they were produced. Table 2 shows the wet yield of the films after 21 days. Cellulose production ranged from 5.33 to 32.33 g. L⁻¹, and the highest cellulose yields were in the PA50 and DV100 media with 32.33 ± 2,21 g.L⁻¹ and 30.82 ± 2,29 g.L⁻¹ respectively.

Table 2 shows that the infusions of the different plants and their active compounds in different concentrations interfered with the yield of BC. The highest yield came from the PA50 medium, suggesting that the association of the *Pereskia aculeata* infusion with *Camellia sinensis* was promising for BC production and that the medium had the right nutrients. These results showed that the plants used in this research can be used as an alternative for low-cost bacterial cellulose production.

About yield, Nascimento et al.^[22], produced BC films using HS medium and added grape residues extract *in situ* and *ex situ* in order to obtain an antioxidant material and after 7 days of fermentation, the highest yield was 134.45 ± 1.92 g. L⁻¹ for hydrated BC incorporated with 25% wt of grape residue extract *in situ* and lowest one was 84.45 ± 2.35 g.L⁻¹ for membrane produced with 100% wt of grape residue extract. They concluded that percentage of HS medium influences directly the yield obtained, higher amount of HS medium, higher the yield. Nývák et al.^[23], produced BC pellicles with different cultive media and analysed the possible interference of these methods on characteristics of the BC and results showed that the highest production of BC (wet mass), after 12 days, was produced by microbial consortium produced in black tea (28.49 ± 13.75 g.L⁻¹). They related the result obtained to the carbon source used in each culture medium and new material presented the

Table 2. Yield of wet pellicle in the different culture media.

Medium	Yield (g.L ⁻¹)
BC	5.33 ± 0.81
CA100	15.79 ± 1.21
CA50	10.13 ± 1.03
IG100	3.47 ± 0.42
IG50	25.44 ± 2.39
PA100	8.41 ± 1.06
PA50	32.33 ± 2.21
DV100	30.82 ± 2.29
DV50	24.80 ± 2.37
DL100	22.51 ± 0.87
DL50	14.18 ± 0.68
PE100	8.00 ± 0.72
PE50	25.05 ± 2.11

same properties as those produced by the pure strain in a synthetic culture medium. Barros et al. (2025)^[24], developed BC using an alternative culture media with mandacaru fruit in different concentration and the highest wet mass was 394.20 ± 17.96g, with 80% wt of fruit, after 15 days of fermentation. They proposal a new and low-cost culture media to replace HS media for BC production.

Several factors may have contributed to the results shown in Table 2; for example, certain xanthenes such as theaflavin, thearubigin and caffeine can stimulate the production of cellulose by bacteria. Bacterial cells are also stimulated by vitamins and other nutrients that are released because of the autolysis of yeast cells^[25]. In other cases, extracts with antimicrobial compounds and infusions produced with leaves that exceed 6,0 g/L can restrict the growth of acetic acid bacteria and decrease cellulose synthesis^[26,27].

3.2 Antimicrobial activity of pellicles

The diffusion of the antimicrobial agent forms a halo of inhibition against the exposed microorganisms, and this method allows the sample to be classified as susceptible (diameters above 18 mm); intermediate (diameters between 9 and 18 mm) or resistant to the antimicrobial (diameters less than 9 mm)^[28].

Kombucha and its residues are known to have antimicrobial activity against Gram-positive and Gram-negative microorganisms due to the presence of active components that facilitate permeation of the cell pellicle, interfere in the synthesis of nucleic acid and essential metabolites, causing damage to the cytoplasmic pellicle, leading to cell death^[7,29,30]. Higher resistance to Gram-negative microorganisms is due to their structure being more complex than that of Gram-positive microorganisms, due to the presence of an additional cell layer of peptidoglycan reinforced by another polysaccharide wall, making it difficult for active agents to attack them^[31]. Figure 2 show the results of the analysis of the pellicles produced with the various plant extracts against the microorganisms evaluated after 24 hours and Table 3 shows the inhibition halos of the films.

Excepting DV100 sample, other samples showed the ability to inhibit microbial growth against *E. coli* and were classified as intermediate (CA100, CA50, IG100, PA100, DV50, DI100, DI50 and PE50) and susceptible (IG50, PA50 and PE100). This result is according to other studies that have tested *Dioclea violacea* extract against *E. coli* and found bacterial growth. The weak antimicrobial activity observed for the DV100 sample (produced with 100% *Dioclea violacea* infusion) may be attributed to multiple factors. Although *Dioclea violacea* contains bioactive compounds such as lectins and phenolics, some studies have shown that its lectin alone is not effective in inhibiting

bacterial growth, especially against *E. coli*, unless used in combination with conventional antibiotics. For example, Santos et al.^[32] evaluated the antimicrobial activity of lectin from *Dioclea violacea* against *S. aureus*, *E. coli* and *P. aeruginosa* and found growth of the bacteria tested at the highest concentration used (1024 µg/mL), indicating that the protein doesn't have antibacterial activity when tested alone. Although microorganisms possess various carbohydrates that interact with plant lectins, preventing initial adhesion and the formation of bacterial biofilms, DV lectin alone wasn't effective, but enhance the action of antibiotics when combined, suggesting a more effective use in conjunction with antimicrobial agents.

Additionally, it is possible that at the tested concentration and under the diffusion conditions of the disc assay, the active compounds did not diffuse efficiently through the agar medium, particularly if they have low polarity or high molecular weight, which limits their availability to act on microbial cells^[33].

Table 3. Pellicles inhibition halo in mm.

Sample	<i>E. coli</i>	<i>S. aureus</i>
CA100	15	34
CA50	16	35
IG100	16	23
IG50	19	28
PA100	15	26
PA50	21	35
DV100	Resistant halo	28
DV50	17	39
DL100	17	40
DL50	16	41
PE100	20	17
PE50	18	29

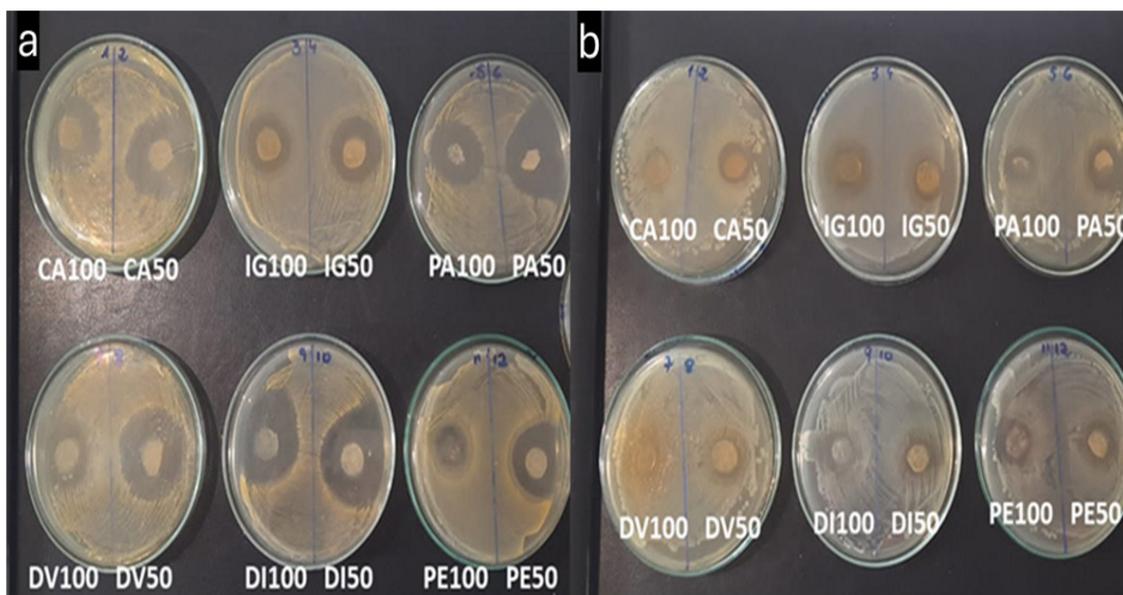


Figure 2. Results of disk diffusion method after 24 hours against a) *S. aureus* b) *E. coli*.

Microorganisms inhibition growth depends on the minimum inhibitory concentration of the active agent present in the extract and the absence of an inhibition halo doesn't mean that the extract is not active against the microorganisms analyzed, but that diffusion has not been completed, as occurs with low-polar compounds where diffusion takes place slowly^[34,35].

The largest inhibition halos against *E. coli* were seen in the PE50 samples, which is explained by the additive interactions between the active agents of *Camellia sinensis* and *Pterodon emarginatus*. In addition to antimicrobial activity, *Pterodon emarginatus* has antioxidant, anti-inflammatory and antibiotic activity^[16,36].

Regarding inhibition against *S. aureus*, all samples were susceptible to inhibition of microbial growth and the largest halo found were in samples DL100 and DL50, both with antimicrobial activity. According to Sousa et al.^[37] the essential oil of *D. lanceolata* bark can inhibit the growth of *S. pyogenes*, *E. coli* and *C. albicans* with a MIC in the range of 20 to 125 µg/mL and *S. aureus* at a concentration of 5 to 10 mg.

3.3 Mid-infrared absorption spectroscopy

The spectra of the BC pellicles and the main characteristic bands are shown in Figure 3 in accordance with the literature^[7,38].

According to Figure 3, for the BC pellicle, there is a peak at 3346 cm⁻¹ which is associated with the vibrational stretching of the hydroxyl group (-OH) of water and polysaccharides. Another peak at 2927 cm⁻¹ is related to the symmetrical and asymmetrical vibrations of the methyl-CH₃ and methylene -CH₂ groups associated with the cellulose matrix^[26,39]. The absorption band at 1632 cm⁻¹ corresponds to the deformation vibrations of the -OH groups of the water bond. The bands in the 1000 to 1200 cm⁻¹ region refer to the C-O-C deformation and C-O vibrations, for example, at 1113 cm⁻¹ the peak is attributed to the stretching of the C-O bond and at 1054 cm⁻¹ the highest absorbance was found due to the vibrations of the carbohydrate rings and the secondary groups (C-O-C; C-OH), confirming the presence of vibrations of the β-1,4 bonds in the polymer matrix^[22,38,39].

The FTIR spectra of the pellicles obtained from different fermentation media did not show significant differences in terms of band position. All samples exhibited the characteristic absorption bands of bacterial cellulose, and no distinct peaks attributable to specific plant extracts were clearly identified. Minor variations in signal intensity were observed; however, these differences may be attributed to experimental factors such as sample contact with the ATR crystal or inherent variability in the measurements. The results suggest that, despite the use of different plant infusions, the chemical structure of the pellicles remains essentially the same, with bacterial cellulose as the predominant component^[22,24,39].

3.4 Scanning electron microscopy

The SEM micrographs of the BC membranes obtained from different kombucha culture media are shown in Figure 4. Overall, the samples exhibit heterogeneous surface morphologies, depending on the type of plant infusion used in the culture medium.

In several samples, notably BC (Figure 4a) and CA50 (Figure 4c), the surface shows a relatively smooth and compact structure, with regions suggesting continuous film-like formation. These characteristics may reflect a dense cellulose layer formed under static fermentation conditions^[22].

In contrast, samples such as IG100 and IG50 (Figures 4d and 4e) display dispersed particulates on the surface, likely corresponding to residual plant components retained during BC formation. The presence of these residues confirms the interaction of plant material with the cellulose matrix during biosynthesis.

In PA50 and DV100 (Figures 4f and 4h), surface irregularities and granular features can be observed, possibly resulting from the incorporation of bioactive compounds or metabolites from the plant infusions^[7]. Some films, particularly DL100 and DL50 (Figures 4j and 4k), exhibit a more homogeneous surface but with visible texture, indicating partial aggregation or rearrangement of the polymer network during drying.

Samples PE100 and PE50 (Figures 4l and 4m) show a denser and more compact morphology, with reduced surface features, suggesting that the plant extract components may have contributed to film compaction or limited the typical fibrillar formation during bacterial cellulose synthesis^[22,26]. While high-resolution nanofibrils are not distinguishable at the current magnification, morphological differences between the samples are evident, supporting the influence of the plant extracts on membrane surface characteristics^[40,41].

3.5 Thermogravimetric analysis (TGA)

Thermal stability of produced films with different culture media was checked by TGA. The thermal decomposition profiles obtained by the analysis are shown in Figure 5, the first derivative curves of mass variation with time (DTG) are shown in Figure 6 and the results for initial degradation temperature (T_{onset}), final temperature (T_{endset}) and maximum degradation temperature (T_{max}) of the pellicles are shown in Table 4.

BC pellicles from kombucha showed two stages of thermal decomposition. All BC films of standard kombucha showed a mass loss due to water evaporation (31.72°C to 86.62°C)

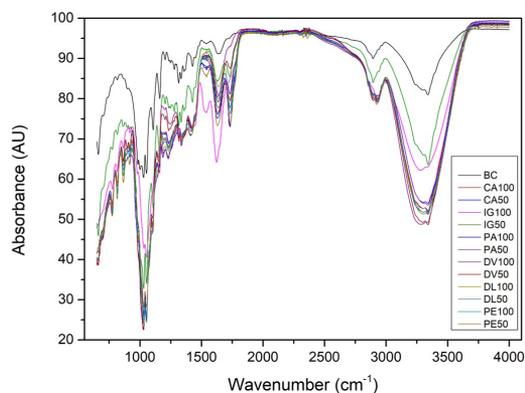


Figure 3. Infrared spectrum of BC pellicles.

and the 2nd stage was in the range of 290.17°C to 357.35°C due to cellulose degradation including depolymerization, dehydration, glucose decomposition and later residue formation^[9,10,26]. BC films obtained from the fermentation various teas presented a loss of volatile compounds, mainly water and the degradation of low molecular weight molecules

from the extracts (29.44 - 155.47°C); a decomposition of micro and macro compounds present in the extracts such as tannins, phenolic compounds, nucleic acids and phospholipids^[26] (109.67 - 283.78°C) and a degradation of non-degradable residues, mainly mineral residues in addition to cellulose degradation itself^[25,40] (265.29 - 387.05 °C).

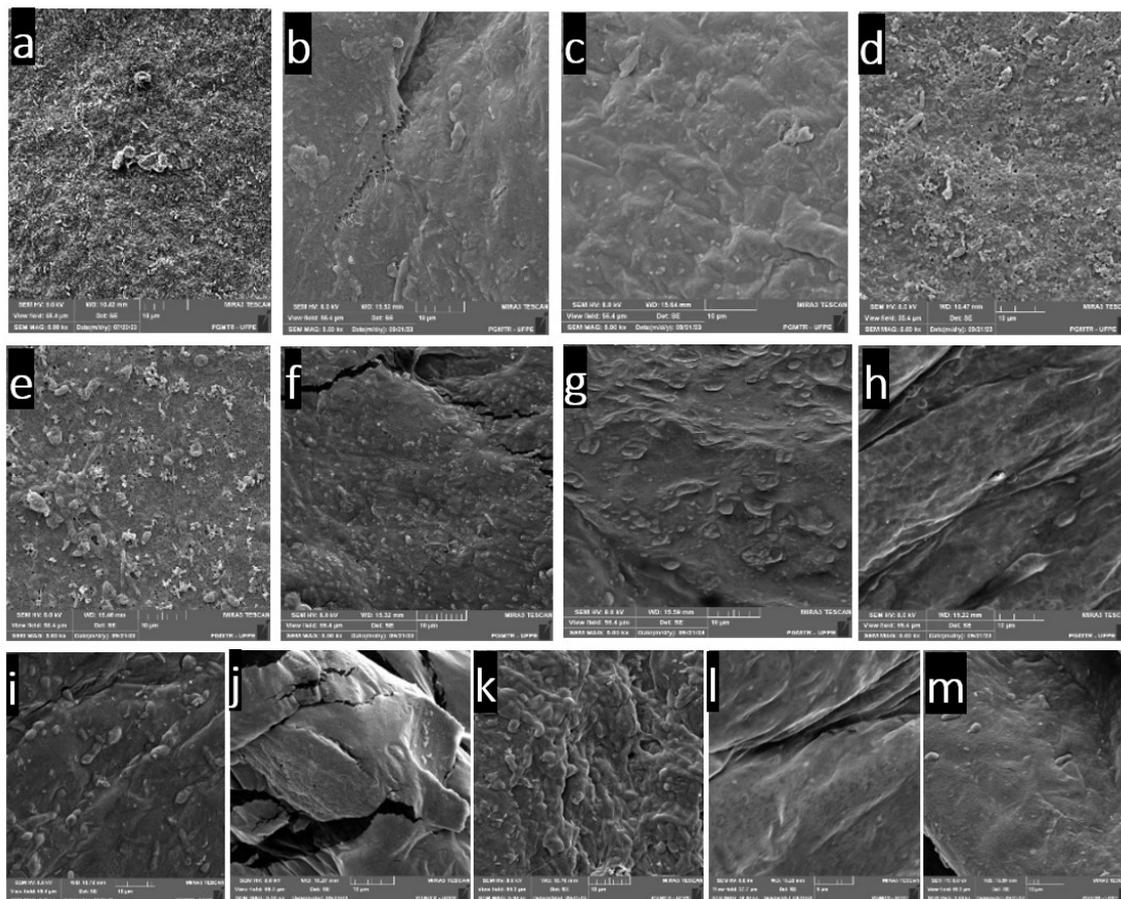


Figure 4. SEM images of the surface of BC membranes from a) kombucha b) CA100 c) CA50 d) IG100 e) IG50 f) PA100 g) PA50 h) DV100 i) DV50 j) DL100 k) DL50 l) PE100 m) PE50.

Table 4. Thermal parameters of kombucha and several teas pellicles.

Sample	1° stage (°C)			2° stage (°C)			3° stage (°C)		
	T _{onset}	T _{endset}	T _{máx}	T _{onset}	T _{endset}	T _{máx}	T _{onset}	T _{endset}	T _{máx}
BC	31.72	86.62	52.77	-	-	-	290.17	357.35	327.99
CA100	29.87	109.67	62.94	127.80	269.70	227.02	317.49	377.24	351.63
CA50	33.07	82.91	55.47	109.67	262.23	227.02	306.82	379.37	352.70
IG100	30.29	78.37	41.96	110.81	219.77	177.38	295.16	371.40	338.40
IG50	30.86	97.43	39.40	113.08	258.17	233.99	265.29	384.49	345.80
PA100	103.05	141.46	118.42	210.81	272.68	241.96	305.76	386.41	356.75
PA50	31.79	113.51	72.33	206.33	270.55	241.53	313.44	387.05	355.68
DV100	29.44	155.47	88.05	160.59	243.95	213.79	296.58	359.74	333.28
DV50	30.72	114.15	64.22	176.88	244.73	209.53	291.89	374.67	346.51
DL100	30.51	123.54	94.95	182.05	283.78	242.81	306.61	380.44	346.72
DL50	29.87	112.44	67.73	166.64	267.78	231.08	300.00	379.80	354.83
PE100	30.93	110.73	54.40	156.61	269.70	230.22	298.29	374.03	339.90
PE50	33.07	110.73	66.14	172.61	273.96	208.89	315.36	381.50	354.83

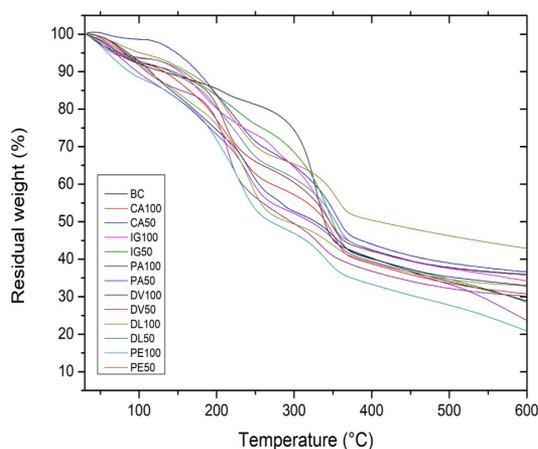


Figure 5. TGA curves for produced pellicles.

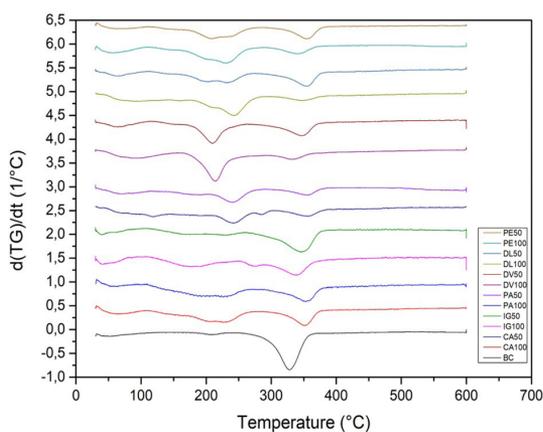


Figure 6. DTG for produced pellicles.

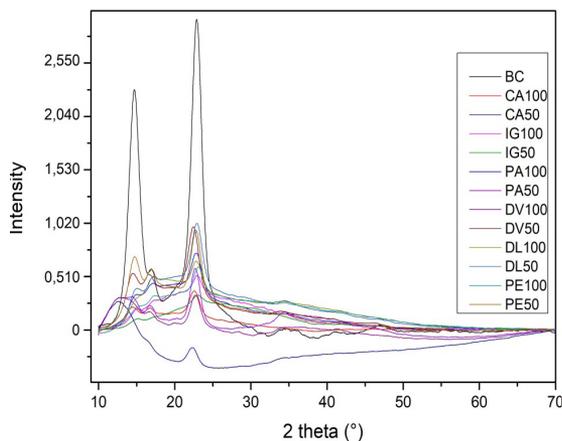


Figure 7. X-ray diffractogram of kombucha and different teas pellicles.

According to Table 4, samples from the different plant extracts have greater thermal stability, except for

Table 5. Crystallinity index of kombucha and teas pellicles.

Samples	Crystallinity index(%)
BC	56.87
CA100	21.28
CA50	11.50
IG100	4.99
IG50	6.15
PA100	18.83
PA50	70.69
DV100	78.12
DV50	16.77
DL100	5.67
DL50	8.94
PE100	4.93
PE50	17.25

IG50 pellicle, due to the onset temperatures (T_{onset}) of the cellulose degradation stage in the pellicles produced from the different teas were higher than those of the standard kombucha pellicles.

3.6 X-ray diffractometry

The diffractograms of produced pellicles are shown in Figure 7 and the analysis was performed to verify if the plant extracts interfered with the crystallinity of the pellicles. All samples showed 2 diffraction peaks, one around $14,6^\circ$ and another more intense one around $22,79^\circ$, corresponding to the (110) and (200) crystal planes respectively. In some samples, a peak can be seen around $16,61^\circ$ corresponding to the (010) crystalline plane, which is also found in bacterial cellulose samples^{[22][25]}. These results show a typical structure of type I cellulose^[7,22,42].

Samples obtained from the various teas showed less intense peaks than the kombucha pellicle (BC), with a reduction in the crystallinity of the pellicles. This reduction in intensity may be related to how phenolics interact during cellulose production^[7]. Based on these results, it was possible to obtain crystallinity index to assess the crystalline and amorphous structure of the pellicles, which are shown in Table 5.

According to Table 5, produced pellicles with different teas have lower crystallinity than kombucha pellicles crystallinity. *In situ* addition can interfere with the crystallinity of the pellicles, modifying the structure and properties of the produced pellicles due to the interaction of plant components in the polymer chain^[22].

4. Conclusion

Bacterial cellulose pellicles were obtained from kombucha and plant extracts from north-eastern Brazil. Thermal analysis showed a reduction in the crystallinity of the pellicles and the appearance of a third stage of degradation. The FTIR showed bands characteristic of bacterial cellulose. In the antimicrobial analysis, all the films showed a halo of inhibition against *S. aureus*. The plant extracts were efficient in producing BC and it could be promising for use in food packaging, given that plant extracts are rich in phenolic compounds that have antimicrobial activity.

5. Author's Contribution

- **Conceptualization** – Glória Maria Vinhas.
- **Data curation** – NA.
- **Formal analysis** – Pâmela Barcelar Ferreira Gomes da Silva de Luna; Karina Carvalho de Souza; Daniella Stepheny Carvalho Andrade; Rhodivam Lucas Mendes Feitosa; Glória Maria Vinhas.
- **Funding acquisition** - Glória Maria Vinhas.
- **Investigation** – Pâmela Barcelar Ferreira Gomes da Silva de Luna; Karina Carvalho de Souza.
- **Methodology** – Pâmela Barcelar Ferreira Gomes da Silva de Luna; Karina Carvalho de Souza; Rhodivam Lucas Mendes Feitosa; Daniella Stepheny Carvalho Andrade; Dayanna Kelly Marques de Oliveira; Fernanda Sobreira Silva; Alexciana Pereira de Melo.
- **Project administration** – Glória Maria Vinhas.
- **Resources** – Glória Maria Vinhas.
- **Software** – NA.
- **Supervision** – Glória Maria Vinhas.
- **Validation** – Glória Maria Vinhas.
- **Visualization** – Pâmela Barcelar Ferreira Gomes da Silva de Luna; Karina Carvalho de Souza; Glória Maria Vinhas.
- **Writing – original draft** – Pâmela Barcelar Ferreira Gomes da Silva de Luna; Karina Carvalho de Souza; Rhodivam Lucas Mendes Feitosa; Daniella Stepheny Carvalho Andrade; Glória Maria Vinhas.
- **Writing – review & editing** – Pâmela Barcelar Ferreira Gomes da Silva de Luna; Karina Carvalho de Souza; Dayanna Kelly Marques de Oliveira; Fernanda Sobreira Silva; Alexciana Pereira de Melo; Glória Maria Vinhas.

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