Characterization of Bacterial Cellulose from Kombucha as a Potential Resource for Its Application on Biodegradable Films

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Abstract The present study is centered on the biosynthesis of bacterial cellulose produced from kombucha inoculum, hereafter referred to as kombucha-derived bacterial cellulose (KBC). For KBC production, Green tea culture media was utilized. *Acetobacter xylinum* cultures were obtained from organic apple vinegar and unpasteurized commercial kombucha. The resulting KBC was characterized by scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and Fourier-transform infrared spectroscopy (FT-IR) in order to verify its morphological characteristics, thermal resistance, and purity. Characterization aimed to evaluate the influence of isolation conditions in this region of the world on the physicochemical properties of KBC and determine its potential as a resource for producing biodegradable films.

Keywords Bacterial cellulose · Kombucha · SCOBY

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Introduction

Due to the growing demand for environmentally sustainable materials, biopolymers represent a viable replacement for synthetic polymers. Extensive research has aimed to obtain high-value-added products from natural polymers, including cellulose, starch, and chitin. Isolated primarily from plants, fungi, trees, and bacteria, cellulose is the most abundant biopolymer on Earth, with approximately 1.5 billion tons generated annually [1]. This material is of great value for producing food packaging, paper, textiles, nanofiber mats, pulp, construction, and fabric materials [2].

Moreover, kombucha is a traditional fermented Chinese beverage whose attributes were treasured by the Qin Dynasty as early as 220 BC. In 414 AD, Dr. Kombu brought kombucha tea from Korea to Japan. Over the following centuries, merchants popularized the drink in Russia, and during the nineteenth and twentieth centuries, it spread throughout Europe. During World War II, shortages of tea and sugar decreased kombucha consumption; however, after the war, it regained popularity in Germany, France, and Italy. Currently, kombucha is one of the most popular fermented drinks produced handcrafted on small scale, and commercial scales [3]; it was valued at 1.84 billion USD in 2019, and the kombucha market forecasts predict strong growth with projections of a 23.2% annual compound growth rate through 2027 [4]. The popularity of kombucha is attributed to its therapeutic effects, such as antimicrobial, antioxidant, anticancer, antidiabetic, detoxifying [5], antifungal, anti-inflammatory, antigenotoxic, and anti-stress properties [6]. It reduces cholesterol and blood pressure levels, promotes weight loss, improves glandular and gastric functions, reduces kidney calcification, combat acne, and inhibits cancer proliferation [7]. The preceding is because it contains different organic acids like gluconic and acetic; additionally, it comprises vitamins, lipids, proteins, polyphenols, minerals (manganese, copper, zinc, iron, cobalt, and cadmium), anions (chloride, fluoride, bromide, iodide, phosphate, and sulfate), etc. [8].

Kombucha is typically produced through the fermentation of sweetened green or black tea using a SCOBY (an acronym for "Symbiotic Culture of Bacteria and Yeast") as a starter culture [9]. The SCOBY is a cellulosic byproduct that forms at the liquid–air interface during fermentation [10]. It consists of a gelatinous membrane composed of pure cellulose [11], generally biosynthesized by *Gram-negative* bacterial strains, including *Acetobacter xylinum, Gluconacetobacter, Agrobacterium, Achromobacter, Sarcina*, etc. [12], whose function is to protect the medium from factors such as ultraviolet light, some fungi, and spores [13]; in the medium there is also yeast such as *Candida kefyr, Candida tropicalis, Dekkera anómala,* to name some of them [14].

Bacterial cellulose (BC) was first reported in 1886 by R. M. Brown, who described it as "a sort of moist skin, swollen, gelatinous and slippery" [15], and has become a valuable biomaterial due to its range of favorable properties; these include exceptional water retention capacity, high degree of polymerization (2000–8000), high crystallinity, mechanical strength, high purity (free from lignin, hemicellulose, and pectin), non-allergenicity, moldability, as well as excellent biocompatibility and biodegradability [16–18] rendering it suitable for applications in tissue engineering,

filtration, electronics, waste treatment, energy production [19], biomedicine, pharmaceuticals, fashion design (for the production of the so-called "vegan leather"), engineering, chemistry, environment; it was also categorized as GRAS (Generally Recognized As Safe) by the FDA (Food and Drug Administration) in 1992, being appropriate for food industry applications such as additive, stabilizer, and gelling in food, and packaging [20].

However, the characteristics, properties, and yield of BC are directly affected by numerous factors such as carbon source (sucrose, glucose, fructose, etc.) [21], incubation period (7–14 days), and temperature of fermentation (28–30 °C) [1]; oxygen pressure and, the amount supplied in the medium; pH (4–6), nitrogen source (yeast) [13]; agitated or static condition of the broth [22], even infusion times and geographical region in which kombucha is elaborated [3].

In this work, kombucha was prepared handcrafted employing a commercial starter inoculum. Then, KBC was characterized by SEM, TGA, and FT-IR to determine its thermal resistance, purity, and morphological characteristics. This is with the objective of obtaining a SCOBY for subsequent cultures, analyzing how these conditions influenced its properties, and determining KBC as a potential resource for its application in the preparation of biodegradable films in future works.

Materials and Methods

Kombucha culture (Vida Bebida), standard sugar, organic apple vinegar (Bragg), green tea, and black tea were purchased from a local market.

Kombucha Started Inoculum

The culture was prepared in a sterilized glass jar where 460 mL of organic vinegar, 235 mL of commercial unpasteurized kombucha, 1 L of water, 80 g of sugar, 2 mL of sugarcane alcohol, and one small homemade kombucha SCOBY were added, with an initial pH of 3. The glass jar was incubated statically at 30 °C for three days.

Preparation of the Infusion of Green Tea

20 g of green tea was boiled at 90 °C in 1 L of purified water and kept under infusion for 10 min, then sachets were removed and let the solution cool until room temperature (20 °C), following the best conditions described by Antolak et al., 2021 [3]. On the fourth day, green tea was added to the glass jar. It was kept incubated statically at 30 °C for 14 days.

Harvesting and Preparation of Kombucha BC

The KBC membrane formed at the liquid–air interface was carefully removed and washed with distilled water. A piece of the sample was placed on a Teflon sheet to dry at room temperature for three days and then cut into smaller pieces to be characterized.

Microbiological Analysis

Microbiological analysis of the kombucha and microscopic observations were conducted using Gram-staining fresh green tea preparation through a Carl Zeiss microscope.

Fourier Transform Infrared (FTIR) Spectrometry

The dried KBC film was characterized using a Perkin Elmer System 2000 Frontier FT-IR spectrophotometer over a wavenumber range of 400 to 4000 cm^{-1}.

Scattering Electron Microscopy (SEM)

The morphology of the BC samples was obtained using a scanning electron microscope (Jeol-IT300) with a voltage of 30 keV.

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was conducted utilizing a Mettler Toledo TGA/SDTA851 thermogravimetric analyzer under the N_2 atmosphere. The sample was heated from 25 to 750 °C at a constant heating rate.

Results and Discussion

A sample of 6 mL of KBC was taken to perform Gram staining and visually analyze the colonies. As observed in Fig. 1a, rod-shaped, reddish homogeneous forms are distinguished, indicating *Acetobacter xylinum,* a Gram-negative bacterium

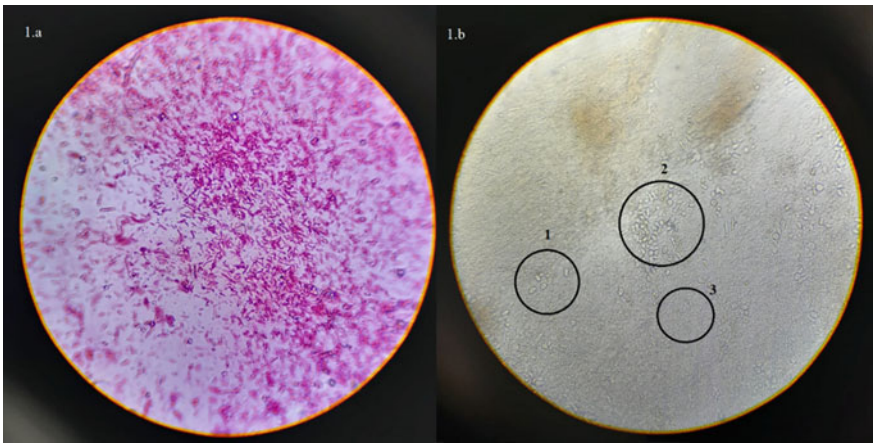

Fig. 1 **a** Gram-negative stain of KBC showing bacteria and **b** native visualization of bacteria and yeast in fresh KBC

that produces BC [23]. Likewise, a smear of the fresh KBC (Fig. 1a) was made, observing symbiosis between yeasts and bacteria (1.b.1), a yeast colony (1.b.2), and homogeneous bacteria (1.b.3) on the surface of the film.

Fourier Transform Infrared (FTIR) Spectrometry

Figure 2, shows the FTIR spectrum where the intensity and position of the absorption peaks are indicated, giving details about the functional groups found in the KBC film, which are similar to the literature and validate the structure of bacterial cellulose.

The band in 3286 cm^{-1} indicates the –OH stretching vibrations [24]. The signal at 2920 cm^{-1} is associated with the –CH stretching [24]. The band at 1645 cm^{-1} corresponds to the –OH bending of the absorbed moisture of the film [25]. Additionally, the signals at 1417 cm^{-1} and 1362 cm^{-1} match with the $-CH_2$ and CH bending, respectively [26, 27]; moreover, the band at 1034 cm^{-1} is associated with either C–O–C and C-O–H stretching vibration of the sugar ring in cellulose [27, 28].

Scattering Electron Microscopy (SEM-E/EDX)

SEM was utilized to analyze the morphological characteristics of the KBC film. As Fig. 3a shows, the SCOBY surface is formed of bacteria in a fibrous configuration and disordered clusters. In Fig. 3b, the bacteria comprising the clusters can be identified, which is consistent with the microbiological observations using optical microscopy. It can be seen that the KBC film presents a high microbial population, as Villarreal

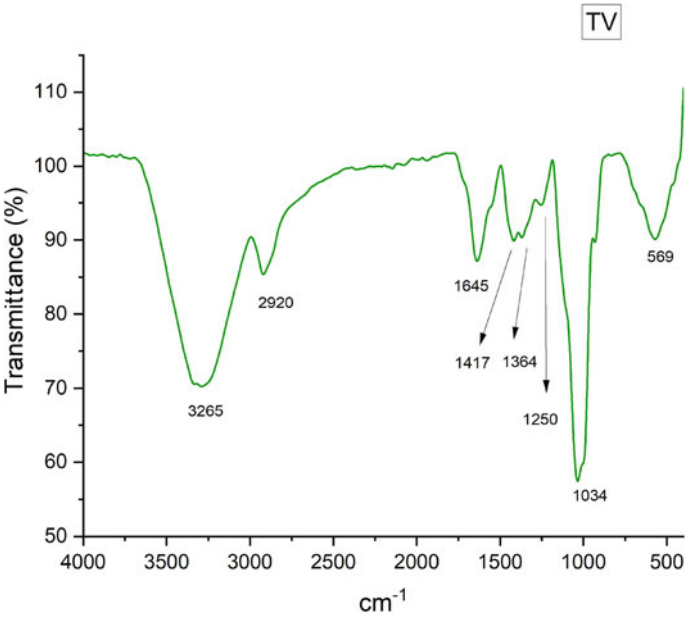

Fig. 2 FTIR spectrum of GTK SCOBY

et al., 2020 indicate, a substantial concentration of microbial cells can obstruct the interaction between fibrils in the network, leading to a decrease in the number of hydrogen bonds and consequently impacting the properties of the biofilm.

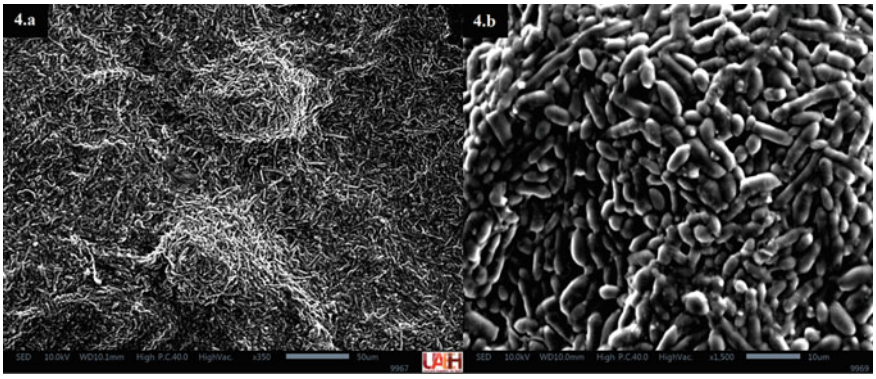

Fig. 3 Scanning electron microscopy (SEM), **a** GTK SCOBY surface (350×), **b** GTK SCOBY surface (1500×)

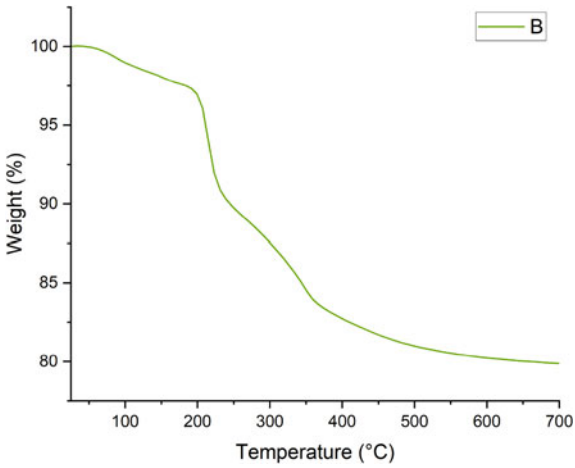

Fig. 4 Thermogram of TGA, KBC film evaluated from 25 to 700 °C under N atmosphere

Thermogravimetric Analysis (TGA)

In Fig. 4, a TGA thermogram is displayed, the first endothermic peak from 25 to 100 °C approximately, corresponds to the evaporation of moisture from the sample resulting in a weight loss of 1.04%, the second peak from 193 to 230 °C consists of the thermal degradation of the KBC sample losing a 15.84% of the weight. From 359 to 700 °C it is observed the decomposition of the KBC corresponds to 20.4% of the weight loss.

In Fig. 4, a TGA thermogram is displayed. The first endothermic peak, occurring approximately between 25 and 100 °C, corresponds to the evaporation of moisture from the sample, resulting in a weight loss of 1.04%. The second peak, ranging from 193 to 230 °C, consists of the thermal degradation of the KBC sample, resulting in a 15.84% weight loss. Between 359 and 700 °C, the decomposition of the KBC is observed, corresponding to a 20.4% weight loss. According to Villarreal et al., 2020, the decline in thermal stability might be associated with a higher crystallinity index.

Conclusions

The green tea kombucha SCOBY film analyzed by SEM shows a high concentration of bacteria, consistent with the microbiological observations, where Gram-negative, rod-shaped, reddish homogeneous forms are observed. As the literature indicates, the properties of the film such as its thermal behavior are influenced by this excess of bacteria on the surface of the SCOBY. Therefore, it is necessary to improve the conditions of the culture and prove other substrates to nourish kombucha, for example, black tea, and carbon sources, apart from improvising other variables such as time of fermentation, temperature, pH, and methods for cleaning the films. Currently, these

variables are under analysis and will be reported in the future; this initial experimentation phase allowed us to obtain the SCOBY, which was subsequently added to the new cultures.

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Conflict of Interest The authors declare that there are no conflicts of interest.

References

1. Lupascu RE, Ghica MV, Dinu Pirvu CE, Popa L, Velescu BS, Arsene AL (2022) An overview regarding microbial aspects of production and applications of bacterial cellulose. Materials 15:1–14. https://doi.org/10.3390/ma15020676
2. Wang J, Tavakoli J, Tang Y (2019) Bacterial cellulose production, properties, and applications with different culture methods—a review. Carbohyd Polym 219:63–76. https://doi.org/10.1016/j.carbpol.2019.05.008
3. Antolak H, Piechota D, Kucharska A (2021) Kombucha tea a double power of bioactive compounds from tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY). Antioxidants 10:1–20. https://doi.org/10.3390/antiox10101541
4. Nyhan LM, Lynch KM, Sahin AW, Arendt EK (2022) Advances in Kombucha tea fermentation: a review. Appl Microbiol 2:73–103. https://doi.org/10.3390/applmicrobiol2010005
5. Dantas Coelho RM, Leite de Almeida A, Gurgel do Amaral RQ, Nascimento da Mota R, de Sousa PH (2020) Kombucha: a review. Int J Gastronomy Food Sci 22:1–12. https://doi.org/10.1016/j.ijgfs.2020.100272
6. Chakravorty S, Bhattacharya S, Bhattacharya D, Sarkar S, Gachhui R (2019) Kombucha: a promising functional beverage prepared from tea. In: Non-alcoholic beverages. Elsevier, pp 285–327. https://doi.org/10.1016/B978-0-12-815270-6.00010-4
7. Laureys D, Britton SJ, De Clippeleer J (2020) Kombucha tea fermentation: a review. J Am Soc Brewing Chem 1–10. https://doi.org/10.1080/03610470.2020.1734150
8. Laavanya D, Shirkole S, Balasubramanian P (2021) Current challenges, applications, and future perspectives of SCOBY cellulose of kombucha fermentation. J Clean Prod 295:1–20. https://doi.org/10.1016/j.jclepro.2021.126454
9. Martínez JL, Ponce García N, Escalante Aburto A (2020) Recent evidence of the beneficial effects associated with glucuronic acid contained in Kombucha beverages. Curr Nutr Rep 1-8. https://doi.org/10.1007/s13668-020-00312-6
10. Amarasekara AS, Wang D, Grady TL (2020) A comparison of kombucha SCOBY bacterial cellulose purification methods. SN Springer Nat J. https://doi.org/10.1007/s42452-020-1982-2
11. Popa L, Ghica MV, Tudoroiu EE, Ionescu DG, Dinu Pirvu CE (2022) Bacterial cellulose—a remarkable polymer as a source for biomaterials tailoring. Materials 15:1–28. https://doi.org/10.3390/ma15031054
12. Chen C, Ding W, Zhang H, Zhang L, Huang Y, Fan M, Sun D et al (2022) Bacterial cellulose-based biomaterials: From fabrication to application. Carbohyd Polym 278:1–14. https://doi.org/10.1016/j.carbpol.2021.118995
13. Rathinamoorthy R, Kiruba T (2020) Bacterial cellulose—a potential material for sustainable, eco-friendly fashion products. J Nat Fibers 1–13. https://doi.org/10.1080/15440478.2020.1842841

14. Gomes MS, de Lima M, Reolon Schmidt VC (2021) Technological aspects of kombucha, its applications and the symbiotic culture (SCOBY), and extraction of compounds of interest: a literature review. Trends Food Sci Technol 110:539–550. https://doi.org/10.1016/j.tifs.2021.02.017
15. Lin D, Liu Z, Shen R, Chen S, Yang X (2020) Bacterial cellulose in food industry: current research and future prospects. Int J Biol Macromol 158:1007–1019. https://doi.org/10.1016/j.ijbiomac.2020.04.230
16. Zhong C (2020) Industrial-scale production and applications of bacterial cellulose. Front Bioeng Biotechnol 8:1–19. https://doi.org/10.3389/fbioe.2020.605374
17. Sperotto G, Stasiak LG, Gongora Godoi JM, Gabiatti NC, Silva De Souza S (2021) A review of culture media for bacterial cellulose production: complex, chemically defined and minimal media modulations. Cellulose 28:2649–2673. https://doi.org/10.1007/s10570-021-03754-5
18. Arruda IAF, Pedro AC, Rampazzo Ribeiro V, Goncalves Bortolini D, Cabral Ozaki MS, Maciel GM, Isidoro Haminiuk CW (2020) Bacterial cellulose: From production optimization to new applications. Int J Biol Macromol 164:2598–2611. https://doi.org/10.1016/j.ijbiomac.2020.07.255
19. Jamsheera CP, Pradeep BV (2021) Production of bacterial cellulose from Acetobacter Species and its application—a review. J Pure Appl Microbiol 15(2):544–555. https://doi.org/10.22207/JPAM.15.2.48
20. Hussain Z, Sajjad W, Khan T, Wahid F (2019) Production of bacterial cellulose from industrial waste: a review. Cellulose 1–17. https://doi.org/10.1007/s10570-019-02307-1
21. Gorgieva S, Trcek J (2019) Bacterial cellulose: production, modification, and perspectives in biomedical applications. Nanomaterials 9:1–20. https://doi.org/10.3390/nano9101352
22. Hamimed S, Abdeljelil N, Landoulsi A, Chatti A, Aljabali AA, Barhoum A (2022) Bacterial cellulose nanofibers. Biosynthesis, unique properties, modification, and emerging applications. In: Handbook of nanocelluloses. Springer Nature Switzerland, pp 1–38. https://doi.org/10.1007/978-3-030-62976-2_15-1
23. Angela C, Young J, Kordayanti S, Partha Devanthi PV, Katherine (2020) Isolation and screening of microbial isolates from kombucha culture for bacterial cellulose production in sugarcane molasses medium. In: The 2019 international conference on biotechnology and life sciences, pp 111–127. https://doi.org/10.18502/kls.v5i2.6444
24. Cazón P, Velázquez G, Vázquez M (2019) Characterization of bacterial cellulose films combined with chitosan and polyvinyl alcohol: evaluation of mechanical and barrier properties. Carbohyd Polym 216:71–85. https://doi.org/10.1016/j.carbpol.2019.03.093
25. Hande NA, Birben M, Bilkay IS (2021) Optimization and physicochemical characterization of enhanced microbial cellulose production with a new kombucha consortium. Process Biochem 108:60–68. https://doi.org/10.1016/j.procbio.2021.06.005
26. Rusdi RAA, Abdul Halim N, Nurazzi Norizan M, Zainal Abidin ZH, Abdullah N, Che Ros F, Azmi AFM (2022) Pre-treatment effect on the structure of bacterial cellulose from Nata de Coco (Acetobacter xylinum). Polimery 67:110–118. https://doi.org/10.14314/polimery.2022.3.3
27. Ghozali M, Meliana Y, Chalid M (2021) Synthesis and characterization of bacterial cellulose by Acetobacter xylinum using liquid tapioca waste. Mater Today Proc 44:2131–2134. https://doi.org/10.1016/j.matpr.2020.12.274
28. Márquez JMR, Rodríguez Quiroz RE, Hernández Rodríguez JP, Rodríguez Romero BA, Flores Breceda H, Napoles Armenta J, Treviño Garza MZ et al (2022) Production and characterization of biocomposite films of bacterial cellulose from Kombucha and coated with chitosan. Polymers 14:1–15. https://doi.org/10.3390/polym14173632