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ORIGINAL RESEARCH

Sustainable bacterial cellulose production by low cost feedstock: evaluation of apple and tea by-products as alternative sources of nutrients

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Abstract The high applicability of Bacterial Cellulose (BC) is often challenging due to its high production costs, which ultimately prevents its widespread use. Therefore, the present study aimed to investigate BC production using alternative feedstock to replace high-cost synthetic carbon and nitrogen sources and to evaluate the physical and structural properties of the produced BC membranes. BC was produced through a microbial consortium from kombucha, and the formulated alternative media sustained promising BC production, especially the association of apple wastes (at 10% (W/V)) with tea mixture, with a yield similar to BC produced on Hestrin–Schramm (HS) control media. Moreover, the BC samples produced in this alternative media also exhibited comparable properties to BC from HS media, with similar water-holding capacity and retention ability, thermal stability, mechanical behavior, and a crystallinity index of 87.61% and 88.08%, respectively. Thus, our findings substantiated that expensive substrates, such as glucose, peptone, and yeast extract, could be successfully replaced by apple wastes, black and green tea, for BC production while maintaining its remarkable physical and structural properties. Furthermore, besides the low-cost advantage, the bioconversion of apple waste also reduces the environmental burden caused by its disposal in landfills.

Keywords Bacterial cellulose · Low-cost substrates · Kombucha · Apple waste · Fermentation

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Introduction

Nowadays, the adoption of global policies, namely bio-economy strategies, to address societal challenges such as global warming, scarcity of natural resources, and unsustainable consumption patterns, have gained prominence to achieve environmental and economic sustainability (le Pera et al. 2022). The fundamental principles of the bio-economy strategy pass through the pursuit of materials from renewable biological resources to replace oil-derived ones. Concerning this, Bacterial cellulose (BC) is an outstanding biodegradable biopolymer, suitable for the replacement of petrochemically derived polymers in many applications, including in the biomedical field, food, and food packaging industries, textile, cosmetic, and even electronics, among others (Ayyappan et al. 2022; Popa et al. 2022; Amorim et al. 2022; Sintharm et al. 2022; Gedarawatte et al. 2022; Płoska et al. 2023).

BC is commonly produced, at the laboratory scale, by isolated acetic acid bacteria, and *Komagataeibacter xylinus* species is the most renowned BC producer from carbon and nitrogen sources (Gorgieva and Trček 2019). Nonetheless, the spontaneous formation of non-producing BC mutants during the fermentation process, as well as the instability of genetically modified BC-producing strains, is one of the bottlenecks in the scale-up process for BC industrial production (Krystynowicz et al. 2002; Skiba et al. 2021). The use of a microbial consortium, a symbiotic culture of bacteria and yeasts, has been appointed as a stable consortium for BC biosynthesis scale-up due to its multispecies composition, the capacity to utilize different carbon sources, high adaptive potential, and tolerance to phage infections, which also allows BC production under non-sterile conditions (Skiba et al. 2020, 2021). This approach has been successfully employed, on a pilot scale, for BC biosynthesis (Skiba et al. 2020) and also at a laboratory scale, using the fermented tea drink kombucha, where the symbiotic culture of bacteria and yeasts are responsible for the fermentation process (Treviño-Garza et al. 2020; Leonarski et al. 2021).

The microbial ecosystems possess enormous diversity and complexity, and the different yeasts and bacterial species process the fermentation substrates in different and complementary manners. Yeasts hydrolyze sucrose into glucose and fructose and produce ethanol, while acetic acid bacteria metabolize glucose and ethanol to produce gluconic acid and acetic acid (Jayabalan et al. 2014; Villarreal-Soto et al. 2018; Coelho et al. 2020; Ramírez Tapias et al. 2022). BC is produced during fermentation and appears as a thin membrane on the surface of the liquid–air interface. Bacteria synthesize cellulose using cellulose synthase to polymerize glucose residues in β1-4-glucan chains. These linear chains, which are secreted extracellularly, after organization and crystallization by hydrogen bonds and Van der Walls forces, form a resistant three-dimensional structure called microfibril, after which cellulose ribbons are assembled, and ultimately form the visible BC pellicle (Jozala et al. 2016). In contrast to obtaining vegetable cellulose, BC contains no undesirable compounds (such as lignin, pectin, and hemicellulose), reducing production costs and environmental impact (Khattab et al. 2017; Faria-Tischer et al. 2019; Mona et al. 2019). Nonetheless, BC production is an expensive process, mainly attributed to the costs of synthetic nutrient media (Raiszadeh-Jahromi et al. 2020; Skiba et al. 2021). Hence, solutions to minimize the cost of the fermentation media are required to produce BC in an economically viable manner. Alternatives have been reported with low-cost fermentation media, using a wide variety of agro-industrial wastes, such as hydrolyzed fruit and vegetable peels (Güzel and Akpınar 2019, 2020; Naeem et al. 2020), palm date and sugarcane molasses wastes (Abol-Fotouh et al. 2020), wheat straw and corn stalk (Chen et al. 2013; Cheng et al. 2017) and even wastes of the most extensively used synthetic polyester, terylene (Zhang et al. 2021). The simultaneous utilization of more than one type of agro-industrial residue to provide all the nutritional requirements for the bioprocess has also been reported. Low-cost date syrup and the waste product of a cheese-making process, cheese whey (Raiszadeh-Jahromi et al. 2020), the combination of two different by-products derived from liquor Baijiu production (He et al. 2020), and cashew apple juice and soybean molasses as carbon and nutrient sources are some of the combined examples explored, for BC production (Souza et al. 2020).

The industrial processing of apples for juice production, cider manufacturing, and pulp for food industry applications generates a vast amount of waste, mainly consisting of peel, core, seeds, calyx, and stems (Vendruscolo et al. 2008; Urbina et al. 2017). Only a limited fraction of this waste is utilized, as fertilizer, fuel, or feed. The majority is disposed of in landfills, causing environmental pollution, besides the extensive resources waste, since apples contain high carbohydrate content, which could be bioconverted into value-added products (Urbina et al. 2017; Zhang et al. 2020). For instance, high fructose content (19 g/100 g of apples dry weight (dw)) has been reported for apples such as Bravo de Esmolfe variety, also containing lower amounts of glucose (8.4 g/100 g dw) and sucrose (1.38 g/100 g dw) (Pires et al. 2018), which could be bioconverted to value-added products, avoiding further depletion of other resources, while minimizing food waste, one of the main challenges of a circular economy (Provin et al. 2021).

Thus, this study aimed to evaluate the suitability of apple processing by-products as a carbon source for BC production using a microbial consortium from kombucha. Moreover, a combination of apple residues with a green and black tea mixture was also evaluated to replace the nitrogen and carbon sources from the commercial media often used for BC production. The impact of these carbon and nitrogen sources on the physical and structural properties of the produced BC membranes was also investigated. To the best of our knowledge, the present study is the first report of BC production by a microbial consortium with apple residues and a mixture of black and green tea as carbon and nitrogen sources.

Materials and methods

Materials

The apple variety Bravo de Esmolfe, green, and black tea were selected from a local wholesale distributor in Portugal. Kombucha Original Bio was provided by Freshness Diagonal, Lda (Montijo, Portugal). Glucose, peptone, yeast extract, disodium hydrogen phosphate (Na_2HPO_4), and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich, and citric acid was obtained from Panreac.

Alternative feedstock preparation from apple residues

Apple residues, consisting of peel, core, seeds, calyx, and stem, were completely dehydrated using a domestic dehydrator at 60 °C foris~8 h. Then, the alternative feedstock was obtained by boiling the dehydrated apple wastes in distilled water (Fennir et al. 2003). After performing the extraction procedure for 30 min with a 1:30 liquor ratio, the solution obtained was filtered to remove solid matter and debris, and oven-dried until a constant weight was achieved.

Inoculum preparation

Kombucha Original Bio commercial beverage containing bacterial and yeast species (Marsh et al. 2014) was used to prepare the inoculum for the experiments. The inoculum was prepared with 10% (v/v) of Kombucha in Hestrin–Schramm (HS) broth (2% (W/V) glucose, 0.5% (W/V) peptone, 0.5% (W/V) yeast extract, 0.27% (W/V) Na_2HPO_4, and 0.15% (W/V) of citric acid). The pH of the medium was adjusted to 6.0 and then incubated at 30 °C in static culture for 120 h to obtain a sufficient number of bacterial cells for inoculation. After this period, the inoculum flasks were vigorously shaken to remove cells embedded in the BC matrix already being formed at the air–liquid interface. The resulting cell suspensions were used for inoculations (Ruka et al. 2012).

Preparation of fermentation medium and BC production

HS was used as control fermentation media and also to produce the control BC samples. Alternatively, various fermentation media were formulated using apple wastes (AW) and a tea mixture (TM) as carbon and nitrogen sources. Briefly, the carbon source from HS media, 2% (W/V) glucose, was replaced with AW (2%, 5%, or 10% (W/V)), and similarly, the nitrogen sources from HS media, 0.5% (W/V) peptone, 0.5% (W/V) yeast extract, were replaced with the same amount of a tea mixture (0.5% (W/V) black tea, 0.5% (W/V) green tea). Once the alternative fermentation media were formulated based on HS media composition, each alternative media also contained 0.27% (W/V) of Na_2HPO_4, 0.15% (W/V) of citric acid, and 10% (v/v) of the previously prepared inoculum to initiate BC biosynthesis, which was accomplished at 30 °C. Table 1 details the carbon and nitrogen content of each formulation.

Harvesting and BC purification

After completing the 7 days of fermentation, each BC pellicle was harvested. The cellular debris and media components were removed by treatment with 0.1 M NaOH at 80 °C for 30 min (Santoso et al. 2020). After this washing procedure BC membranes were thoroughly rinsed with distilled water until reaching a neutral pH.

Determination of BC dry and wet weights

The wet weight of BC was measured, after washing it with distilled water, after only removing moisture excess from its surface. After drying, until a constant weight was achieved, BC dry weight was determined

Table 1 Carbon and nitrogen content in the formulated HS-based media

Media designation	Carbon source	Nitrogen source
HS	2% (W/V) glucose	0.5% (W/V) peptone 0.5% (W/V) yeast extract
HS-AW	2% (W/V) apple wastes	0.5% (W/V) peptone 0.5% (W/V) yeast extract
HS-TM	2% (W/V) glucose	0.5% (W/V) black tea 0.5% (W/V) green tea
TM-AW	2% (W/V) apple wastes	0.5% (W/V) black tea 0.5% (W/V) green tea
HS-5AW	5% (W/V) apple wastes	0.5% (W/V) peptone 0.5% (W/V) yeast extract
HS-10AW	10% (W/V) apple wastes	0.5% (W/V) peptone 0.5% (W/V) yeast extract
TM-5AW	5% (W/V) apple wastes	0.5% (W/V) black tea 0.5% (W/V) green tea
TM-10AW	10% (W/V) apple wastes	0.5% (W/V) black tea 0.5% (W/V) green tea

using an analytical balance (with readability to 0.01 mg, Mettler Toledo, Switzerland).

Production yield

Production yield is an important criterion to measure the process performance and to establish comparisons with values reported in the literature, given the importance of increasing BC productivity while decreasing production costs. The production yield was obtained with the ratio of BC dry weight to carbon source weight (Han et al. 2019), as well as with the nitrogen source weight (Yim et al. 2017) in each fermentation media used. Moreover, BC productivity per volume of fermentation media was also calculated (Han et al. 2020). Thus, the production yield of BC was calculated accordingly with Eqs. (1), (2), and (3):

$$\text{Yield by carbon source}(\%) = \frac{W_{dry}}{C} \times 100 \tag{1}$$

$$\text{Yield by nitrogen source}(\%) = \frac{W_{dry}}{N} \times 100 \tag{2}$$

$$\text{BC Yield}(\text{g/L}) = \frac{W_{dry}}{V} \tag{3}$$

where W_{dry} is BC dry weight, C and N represent the weight of carbon and nitrogen source, respectively, and V is the medium volume used in BC production. For further comparison purposes, the BC production rate was also determined using BC yield (g/L) and the production time in days (D), accordingly to Eq. (4):

$$\text{BC production rate}(\text{g/L/}day) = \frac{BC\ Yield}{D} \tag{4}$$

BC thickness and opacity

A micrometer (Adamel Lhomargy MI20, France) was used to measure BC thickness by averaging ten different points of each BC sample.

The opacity of BC membranes was measured using a UV–Vis spectrophotometer (UV-300 Unicam), as previously described (Han and Floros 1997; Salari et al. 2018). BC dry samples were cut to fit perpendicularly into a glass cuvette, whereas an empty glass cuvette was used as a reference. The opacity of BC membranes was determined by the following Eq. (5):

$$\text{Opacity} = \frac{Abs_{600}}{T} \tag{5}$$

where Abs_{600} is the absorbance of BC dry samples at 600 nm and T is the BC membranes thickness (mm).

BC surface color evaluation

The surface colors of BC membranes, obtained using different carbon and nitrogen sources, were evaluated, and their whiteness index (WI) was calculated (Jipa et al. 2012; Han et al. 2019). The values of L^*, a^*, and b^* were recorded using a Datacolor 110 spectrophotometer (Datacolor company, USA) with the CIELAB color system, under illuminant D65, by a 10° standard observer. These values were then used to calculate the WI shown in Eq. (6):

$$WI = 100 - \left[(100 - L^*)^2 + (a^{*2} + b^{*2})\right]^{1/2} \tag{6}$$

where L^* indicates lightness, a^* represents samples' redness/greenness, and b^* the yellowness/blueness. Values were measured at five random points per sample, at least, and averaged.

Scanning electron microscopy (SEM)

The morphological analysis of BC samples was accomplished using a scanning electron microscope (S-3400N, Hitachi, Tokyo, Japan). The accelerating voltage was 10 kV, and all BC samples were sputter-coated with a thin layer of gold (Q150R ES, Quorum Technologies Ltd, Laughton, UK) before scanning, and the images were captured at 5000× magnification.

Water holding capacity (WHC) and water retention rate (WRR)

To determine the WHC, the sieve shake method was used (Ul-Islam et al. 2012; Barshan et al. 2019). Briefly, dried BC membranes were immersed in distilled water and, after complete rehydration, placed in a sieve, quickly shaken twice (in order to remove the surface water), and then weighed. Afterward, the BC membranes were dried until complete water removal and thus constant weight. WHC was calculated as shown in Eq. (7):

$$WHC\ (g/g) = \frac{W_{wet}}{W_{dry}} \tag{7}$$

where W_{wet} is BC wet weight after applying the sieve shake method, and W_{dry} represents BC dry weight.

The sample's water retention rate (WRR) was determined by measuring their weights every 30 min while drying at room temperature until constant dry weight. The weight values were plotted as percentages of wet weight versus time (Ul-Islam et al. 2012).

Analysis by Fourier transform infrared spectroscopy (FTIR)

The spectroscopy analysis was used to compare functional groups of BC obtained from the different fermentation media formulations. FTIR spectra were collected at room temperature using a Thermo-Nicolet is10 FT-IR spectrometer equipped with an attenuated total reflection (ATR) accessory. The scanning was performed at wavenumbers between 4000 and 500 cm^{-1}, with 4 cm^{-1} resolution, and an average of 64 scans per sample.

X-ray diffractometry (XRD)

X-ray diffraction was performed using Rigaku DMAX III/C diffractometer with a copper x-ray source (1.54 Å) at filament emission of 30 mA and voltage of 40 kV. Radiation reflection of the samples, the dried BC membranes, was measured over a range of 2θ angle of 5–60° and a test speed of 1.2°/min. Background subtraction and smoothing were not applied to the diffraction patterns. The crystallinity index of the produced BC samples was determined by the following equation:

$$CrI\ (\%) = \left[I_{110} - I_{am}/I_{110}\right] \times 100 \tag{8}$$

where the maximum intensity value I_{110}, which corresponds to $I_{110,}$[1] is found between the scattering angles of 2θ between 22° and 23°, and I_{am} is the intensity minimum between the (010) and (110) peaks (Segal et al. 1959).

Differential scanning calorimetry (DSC) measurements

Thermal analysis of BC membranes obtained from the fermentation media with different compositions was carried out on a DSC analyzer (DSC 204 Phoenix Netzsch, Germany). Small quantities (5 mg) of dry BC samples were placed into aluminum pans, sealed, and heated from 30 to 400 °C at a heating rate of 5 °C/min. An empty aluminum pan was used as a reference.

[1] Segal's original (200) indices were for a convention different than that required for *Cellulose*, and the (110) indices here are specific for cellulose I_α as per (French 2014).

Tensile properties evaluation

The mechanical properties (tensile strength, Young's modulus, and elongation at break) of BC samples obtained from fermentation with alternative feedstock and commercial media (HS) were characterized by a Universal tensile test machine (Adamel Lhomargy Division d'Instruments Model DY-35, France). BC membranes were cut into rectangular shape specimens with 5 × 30 mm, the cell preload was 100 N, and a testing speed of 1 mm/min was applied at room temperature, as previously reported (Gea et al. 2011). The testing was performed for at least 5 specimens from the same production batch, stored in desiccators for 24 h before testing, and tensile strength, Young's modulus, and elongation at break were evaluated.

Statistical analysis

All experiments were conducted in triplicate unless otherwise stated. The data are presented as the mean value of the triplicate with standard deviation (SD). Significant differences were determined according to one-way or two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test at $p < 0.05$, using GraphPad Prism 6 software.

Results and discussion

Alternative feedstock as carbon and nitrogen sources

BC production costs are mainly attributed to synthetic media components, which are quite expensive (Ul-Islam et al. 2020). High amounts of carbon source are characteristic of synthetic media for BC production to improve cell growth and metabolism, being glucose the sole carbon source in HS media (Güzel and Akpınar 2020; Treviño-Garza et al. 2020). Alternative carbon sources have been explored for BC production, with agro-industrial residues being a promissory approach to reduce production costs (Fernandes et al. 2020).

In this work, apple waste from *Malus domestica* Borkh. cv Bravo de Esmolfe, a typical Portuguese apple cultivar, was tested as a carbon source for BC production. In 100 g of apples, around 34 g of waste was collected, which includes peels, stems, seeds, calyx, and some soft tissue that remained attached to the peels. After the dehydration procedure, the apple's residues weight was reduced to 6.5 g since 81% of the apple residues was water, and the final AW crude extract obtained from this amount of dehydrated apple residues was 3.19 g, which corresponds to 49.15 g/100 g dw. The nutritional and chemical composition of Bravo de Esmolfe apples has already been reported, showing that carbohydrates are the most abundant macronutrients in their composition. Fructose was detected in higher amounts although glucose and sucrose were also identified (Feliciano et al. 2010; Pires et al. 2018). During BC production, nitrogen sources also play a major role in microorganisms' growth and cell construction (Yim et al. 2017; Fernandes et al. 2020). Synthetic media for BC production, HS, contains yeast extract and peptone for nitrogen supply. Nonetheless, sodium glutamate, glycine, ammonium sulfate, hydrolyzed casein, and several tea extracts have also been explored as suitable nitrogen sources for BC production (Yim et al. 2017; Song and Kim 2019; Fernandes et al. 2020). The tea extracts evaluated include corn silk tea, rooibos tea, and, more commonly, green and black tea (Yim et al. 2017; Kamiński et al. 2020; Treviño-Garza et al. 2020; Leonarski et al. 2021). Besides nitrogen, tea extracts contain polyphenols, flavonoids, amino acids, and proteins, among other essential components in substrates for acetic acid bacteria (Łuczaj and Skrzydlewska 2005; Yim et al. 2017; Musial et al. 2020; Treviño-Garza et al. 2020).

After 7 days of static culture, the BC membranes produced with HS media (control) and the different media formulations containing apple wastes and tea mixture (as shown in Table 1) were harvested and washed (Santoso et al. 2020). The BC production yields in the modified media were compared with the HS production yield, and the results are summarized in Fig. 1. The production yield was calculated using the ratio of the dry BC weight to that of each carbon source and each nitrogen source weight, Fig. 1a, and clearly shows that the yields obtained using alternative feedstock were lower when compared with HS medium, which was expected due to the high purity of chemical-grade carbon and nitrogen sources present in HS media. Figure 1b presents the same pattern regarding the BC productivity per volume of fermentation, the yield obtained using HS-AW (0.21 ± 0.02 g/L) and TM-AW (0.24 ± 0.02 g/L) is very similar, whilst

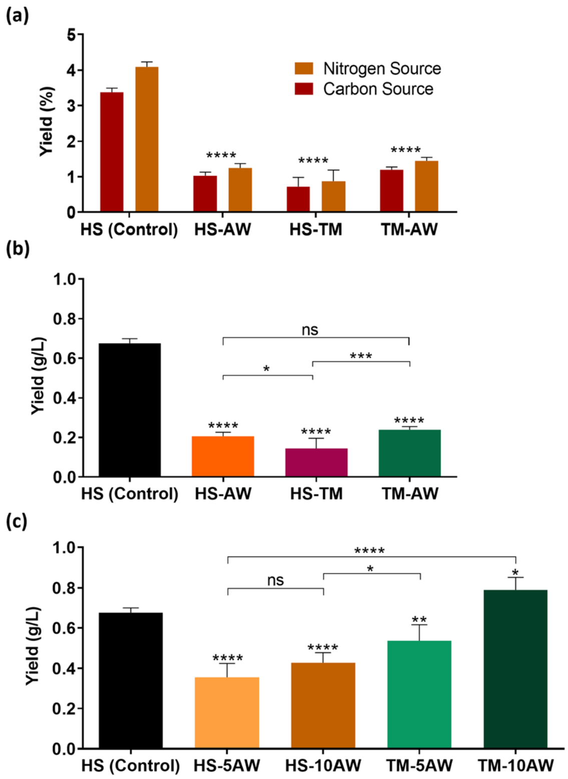

Fig. 1 Yield obtained with the different media formulations. **a** Production yield calculated using the ratio of the dry BC weight to that of each carbon source weight (glucose or apple wastes) and each nitrogen source weight (yeast extract and peptone or black and green tea mixture); **b** BC productivity per volume of fermentation media, with the same amount of carbon and nitrogen sources as the control media (HS); **c** BC productivity per volume of fermentation, with increasing carbon source concentration, 5 and 10%. Data presented as mean ± SD, ns indicates non-significant, * $p<.05$, ** $p<.01$, *** $p<.001$, and **** $p<.0001$. Different asterisks above bars indicate significant differences for comparisons between control (HS media) and the different media formulations, comparisons between media formulations are also shown with the connecting lines

the tea mixture with glucose (HS-TM media) had the lowest yield, 0.14 ± 0.05 g/L. The highest yield was obtained with the HS control media (0.68 ± 0.02 g/L), as reported in previous works when comparing BC productivity in HS medium with alternative feedstocks (Costa et al. 2017; Zhao et al. 2018; Zhang et al. 2021). Therefore, apple waste concentration was increased from 2% (corresponding to glucose amount in HS media) to 5% and 10%. This increase provided a higher BC yield (Fig. 1c), as expected, since the first evaluation compared the same amount, 2% (W/V), of laboratory-grade glucose, with high purity, with apple wastes crude extract as carbon sources. Despite the higher yield obtained, no significant differences were observed between 5 and 10% of apple waste extract concentration with yeast extract and peptone (HS-5AW and HS-10AW). In this case, the higher apple wastes concentration may cause an imbalance between the carbon source and cellular materials, since the higher availability of carbon source for the biochemical processes, also results in a great amount of gluconic acid produced, which acidifies the fermentation medium and could impair BC production (Yim et al. 2017; Abol-Fotouh et al. 2020). On the other hand, regarding the TM-AW media, increasing apple wastes concentration resulted in better yields, especially with 10% apple waste (0.79 ± 0.06 g/L), the BC yield was even higher than the yield obtained in the HS commercial medium (0.68 ± 0.02 g/L), used as control. Hence, increasing carbon source concentration revealed the influence of nitrogen sources on BC productivity, the same effect was previously reported by Yim and collaborators, using tea as a nitrogen source influenced the effectiveness of the carbon source (Yim et al. 2017), and previously Ramana et al. have also stated that the optimum nitrogen substrate varied accordingly to the carbon source of the medium (Ramana et al. 2000).

Due to all the experimental variables and differences between studies, it is difficult to compare BC productivity with other works using alternative feedstock. Nonetheless, from the possible comparisons and comparing BC production rate, the maximum productivity was obtained for TM-10AW medium with 0.11 g/L/day, superior to previous reports (Çakar et al. 2014; Erbas Kiziltas et al. 2015) and quite similar to the production rate obtained by Dórame-Miranda and coworkers, using pecan nutshell as carbon source, nonetheless, their fermentation process was performed during 28 days, which may imply higher process costs (Dórame-Miranda et al. 2019). Moreover, the aforementioned studies were performed with isolated bacterial species, whereas BC production in TM-10AW medium was obtained using a microbial consortium from kombucha. Previous results using a symbiotic culture of bacteria and yeast species from different origins revealed a lower production rate (0.04 g/L/day, and 0.05 g/L/day, for

Tibetan and European origin, respectively) and a similar production rate as in our study when BC was produced using kombucha from Oriental origin as inoculum (0.12 g/L/day) (Laavanya et al. 2021).

Regardless of the promissory results obtained using apple wastes and a tea mixture as an economically viable alternative for BC production, the impact of seasonal and geographic variations in the feedstock could affect the cellulose yield and quality. For instance, variations in the chemical composition of the apple Bravo de Esmolfe by-products, and black and green tea, such as the sugar content and nitrogen availability, could impact the growth and metabolic activity of the microbial consortium used for BC production. Therefore, the potential impact of these factors should be taken into account in further studies.

BC optical properties

Opacity and whiteness index were analyzed to compare the optical properties of BC membranes obtained in the commercially defined media with the membranes produced with alternative feedstock.

In this work, the differences in opacity values obtained for BC membranes produced from the control media HS (6.04 ± 0.50 Abs 600 nm mm^{-1}) were not significant when compared with the opacity values obtained from BC membranes harvested from the alternative media formulations evaluated. Moreover, the desirable BC opacity depends on the intended application. For instance, materials with higher opacity values limit the amount of light that can pass through and are convenient for packaging applications, to protect food from oxidative deterioration (Salari et al. 2018). On the other hand, the visible light transmission may be required, for certain applications, namely in electronics (Legnani et al. 2019).

Overall, a high whiteness index was obtained for all the BC membranes produced in the different media formulations, applying only an alkaline treatment for BC purification (Santoso et al. 2020). No further purification steps, such as bleaching, were required to achieve a whiteness index comparable to the work of Han and coworkers, where hydrogen peroxide was used as a bleaching agent, which in high concentrations may cause deformation of BC structure (Han et al. 2019). No significant differences were observed between the whiteness index of BC membranes produced with HS fermentation medium and HS-AW, HS-TM, and TM-AW media, as shown in Table 2. Similar results were previously reported by Fan et al., regarding the whiteness index of BC samples produced with HS media and modified media of citrus peel and pomace enzymolysis, where the membranes obtained were practically indistinguishable (Fan et al. 2016). Nonetheless, a lower whiteness index was obtained for the BC samples produced in alternative media with increased concentration of apple wastes (HS-5AW, HS-10AW, TM-5AW, and TM-10AW). This outcome may be a result of the synergic effect of the dark brown coloration of the medium, from the apple wastes as well as the tea mixture, and residual impurities from bacterial cells that could remain in the membranes (Han et al. 2019), once the alkaline procedure performed was the same for all the membranes, independently of their thickness.

Table 2 Thickness, opacity, and whiteness index of BC samples recovered from each fermentation media. Data presented as mean ± SD

	Thickness (mm)	Opacity (Abs 600 nm mm^{-1})	Whiteness index
HS	0.274 ± 0.030	6.04 ± 0.50	82.41 ± 0.66
HS-AW	0.153 ± 0.065	7.06 ± 0.79	83.27 ± 1.13
HS-TM	0.063 ± 0.003	6.80 ± 0.38	81.27 ± 1.33
TM-AW	0.158 ± 0.029	6.78 ± 0.43	82.01 ± 0.77
HS-5AW	0.172 ± 0.017	7.00 ± 0.61	78.63 ± 0.91
HS-10AW	0.132 ± 0.055	7.45 ± 0.72	76.34 ± 0.50
TM-5AW	0.143 ± 0.023	7.18 ± 0.90	77.76 ± 0.91
TM-10AW	0.328 ± 0.025	5.98 ± 0.21	68.01 ± 0.79

Morphologic properties

The morphology of BC samples, synthesized under different media formulations, was examined using scanning electron microscopy (SEM). Even though the BC pellicles were subjected to an alkaline treatment after recovery from the alternative media formulations, it is evident from the SEM images (Fig. 2) that there are residual remnants of cellular debris that were not fully eliminated from the BC samples. This remaining debris may hinder

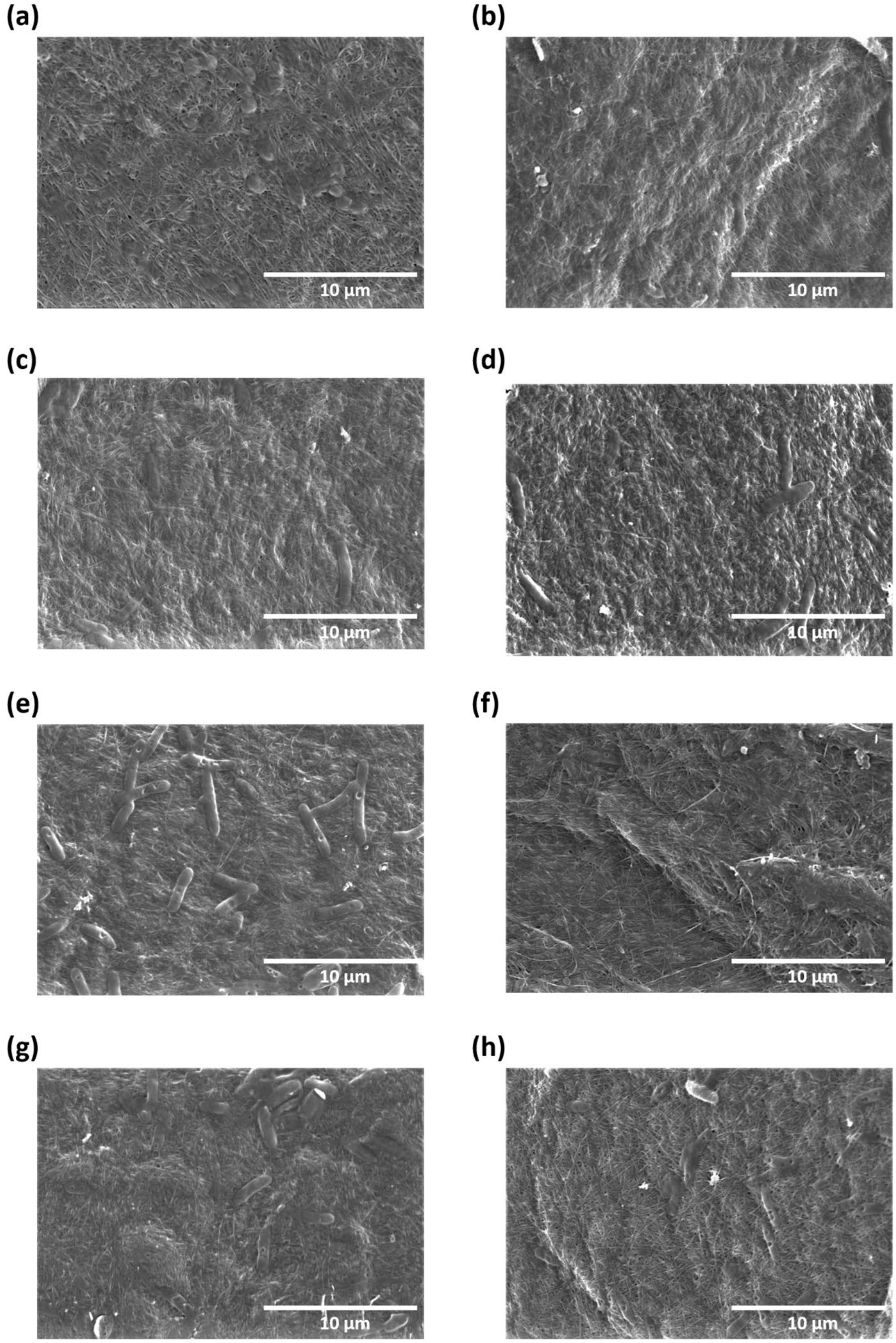

Fig. 2 Scanning Electron Microscopy (SEM) of BC membranes produced on: **a** HS, **b** HS-AW, **c** HS-TM, **d** TM-AW, **e** HS-5AW, **f** HS-10AW, **g** TM-5AW, and **h** TM-10AW

the morphologic comparison between BC samples produced from different media formulations, and additionally, the exact dimensions of the fibrils are challenging to estimate. Nonetheless, the gross morphological structure of the BC samples exhibited similar trends with a dense interwoven mesh of fibrils and with minimal inter-fibrillar space.

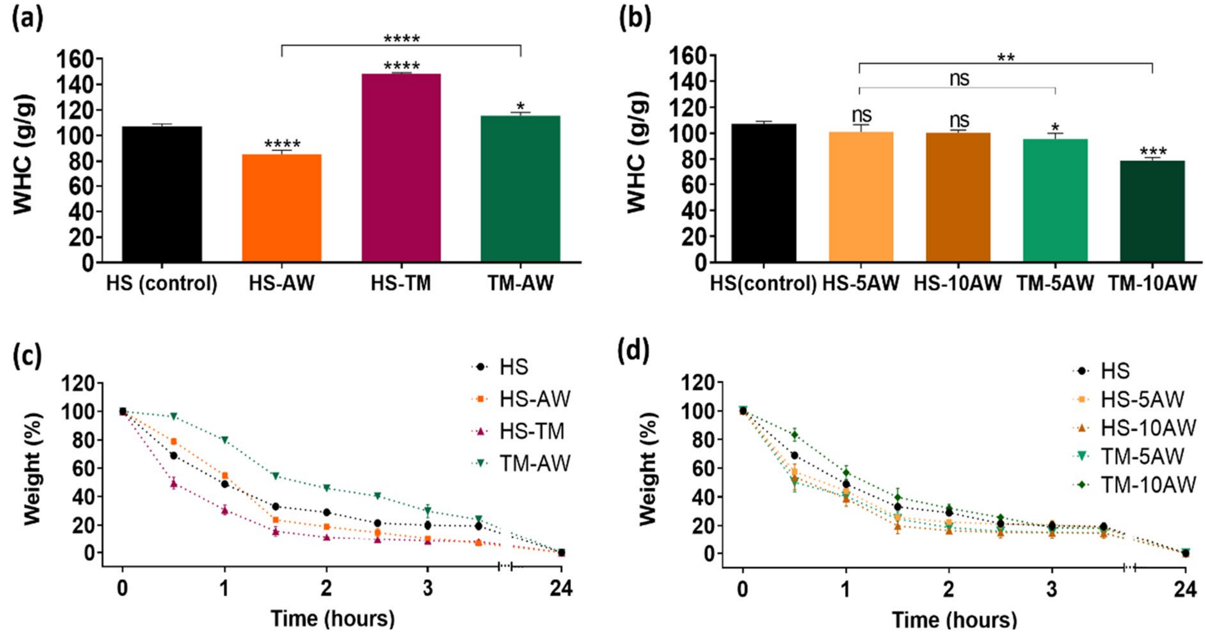

Fig. 3 Water holding capacity (WHC) of BC membranes produced on: **a** HS, HS-AW, HS-TM, and TM-AW; **b** HS, HS-5AW, HS-10AW, TM-5AW, and TM-10AW. Water retention rate (WRR) of the BC membranes produced on: **c** HS, HS-AW, HS-TM, and TM-AW; **d** HS, HS-5AW, HS-10AW, TM-5AW, and TM-10AW. Data presented as mean ± SD, ns indicates non-significant, * $p<.05$, ** $p<.01$, *** $p<.001$, and **** $p<.0001$. Different asterisks above bars indicate significant differences for comparisons between control (HS media) and the different media formulations, comparisons between media formulations are also shown with the connecting lines

Analysis of BC water holding and retention ability

The effects of different medium compositions on WHC and WRR of the produced BC membranes were investigated since the highly porous BC matrix may differ, within limits, according to the composition of the fermentation media (Kaewnopparat et al. 2008). Besides, the high water retention of BC, due to its high hydrophilicity and high surface area to mass ratio, is an indispensable feature for several application fields, especially in biomedical applications, where, for example, moisture retention and exudate absorption are crucial features for its successful use in wound dressing and tissue engineering materials (Portela et al. 2019; Pang et al. 2020).

The WHC of BC membranes produced in HS and alternative media are shown in Fig. 3. The highest WHC was recorded for BC membranes produced in HS-TM media, with 148.20 ± 0.87 g/g, followed by BC from TM-AW, with 115.50 ± 2.61 g/g (Fig. 3a). The WHC of membranes from alternative TM-AW media with increasing concentration of apple wastes, TM-5AW and TM-10AW, exhibited lower WHC, especially BC membranes from TM-10AW, with only 79.06 ± 2.40 g/g. However, BC from HS-5AW and HS-10AW exhibited similar WHC as the control samples produced in HS media, with membranes capable of absorbing approximately 100 times their dry weight of water (Fig. 3b).

The rate of water evaporation from the produced BC materials was also evaluated and the results are shown in Fig. 3c, d. The lowest WRR was observed for BC produced in HS-TM media, Fig. 3c, whilst TM-10AW exhibited the highest WRR (Fig. 3d). Since WHC and WRR values are greatly influenced by available surface area and pore size distribution (Portela et al. 2019), the results obtained indicate that surface area, porosity, and fibril arrangement of BC samples, produced with different fermentation media, could have slight variations. BC's high surface area, hydrophilicity, and porous nature, in addition to a looser fibril arrangement, are usually associated with high WHC. On the other hand, a more compact structure, with denser fibril arrangements and

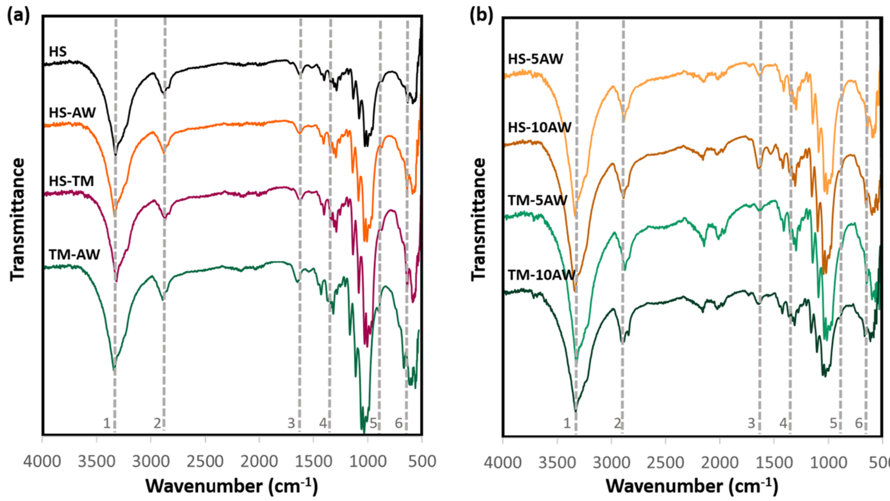

Fig. 4 FTIR spectra of BC membranes produced on: **a** HS, HS-AW, HS-TM, and TM-AW; **b** HS-5AW, HS-10AW, TM-5AW, and TM-10AW. For corresponding band assignments, indicated by the vertical dotted lines (- - -), see Table 3

Table 3 FTIR band assignments of the BC produced in HS, HS-AW, HS-TM, TM-AW, HS-5AW, HS-10AW, TM-5AW, and TM-10AW media. Numbers preceding the band assignment correspond to dotted lines in Fig. 4

	HS	HS_AW	HS_TM	TM_AW	HS_5AW	HS_10AW	TM_5AW	TM_10AW
(1) O–H stretching	3340	3343	3339	3339	3340	3340	3339	3340
(2) C–H stretching	2890	2893	2893	2893	2893	2894	2892	2893
(3) H_20 bending	1640	1640	1644	1645	1640	1643	1640	1640
(4) C–H bending	1360	1360	1363	1360	1362	1362	1362	1363
(5) C–O–C stretching	898	893	894	895	895	897	894	894
(6) C–O–H out-of-plane bending	662	662	662	661	660	661	661	662

reduced pore volume and surface area, which results in reduced available space and trapping sites to capture water molecules, is associated with a reduced WHC. Nonetheless, this compact BC structure is also associated with superior WRR, once the formation of hydrogen bonds in a denser fibril structure, results in higher water retention and smaller amounts of free bulk water (Gelin et al. 2007; Ul-Islam et al. 2012; Portela et al. 2019).

FTIR analysis

Figure 4 shows the FTIR spectra of BC samples produced under different fermentation media compositions, and the band assignments for each BC sample are detailed in Table 3. Briefly, the broad bands centered around 3339–3343 cm^{-1} are assigned to O–H stretching, the sharp absorption bands at 2892–2890 cm^{-1} correspond to C–H stretching vibrations, while the bands at 1360–1363 cm^{-1} correspond to C–H bending vibrations. The bands arising around 1640–1645 cm^{-1} were attributed to the bending vibrations of absorbed water H–O–H, while the band at 898–894 cm^{-1} may be associated with the stretching vibrations of the C–O–C bond of the β-1,4-glycosidic linkages, and below 660–662 cm^{-1} C–O–H out of plan bending was observed (Castro et al. 2011; Fan et al. 2016; Barshan et al. 2019; Han et al. 2020; Abol-Fotouh et al. 2020). The bands around 2100 cm^{-1}, which only appear in the FTIR spectrum of BC produced from fermentation medium with increasing apple wastes concentration (Fig. 4b), have been attributed to carbohydrates and proteins (Barrios-Rodríguez et al. 2021) and, therefore, may be a result of impurities remaining in BC samples.

Nonetheless, the results indicated that the different medium compositions, with distinct nitrogen and carbon sources, revealed no effect on the chemical groups of the obtained BC samples. Similar outcomes have also been reported by other authors, using

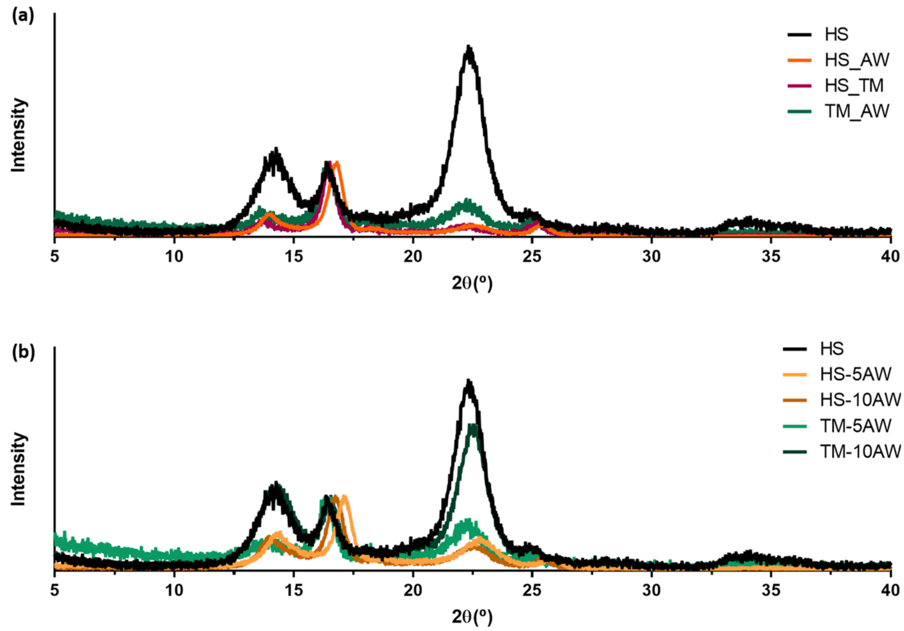

Fig. 5 X-ray diffraction patterns of the BC samples produced on membranes produced on: **a** HS, HS-AW, HS-TM, and TM-AW; **b** HS, HS-5AW, HS-10AW, TM-5AW, and TM-10AW media

alternative fermentation media for BC production, for example, using citrus peel and pomace enzymolysis (Fan et al. 2016) or with a mixture of date syrup and cheese whey supplemented with ascorbic acid (Raiszadeh-Jahromi et al. 2020), and even using terylene waste (Zhang et al. 2021), with all resulting in no drastic effect on the chemical conformation of the BC produced.

X-ray diffraction

X-ray diffraction (XRD) was used to evaluate the crystallinity of BC samples produced, once several parameters influence BC crystallinity, including the fermentation media and the concentration of the media components (Dórame-Miranda et al. 2019; Güzel and Akpınar 2020).

Figure 5 shows the X-ray diffractograms of BC samples produced from the fermentation media evaluated. BC membranes obtained from HS media exhibited the typical XRD pattern of cellulose I, with two apparent diffraction peaks at 2θ of 13.68° and 22.02° with strong intensity and a weak peak at 2θ of 16.06° (Dórame-Miranda et al. 2019; Han et al. 2020). The peak at 2θ of 13.68° could be assigned to the (100) crystallographic plane of I_α, whilst the peak at 2θ of 16.06° could be attributed to the (010) plane of I_α, and the peak with the highest intensity at 2θ of 22.02° could correspond to (110) plane of I_α (French 2014). The BC samples also exhibit unidentified peaks at 2θ between 25° and 27°, which could be related to impurities. In the work of George et al. (2008) a similar peak was attributed to non cellulosic materials that remained attached to the samples.

The crystallinity index obtained for the produced BC samples ranged from 57.26 to 88.08% for BC membranes obtained from HS-TM and HS, respectively (Table 4). Moreover, BC samples produced on TM_10AW (87.61%) exhibited a comparable

Table 4 Crystallinity index, and Herman´s orientation parameter of (110) crystal plane of the BC membranes produced on HS, HS-AW, HS-TM, TM-AW, HS-5AW, HS-10AW, TM-5AW, and TM-10AW media

Samples	Crystallinity index (%)	Herman's orientation
HS	88.08	0.6795
HS-AW	60.53	0.6007
HS-TM	57.26	0.6884
TM-AW	70.59	0.6832
HS-5AW	67.79	0.6917
HS-10AW	67.39	0.6819
TM-5AW	73.33	0.6803
TM-10AW	87.61	0.6813

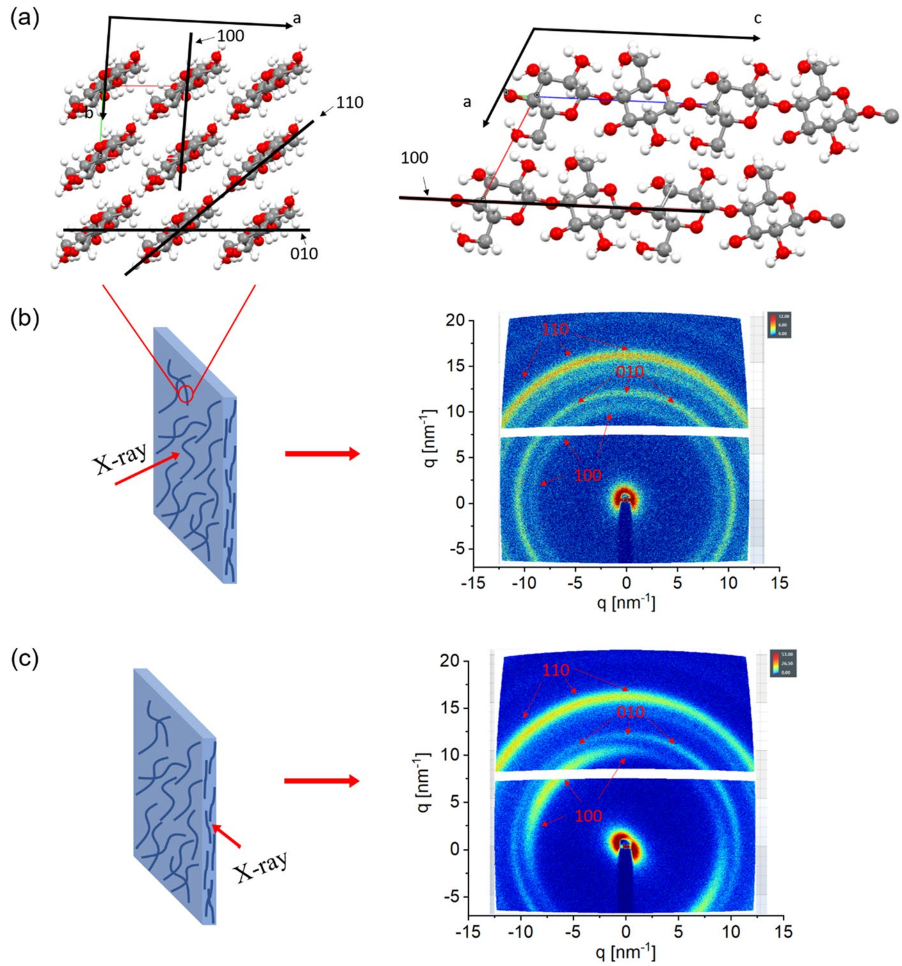

Fig. 6 **a** Crystallographic structure of cellulose I_α (Nishiyama et al. 2003; French 2014). **b** X-ray perpendicular to the film surface and X-ray pattern (Example of TM-10AW sample). **c** X-ray parallel to the film surface and X-ray pattern (Example of TM-10AW sample). The red arrows indicate the (100), (010), and (110) crystal planes

crystallinity index to BC samples produced on HS control media.

Nonetheless, once the diffraction patterns appear to have varying types and extents of preferred orientation, the impact of the different media compositions on the BC samples was further evaluated by wide-angle X-ray diffraction with a 2D detector (Fig. S1). The red arrows in Fig. S1 indicate the position of ring patterns that represent (100), (010), and (110) crystal planes, where some extra patterns might be due to the presence of large crystallites in the samples. The crystallographic structure of cellulose I_α is depicted in Fig. 6a. Cellulose thin films typically have the molecular axes lying in the plane of the film, the c-axis of cellulose pointing everywhere in the plane, so the crystallites in the film plane is random in statistic (Fig. 6b). When the X-ray beam is perpendicular to the film surface (Fig. 6b), (100), (010), and (110) patterns show mostly full circles. However, the (100) pattern is relatively weak as it has less chance to be in the Bragg condition for transmission mode. By contrast, when the X-ray beam is parallel to the film surface (Fig. 6c), intense arc patterns are observed, which is evidence for the preferred orientation in the out-of-plane direction. The (100) and (110) planes have more chance of being in the Bragg condition, so the intensity is stronger than (010).

Here we extracted the orientation factors of (110) to quantify the orientation. All the tested samples revealed a similar orientation factor in the (110) crystal plane, with the HS-AW sample as the only exception (Table 4). Our explanation is the elementary packing of HS-AW along the (110) plane direction could be limited due to factors related to the media composition. This sample was produced with nitrogen sources from the HS medium (peptone and yeast

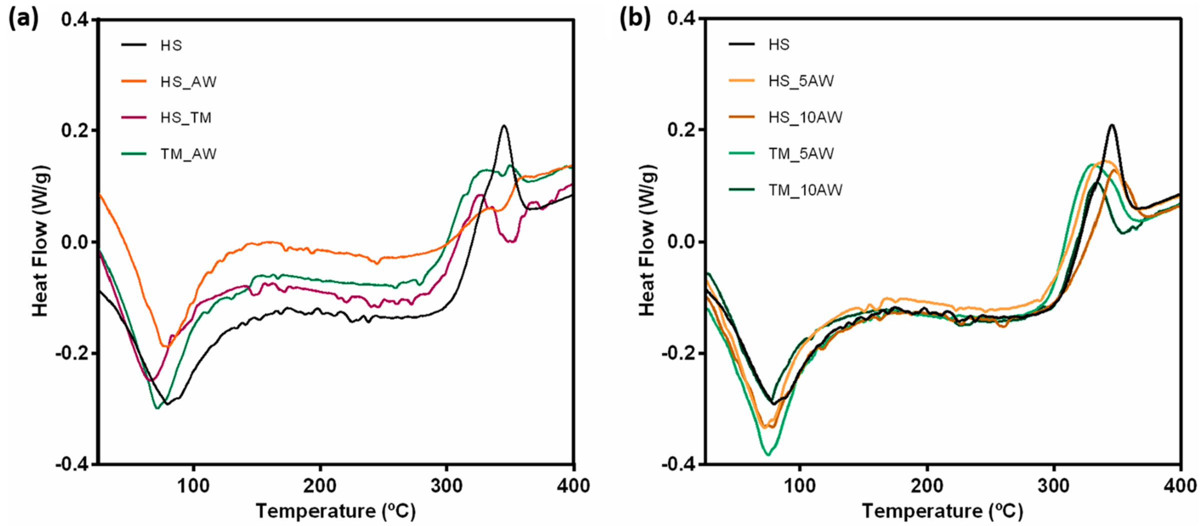

Fig. 7 DSC curves of BC membranes produced on: **a** HS, HS-AW, HS-TM, and TM-AW; **b** HS, HS-5AW, HS-10AW, TM-5AW, and TM-10AW

Table 5 Tensile properties of the BC membranes produced on HS, HS-AW, HS-TM, TM-AW, HS-5AW, HS-10AW, TM-5AW, and TM-10AW media. Data presented as mean ± SD

	Young's modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)
HS	217.77 ± 17.27	17.09 ± 2.32	7.87 ± 0.98
HS-AW	171.48 ± 12.70	15.19 ± 1.38	8.85 ± 0.15
HS-TM	58.01 ± 7.40	4.98 ± 1.49	8.40 ± 1.50
TM-AW	102.02 ± 22.51	11.12 ± 0.20	11.37 ± 2.13
HS-5AW	151.56 ± 23.38	13.93 ± 2.83	9.20 ± 1.25
HS-10AW	146.27 ± 19.27	15.29 ± 2.29	10.43 ± 0.19
TM-5AW	149.23 ± 10.65	13.62 ± 2.33	9.07 ± 1.07
TM-10AW	217.35 ± 29.60	19.68 ± 3.47	9.23 ± 1.93

extract) and with AW, since the AW concentration on this formulation was only 2%, and it's not a highly purified carbon source, there could be an imbalance due to the presence of higher amounts of nitrogen in the medium, that could ultimately disturb the aggregation of the sub-elementary fibrils.

Differential scanning calorimetry

The DSC curves from the BC samples produced in the HS and alternative media, Fig. 7, show a very similar profile. A first endothermic peak is observed at a temperature of 66 to 80 °C, which could be attributed to the loss of water in BC, as previously reported by other authors (Liu et al. 2020; Leonarski et al. 2022). The DSC curves of all samples also show an exothermic peak around 331 up to 347 °C, corresponding to exothermic reactions in the oxidation of BC. In BC samples from HS-AW, HS-TM, and TM-AW media, further heating yielded a second exothermic peak, appearing in temperatures of 351–369 °C, suggesting an exothermic reaction in the oxidation of char residues (Villarreal-Soto et al. 2021), Fig. 7a. Overall, in BC membranes produced with modified media containing increasing apple wastes concentration (samples HS-5AW, HS-10AW, TM-5AW, and TM-10AW, Fig. 7b), melting temperatures shifted to slightly higher values and the samples appear to degrade at higher temperatures, indicating increased thermal stability of these samples.

Mechanical properties evaluation

The intrinsic mechanical properties of BC samples, produced from the different media formulations, were investigated and the values of tensile strength, Young's modulus, and elongation at break are summarized in Table 5.

The highest Young's modulus and tensile strength were obtained for BC membranes generated from TM-10AW media (217.35 ± 29.60 MPa and 19.68 ± 3.47 MPa, respectively) and from HS media (Young's modulus of 217.77 ± 17.27 MPa and tensile strength of 17.09 ± 2.32 MPa). The higher tensile properties obtained for these membranes could be related to denser fibril arrangements and a more compact structure, which provides better resistance against the force applied.

Moreover, the tensile properties of BC membranes from different media formulations presented a significant correspondence between the results obtained in the WHC and WRR evaluation regarding the indications of fibril arrangement, which is also supported by the sample's crystallinity indices. Similar behavior was also previously reported using other alternative media, namely black strap molasses and molasses from the condensation unit of a brewery industry (Khattak et al. 2015).

Conclusion

This study showed that apple wastes and a mixture of black and green tea could be efficiently bioconverted to BC using a microbial consortium from the kombucha beverage. Moreover, the higher BC productivity is attributed to the combined effect and compatibility of both: the type of carbon and nitrogen source. Herein, we report a mixture of 0.5% (W/V) black and 0.5% (W/V) green tea as a suitable nitrogen source to combine with 10% (W/V) of apple wastes to achieve higher yield while reducing the production costs and at the same time decreasing organic disposal in the environment. Moreover, BC membranes obtained from the alternative TM-10AW media exhibited comparable, or even superior, physical and structural properties when compared with BC membranes produced on synthetic HS media, including water holding and retention ability, crystallinity index, thermal stability, and mechanical performance. Thus, the low-cost TM_10AW medium is a promising alternative for large-scale production of BC, and to enable its unrestricted use in various fields, for example, in the biomedical field, where BC has been utilized in the development of wound dressings, tissue engineering scaffolds, and artificial blood vessels, or the food and packaging industries as a fat replacer, thickener, or film-forming agent, or even in the textile and electronics industries, and many more, where its widespread use has been limited by the high production costs.

Nonetheless, seasonal and geographic variations in the feedstock that could affect the cellulose yield and quality should be evaluated in further studies. The potential antioxidant properties of BC, produced with tea mixture as an alternative nitrogen source, could also be further evaluated due to the polyphenols released from tea during the fermentation, which could provide low-cost BC with value-added properties.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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