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UNVEILING THE IMPACT OF BACTERIAL CELLULOSE DERIVED FROM NATA DE COCO AND SORBITOL ON BIOCOMPOSITE FILM PROPERTIES FOR POTENTIAL FOOD PACKAGING

(Menyingkap Kesan Selulosa Bakteria Diperoleh daripada Nata De Coco dan Sorbitol Terhadap Sifat-Sifat Filem Biokomposit Sebagai Bahan Pembungkus Makanan Alternatif)

Sharifah Fathiyah Sy Mohamad*, Nur Fikriyah Yaacob, and Intan Anak Ekong

Faculty of Chemical and Proces Engineering Technology, Universiti Malaysia Pahang Al-Sultan Abdullah
Lebuh Persiaran Tun Khalil, Yaakob, 26300 Kuantan, Pahang, Malaysia

**Corresponding author: fathiyah@umpsa.edu.my*

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Abstract

The surge in plastic production in recent years has demonstrated the urgent need for eco-friendly alternatives. Bacterial cellulose (BC) has emerged as a promising candidate for replacing petroleum-based materials. However, the inherent rigidity, fragility, and brittleness of BC have posed formidable challenges, particularly in food packaging. This study investigates the impact of combining BC (1.0g, 2.5g, 5.0g, 7.5g, 10.0g) derived from nata de coco and sorbitol (0.5g, 1.0g, 1.5g, 2.0g, 2.5g) on the physical, mechanical, chemical, and thermal properties, as well as the surface morphology of the biocomposite films. Films were produced by varying BC and sorbitol quantities via the solvent casting method. Evaluations encompassed film thickness, moisture content, water solubility, mechanical characteristics, FTIR, TGA, and FE-SEM. Tensile strength increased with BC content but decreased with added sorbitol. FTIR confirmed the consistency of functional groups in the BC/Chitosan/Sorbitol biocomposite film with those in BC. TGA indicated improved thermal stability with increased BC and decreased sorbitol content. FE-SEM images revealed a smoother biocomposite film surface with sorbitol. The optimal composition was determined to be 10.0BC and 0.5S due to its low moisture content, low water solubility, high tensile strength, and minimal TGA weight loss. Overall, this study highlights the potential of BC/Chitosan/Sorbitol films as environmentally friendly and sustainable alternatives to traditional synthetic food packaging materials.

Keywords: bacterial cellulose, nata de coco, chitosan, sorbitol, biocomposite film

Abstrak

Peningkatan pengeluaran plastik dalam beberapa tahun kebelakangan ini telah menghasilkan keperluan mendesak untuk pembangunan pengganti plastik yang mesra alam. Dalam konteks ini, selulosa bakteria (SB) mempunyai potensi yang memberangsangkan sebagai pengganti bahan berasaskan petroleum. Walau bagaimanapun, ketegaran dan kerapuhan SB telah menimbulkan pelbagai cabaran, terutamanya dalam konteks kegunaannya sebagai pembungkus makanan. Dalam kajian ini, kami mengkaji kesan gabungan BC dan sorbitol terhadap sifat fizikal, mekanikal, kimia, termal, dan morfologi permukaan filem biokomposit berdasarkan BC. Filem-filem ini dihasilkan dengan mengubah kuantiti BC (1.0g, 2.5g, 5.0g, 7.5g, 10.0g) dan sorbitol (0.5g, 1.0g, 1.5g, 2.0g, 2.5g) menggunakan kaedah pelarut. Sampel filem yang terhasil dinilai berdasarkan ketebalan filem, kandungan lembapan, kelarutan dalam air, sifat-sifat mekanik, FTIR, TGA, dan FE-SEM. Hasil kajian menunjukkan bahawa nilai kekuatan regangan meningkat seiring dengan peningkatan BC, tetapi berkurangan apabila kepekatan sorbitol bertambah. FTIR menunjukkan bahawa kumpulan berfungsi yang wujud dalam filem biokomposit BC/Chitosan/Sorbitol sepadan dengan kumpulan berfungsi BC. TGA pula mendedahkan bahawa kestabilan termal filem komposit meningkat dengan peningkatan BC dan penurunan sorbitol. Peningkatan kuantiti BC menyebabkan filem mengalami kehilangan berat yang minimum, tetapi apabila tahap sorbitol meningkat, filem mengalami kehilangan berat yang lebih besar FE-SEM menggambarkan bahawa permukaan filem komposit menjadi lebih licin dengan penambahan sorbitol. Komposisi optimum ditentukan sebagai 10.0BC dan 0.5S, disebabkan oleh kandungan lembapan yang rendah, kelarutan dalam air yang rendah, kekuatan regangan yang tinggi, dan kehilangan berat yang minima dalam TGA. Keseluruhannya, kajian ini membuktikan bahawa filem komposit berdasarkan BC mempunyai potensi untuk digunakan sebagai alternatif yang boleh dikompos secara biodegradasi dan mampan kepada bahan pembungkusan makanan sintetik tradisional.

Kata kunci: selulosa bakteria, nata de coco, kitosan, sorbitol, filem biokomposit

Introduction

The packaging industry plays a crucial role in the global economy, particularly in the food and beverage segment, which holds the majority market share of around 85% [1]. However, the reliance on conventional plastic packaging materials has led to significant environmental concerns due to their non-biodegradable nature [2]. The immense volume of plastic waste generated worldwide is expected to triple by 2060, surpassing one billion metric tons. Alarmingly, only a minimal fraction (9%) of this plastic waste is recycled, leading to exacerbated resource depletion, contributing to global warming, acidification, eutrophication, and posing risks to human health [3]. Consequently, there is an increasing global demand for environmentally friendly alternatives to traditional packaging materials. Traditional approaches to biodegradable food packaging, which use proteins, polysaccharides, and lipids from agricultural byproducts or synthetic biopolymers such as PLA and PHA, encounter significant challenges because of their reduced tensile strength and insufficient moisture and oxygen barriers. These properties are essential for preserving food safety and quality. Despite their eco-friendly nature, these methods struggle with scalability and cost-effectiveness, primarily due to the elevated price of raw materials, energy-intensive processing, and advanced equipment requirements [4]. Moreover, the environmental advantages of polymers, such as PLA, are mitigated by their dependence on industrial composting facilities, which are scarcely available. On the other hand, naturally degradable materials such as chitosan show promise but are plagued by sourcing and quality consistency issues [5]. These limitations highlight the urgent necessity for new, sustainable, and economically viable food packaging solutions that effectively balance environmental benefits with performance requirements for widespread commercial adoption.

In this context, Nata de coco (NDC) emerges as an environmentally sustainable and promising alternative to conventional plastic packaging materials, primarily due to its role as a BC source. BC, derived from Nata de coco, is a natural and renewable biopolymer primarily produced by *Acetobacter xylinum* through the fermentation of coconut water [6]. This unique production process involves the metabolic conversion of glucose found in coconut water, enriched with carbon and nitrogen, by *Acetobacter xylinum*, resulting in the extracellular production of cellulose as a valuable metabolite [7]. BC shares a chemical composition identical to plant cellulose but distinguishes itself by the absence of lignin or hemicelluloses, leading to superior physical characteristics compared to conventional plant cellulose [8]. Its high tensile strength and water-holding capacity make BC stronger, more resistant to breaking, and capable of absorbing more water. Furthermore, BC exhibits high purity and crystallinity, contributing to excellent transparency and barrier properties [9]. These properties make BC an attractive potential replacement for synthetic food packaging materials [10]. Nevertheless, despite its potential, using BC alone for food packaging applications is not ideal due to its natural brittleness when dried. Dried BC has a rigid and less pliable structure, resulting in a rough appearance and reduced flexibility. To overcome this limitation, BC is often combined with other biopolymers or plasticizers, such as chitosan or glycerol, to enhance its pliability, achieve a more plastic-like appearance, and improve overall properties while minimizing brittleness [11,12].

Chitosan, the second most abundant natural biopolymer after cellulose, is primarily sourced from the byproducts of the shellfish industry [5]. It has demonstrated remarkable versatility in various applications, notably in food packaging, due to its non-toxicity, biodegradability, film-forming attributes, solubility, and inherent antimicrobial properties. Furthermore, chitosan and bacterial cellulose exhibit similar chemical structures and mutually complementary characteristics, fostering enhanced molecular interactions among their polysaccharide chains [13]. Consequently, chitosan emerges as a highly promising candidate for utilization as a filler in the production of BC-based films, offering the prospect of enhanced mechanical properties and film-forming capability [14]. In contrast to films composed solely of chitosan, BC/chitosan composite films present distinct advantages in terms of mechanical resilience, including improved thermal stability and reduced oxygen permeability, making chitosan a highly viable candidate for augmenting BC-based films [15].

Moreover, incorporating plasticizers such as sorbitol, recognized for its low molecular weight and non-volatile nature, into film formulations can significantly enhance their flexibility, elongation, and toughness, thereby effectively addressing the inherent brittleness of BC [16]. The materials can also reduce film stiffness and brittleness, enhance transparency, and decrease water vapor transmission rate. One commonly used plasticizer is sorbitol, which has a low molecular weight, non-volatile nature, and high boiling point. Sorbitol exhibits significant intermolecular interaction with polymers, reducing polymer strength while increasing flexibility and extensibility. It is a polyhydric alcohol monosaccharide molecule, also known as hexitol or glucitol, with a chemical formula of $C_6H_{14}O_6$ [17]. Incorporating sorbitol as a plasticizer into film formulations can influence film thickness, flexibility, density, and water content [15], offering a means to enhance the properties of BC and make it more suitable for food packaging applications.

In the past few years, numerous studies have explored the incorporation of BC and chitosan for food packaging applications [18–20]. BC showed a reinforcing effect and can improve the physical properties of whey protein concentrate films [21]. However, despite these advancements, comprehensive studies considering film formulations and interactions between BC from NDC, chitosan, and sorbitol are still lacking in the literature. Building on this gap of knowledge, this study aimed to investigate the impact of varying BC and sorbitol concentrations on the physical, mechanical, and thermal properties of BC-based biocomposite films for potential use in sustainable food packaging. The study aims to determine the ideal composition of BC and sorbitol that ensures low moisture content, high tensile strength, and improved thermal stability. This research is expected to contribute to the advancement of an environmentally friendly substitute for traditional plastic packaging materials.

Materials and Methods

Materials

1.5 kg of food-grade nata de coco (Brand: Desa Alpha, Manufactured by Rico Food Ind. Sdn. Bhd.) in the form of cubes was purchased from the local supermarket. Chitosan of medium molecular weight and sodium hydroxide were obtained from Sigma-Aldrich. Sorbitol and acetic acid were purchased from R&M Chemicals. All other chemicals used were of the analytical grade.

Purification of bacterial cellulose (BC) from nata de coco

The nata de coco cubes were thoroughly washed and soaked in distilled water twice daily for 10 days to ensure the pH reached 7. This step was done to neutralize the acidity and remove any impurities from the nata de coco cubes. After that, an alkaline treatment was performed by stirring the nata de coco cubes in 0.01 M NaOH at 80 °C for 90 min to remove any remaining bacterial cell debris, microorganisms, and other dissolved substances. Subsequently, the nata de coco cubes were washed multiple times with distilled water at room temperature until the pH reached 7 [22]. The purified nata de coco was then ground and mechanically blended for 1 hour using a kitchen blender (ProMix hand blender, Philips). The resulting BC slurry was collected and stored at room temperature for future use.

Preparation of BC/Chitosan/Sorbitol biocomposite films

The biocomposite films were prepared using a solvent casting method, following the procedure by Yanti et al. [23]. The film-forming solution consisted of BC suspension, chitosan, and sorbitol as plasticizers, as shown in Table 1. The chosen ranges for BC and sorbitol were established through preliminary experiments aimed at identifying levels that yield films with flexibility and without brittleness, suitable for further testing and application. Chitosan solution was first prepared by dissolving 0.25g of chitosan powders in 25 mL of 1 %v/v acetic acid. All film formulations were homogenized using an ultrasonic homogenizer for 5 min with a pulse of 5 sec and amplitude of 60%. The resulting film solutions were then cast onto an acrylic mold (8 cm × 12 cm) and dried at 50 °C for 12 hours in an oven. The dried films were peeled off from the petri dishes and stored in zip-lock bags for further analysis. The biocomposite films were previously conditioned for 48 h at 25 °C and 54% RH before evaluating their physicochemical properties.

Table 1. Composition of BC and sorbitol in film formulations.

Sample Name	Bacterial Cellulose (g)	Sorbitol (g)
0.5S	10.00	0.50
1.0S	10.00	1.00
1.5S	10.00	1.50
2.0S	10.00	2.00
2.5S	10.00	2.50
1.0BC	1.00	0.50
2.5BC	2.50	0.50
5.0BC	5.00	0.50
7.5BC	7.50	0.50
10.0BC	10.00	0.50

Characterization of BC/Chitosan/Sorbitol films: Film thickness

A digital micrometer (3109-25A, InSize, China) was used to measure the thickness of the formed film with an accuracy of 0.001 mm. The thickness of the film was measured at five different spots, and the average thickness was calculated and recorded.

Moisture content (MC)

Test specimens (2 cm × 2 cm) at three random positions of each film type were uniformly cut and placed on glass Petri dishes. The moisture content of the films was determined by drying test specimens in an oven at 105 °C for 24 h until a constant dry weight was obtained according to the method briefly explained by Azaza et al. [24]. The dry weight of the film was measured after drying, and the MC was calculated using Equation (1) below:

$$\text{Moisture Content (\%)}=\frac{\text{Initial sample weight (g) - Dried sample weight (g)}}{\text{Initial sample weight (g)}}\times 100 \tag{1}$$

Water solubility (WS)

The water solubility of the film was measured according to the method described by Lee et al. [25]. Briefly, to determine the initial dry weight, samples were cut to sizes (2 cm × 2 cm) in triplicate and dried at 105 °C for 24 hours. The dried films were then immersed in 50 mL of distilled water and stirred at room temperature for 24 hours at 100 rpm. After 24 hours, any remaining films were removed and dried at 105 °C for 24 hours to obtain the final dry weight. The percentage of water solubility in samples was calculated using the following Equation (2):

$$\text{Water Solubility (\%)}=\frac{\text{Dried sample weight (g) - Final dried sample weight (g)}}{\text{Dried sample weight (g)}}\times 100 \tag{2}$$

Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of the film samples were measured according to the ASTM D882-00 method (ASTM., 2000) using a texture analyzer (Stable Micro Systems TA-XT plus, Surrey, UK). Each BC and BC/Chitosan/Sorbitol film was cut into a rectangular shape (10 cm × 1 cm). All the tests were carried out at least twice with different strips. TS was calculated using the following Equation (3):

$$\text{TS} = \frac{\text{Force Maximum (Fmax)}}{\text{Area (A)}} \quad (3)$$

where Fmax is the maximum load, and A is the cross-sectional area.

EAB was calculated using the following Equation (4):

$$\text{EAB} = \frac{\Delta L}{L_0} \times 100 \quad (4)$$

where ΔL is the film extension, and L_0 is the initial length of the film sample.

Structure by Fourier-transform infrared (FTIR)

The film samples were cut into small pieces (2 cm × 2 cm) and analyzed using an FTIR Spectrophotometer (Nicolet iS5, Thermo Fisher Scientific, USA). For each sample, 32 scans with a resolution of 4 cm^{-1} were collected over the wavenumber ranging from 600 – 4000 cm^{-1} in transmittance mode.

Thermogravimetric analysis (TGA)

TGA was carried out on selected film samples to evaluate their respective mass losses when it is subjected to continuous heating. TGA was performed using TGA Instrument Q500 V 6.7 (TA Instruments, USA) with a 10 °C/min heating rate from 25 to 600 °C under nitrogen gas purging with a flow rate of 40 ml/min.

Surface morphology by field emission scanning electron microscopy (FE-SEM)

The surface morphology of the pure BC and selected BC/Chitosan/Sorbitol films were visualized using a Field Emission Scanning Electron Microscope (FE-SEM) JEOL JSM 7800 F with an accelerating voltage of 15 kV.

Results and Discussion

Physical appearances of BC/Chitosan/Sorbitol films

The physical appearance of the films was evaluated by analyzing their opacity and transparency, as shown in Figure 1. The results indicated that the opacity of the pure BC film was nearly 100% due to the high concentration of BC fibers. The higher value of opacity signified smaller transparency. BC is a polymer composed of ultrafine nanofibrils that form a three-dimensional web-shaped structure, held together by intermolecular and intramolecular hydrogen bonding [26]. As the BC content increased, the film became rougher and less homogenous. Conversely, the addition of sorbitol improved homogenization and gave the film a smoother appearance due to its high hydrophilicity. These findings align with previous research by Wang et al. [27], which also observed a significant increase in film opacity with increasing BC concentrations from 0% to 10%.

Film thickness, moisture content, and water solubility

Table 2 presents valuable insights into the characteristics of the film formulations with varying proportions of bacterial cellulose and sorbitol. The thickness of these films ranged from 0.0384 to 0.2507 mm and can be adjusted by modifying the amounts of BC and sorbitol. The results suggest that adding BC and sorbitol can improve film thickness. The film with a greater sorbitol concentration (2.5S) formed a thicker (0.2526 mm) film than the film with a lower amount of BC and sorbitol (1.0BC), which produced a thinner (0.0718mm). These findings are comparable to similar reports on the effects of sorbitol on the characteristics of carrageenan edible film. Edible film features and basic material composition are two aspects that impact the thickness of edible film. As the sorbitol concentration increased, the edible coating showed a corresponding thickness increment. This is because when the total solids in the edible film solution increased, the formation of the edible film occurred. Therefore, the higher the concentration of sorbitol, the higher the thickness of the film due to the solid content and characteristics of sorbitol [28].

In contrast, the moisture content exhibited less significant variations upon the introduction of BC but demonstrated a substantial increase when sorbitol concentration was augmented, ranging from 4.82% to

89.51%. This phenomenon mirrors findings from a study by Ghamari et al. [29], which investigated active edible film based on milk proteins infused with Nigella sativa essential oil. The study emphasized the crucial role of sorbitol concentration in moisture content, highlighting that its increment can affect the hydrophilic nature of sorbitol. This is because sorbitol has hydroxyl groups in its structure that can actively form hydrogen bonding with water.

Furthermore, the water solubility of the films exhibited a notable decline, decreasing from 32.88% to 5.29%, as BC and sorbitol were incorporated in greater quantities. The reduction in water solubility can be attributed to the enlarged surface area resulting from BC and the formation of intermolecular interactions between the agar matrix and BC. These interactions enhance the cohesion of the biopolymer matrix, leading to decreased water solubility [27]. Therefore, it is evident that as the proportions of BC and sorbitol increased, the water solubility of the films decreased significantly, an essential property for their potential applications

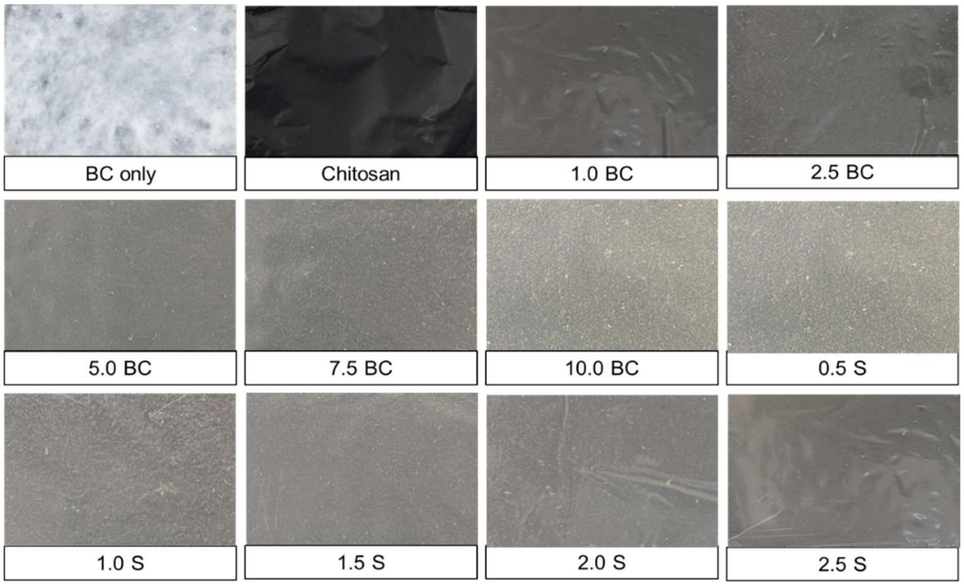

Figure 1. Visual appearances of BC/Chitosan/Sorbitol films. Digital photographs are taken under natural light to show color, transparency, and texture differences. No magnification was applied

Table 2. Thickness, moisture content, and water solubility of the BC/Chitosan/Sorbitol films

Sample Name	Thickness (mm)	Moisture Content (%)	Water Solubility (%)
BC	0.0384	8.31	64.88
0.5S	0.0822	58.57	8.87
1.0S	0.1620	66.76	6.93
1.5S	0.1580	74.72	5.29
2.0S	0.1896	89.51	18.60
2.5S	0.2526	78.42	19.21
1.0BC	0.0718	4.82	27.95
2.5BC	0.0774	11.32	26.98
5.0BC	0.0774	4.86	16.23
7.5BC	0.0760	8.37	32.63
10.0BC	0.0886	16.67	12.46

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was employed to investigate the intricate chemical interactions occurring between BC and sorbitol. This analytical approach allowed for the observation of shifts and alterations in the intensity of characteristic peaks within the spectra, providing valuable insights into both the physical and chemical interaction between these substances and shedding light on their compatibility [30].

Figure 2 compares FTIR spectra between BC and BC/Chitosan/Sorbitol films, varying in the amounts of BC and sorbitol. Figure 2(a) notably highlights the characteristic peaks of BC present in all the samples. The broad absorption band in the 3200-3500 cm^{-1} range was attributed to the stretching vibrations of hydroxyl groups (-OH) of BC and sorbitol [31]. The existence of hydroxyl groups within BC facilitates the formation of different intermolecular and intramolecular hydrogen bonds. The establishment of these hydrogen bonds, both between molecules and within individual molecules, plays a fundamental role in shaping the physical characteristics of cellulosic materials [12]. Meanwhile, the peak around 2925 cm^{-1} corresponds to C-H stretching vibrations, while the peak at 1637 cm^{-1} can be attributed to the –CO group. The peaks at 1411 and 1314 cm^{-1} signify the –CH asymmetric deformation and –CH bending vibrations, which serve as critical signatures for samples with chitosan [32]. Furthermore, the peak around 1056 cm^{-1} corresponds to C–O symmetric stretching. In contrast, the presence of the hydroxyethyl group is indicated by the shift of the C-O-C stretching vibration peak to 888 cm^{-1} [15].

The introduction of sorbitol as a plasticizer induced a noticeable shift in the peak positions, transitioning from 3500 cm^{-1} (as observed in pure BC in Figure 2(b)) to lower wavenumbers, typically around 3300 cm^{-1}. This shift is primarily attributable to the formation of hydrogen bonding interactions involving BC, chitosan, and sorbitol [27]. Interestingly, the FTIR spectra of plasticized films (Figure 2(b)) displayed only marginal reductions in intensity. This observation suggests the presence of intermolecular interactions facilitated by the formation of hydrogen bonds. Consequently, it can be inferred that the plasticizer did not induce significant structural alterations in BC; instead, it engaged in cooperative interactions with the material.

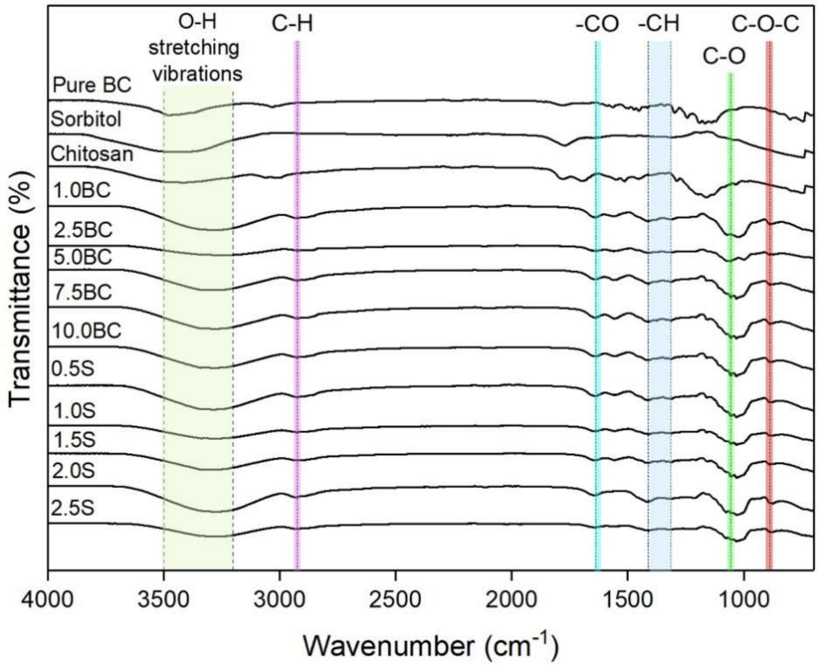

Figure 2. FTIR spectra for BC/Chitosan/Sorbitol films containing various amounts of BC and sorbitol

Mechanical properties

The evaluation of mechanical properties (Table 3), including tensile strength (TS), Young's modulus (YM), and elongation at break (EAB), is fundamental in assessing the potential of BC/sorbitol biocomposite films for food packaging applications. The control sample, which consisted solely of BC, served as the baseline for subsequent comparisons, recording a TS of 10.60 MPa, a YM of 629.91 MPa, and an EAB of 2.36%. As the BC concentration incrementally increased, these mechanical properties were affected. The findings align with previous analysis suggesting that a BC reinforcing effect within composite matrices can ultimately enhance the TS [33,34]. However, the phenomenon of agglomeration at higher BC concentrations observed in the 10.0BC formulation resulted in diminished TS, possibly due to impaired stress distribution [35]. On the other hand, adding sorbitol reduced the sample's YM due to its function as a plasticizer. Notably, the 2.5S formulation showed the lowest YM at 44.55 MPa, suggesting a significant improvement in film flexibility. This reduction aligns with theoretical expectations, where plasticizers weaken intermolecular hydrogen bonds in cellulose, thereby enhancing chain mobility and reducing stiffness [18].

Moreover, the YM analysis delineated a decrease with incremental BC and sorbitol additions. Initially, the BC-only films displayed considerable stiffness, a characteristic trait of materials with high crystallinity [23]. As BC concentrations were increased, stiffness initially rose due to the additional cellulose fibers reinforcing the structure of the films. However, similar to TS findings, excessive BC countered stiffness due to ineffective stress distribution within the matrix [35]. In contrast, the systematic addition of sorbitol to the film formulations markedly decreased the YM, reflecting its function as a plasticizer. For instance, the 2.5S formulation showed the lowest YM at 44.55 MPa, indicating substantial increases in film flexibility. This reduction in stiffness with added sorbitol supports the anticipated outcome where plasticizers disrupt hydrogen bonding within cellulose, thereby increasing chain mobility and decreasing material rigidity [36].

EAB further elucidates the ductility and adaptability of the films under strain. Initially, BC-only films displayed low EAB values, signifying limited elasticity, which is typical for rigid polymer matrices. As BC content was increased, there was a moderate increase in EAB, suggesting that higher levels of BC might slightly enhance the flexibility due to the distribution of stress across a denser network of cellulose fibers. On the other hand, increasing amounts of sorbitol had a more pronounced effect on EAB, substantially improving the stretchability of the films. Particularly, films with higher sorbitol content, such as the 2.5S sample, demonstrated significant increases in elongation capabilities. This behavior, indicative of enhanced film plasticity, highlights the efficacy of sorbitol in facilitating polymer chain slippage that induces more deformable film structures [36]. Such properties are desirable in packaging applications where material flexibility and resistance to breakage under load are essential.

Comparatively, the range of TS in this study, between 5.02 and 17.47 MPa, aligns closely with that of the modified BC films derived from sago liquid waste. However, it is noteworthy that the YM and EAB parameters observed in this study were slightly lower compared to those reported for modified BC derived from sago liquid waste. The modified BC displayed a YM range of 2.93-6.54 GPa and an EAB range of 10.27-29.67%, suggesting opportunities for further refinement of materials and tailored adjustments for specific applications.

Thermogravimetric analysis (TGA)

The thermal behavior of selected BC/Chitosan/Sorbitol films was investigated using TGA to unravel their thermal stability and degradation profiles. The obtained TGA curves and their first derivative (DTG) are depicted in Figure 3. DTG was the first-order differential of the TG curve; that is, the upward peaks in the DTG curve were consistent with the maximum weight loss of the TG curve. Notably, the findings unveiled an intriguing similarity in the thermal degradation behavior among all BC/Chitosan/Sorbitol films, aligning closely with the patterns observed in BC and sorbitol. The TGA curves revealed a multi-stage thermal degradation process, shedding light on the intricate transformations that occur within these materials when subjected to high temperatures [27]. Initially, a subtle weight loss, occurring around 100 °C, was linked to the evaporation of water and the presence of residual acetic acid in the polymer matrix [33]. Subsequently, a distinct and substantial weight loss phase unfolded within the temperature range of 200 °C to 350 °C, marking the second thermal event that is closely associated with polymer decomposition [30]. It is worth noting that cellulose typically undergoes thermal degradation within the 300-450 °C temperature range. Incorporating chitosan and sorbitol into the film composition influenced the second stage of thermal degradation, with the primary thermal degradation step for chitosan and sorbitol initiating at approximately 300 °C [37]. Overall, the results regarding thermal stability suggest that the BC/Chitosan/Sorbitol biocomposite films remain stable when subjected to temperatures below 200 °C. As such, this biodegradable composite material is better suited for use with food products following sterilization or pasteurization processes.

Table 3. Tensile strength (TS), Young's modulus (YM), and elongation at break (EAB) of the BC/Chitosan/Sorbitol films

Sample Name	Tensile (MPa)	Young Modulus (MPa)	Elongation at Break (%)
BC	10.60±1.00	629.91	2.36±0.04
0.5S	41.08±2.58	1054.43	4.14±0.05
1.0S	14.95±1.09	426.03	3.71±0.64
1.5S	25.32±2.33	396.34	6.76±0.20
2.0S	15.32±0.76	248.37	6.47±0.04
2.5S	3.83±0.20	44.55	9.21±0.94
1.0BC	6.10±0.14	104.42	6.45 ± 0.05
2.5BC	19.20±0.38	255.99	8.60 ± 0.27
5.0BC	7.90±0.24	54.26	12.91 ± 0.09
7.5BC	20.65±0.85	318.89	11.29 ± 0.45
10.0BC	29.59 ±0.26	403.46	7.78 ± 0.63

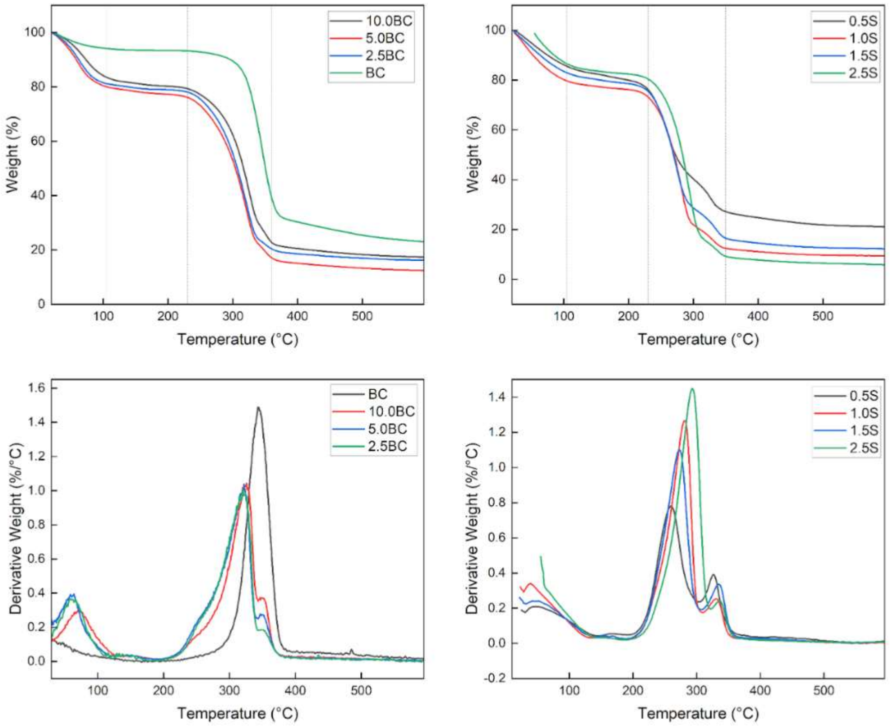

Figure 3. TG and DTG curves of selected BC/Chitosan/Sorbitol films

Morphology by FE-SEM

Figure 4 presents the surface morphology of pure BC film and selected BC/Chitosan/Sorbitol film samples (pure BC, 1.0BC, 10.0BC, 0.5S, 2.5S). The FE-SEM image of the pure BC film reveals the fibrous 3-D ultrafine networks of well-arranged nanofibers stabilized by hydrogen bonds existing in cellulose units [12,22]. However, the addition of sorbitol to the film formulation resulted in slightly smooth and homogeneous surfaces because of an even distribution of the material in the BC-based film [27]. Due to the hydrophilicity properties of sorbitol, an increase in the amount of plasticizer (from 0.5S to 2.5S) resulted in the least appearance of BC fibers and a wetter texture. In contrast, the 1.0BC film containing 0.5g of sorbitol had a dry texture but the same surface morphology.

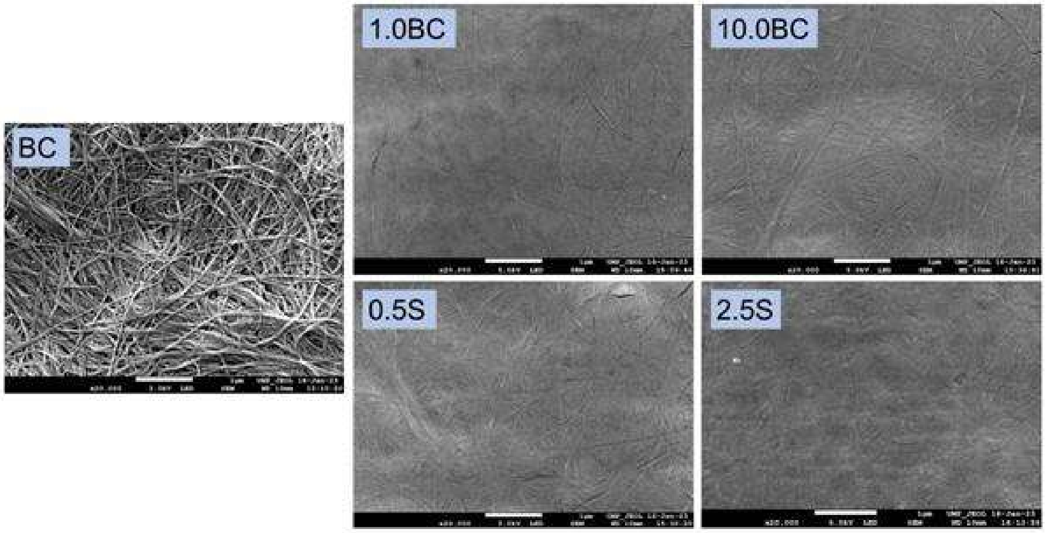

Figure 4. FE-SEM images of selected BC/Chitosan/Sorbitol films at 20000x magnifications

This study presents a comprehensive exploration of BC/Chitosan/Sorbitol films, which were developed using nata de coco as a key resource. Various amounts of BC and sorbitol were added to the films to improve their properties and compare their characterization. The results showed that an increase in BC content led to a significant improvement in the tensile strength, elongation at break, and thermal stability of the films due to hydrogen bonding interactions between the macromolecule chains of BC and the plasticizers. Conversely, the addition of sorbitol influenced moisture content and TGA weight loss. The optimum film composition emerged as 10.0BC and 0.5S, characterized by low moisture content, low water solubility, high tensile strength, and minimal TGA weight loss. These properties position this film as an excellent candidate for extended food packaging, offering a shield against physical, chemical, and biological contaminants while facilitating long-term content preservation. This research not only advances our understanding of biocomposite films but also underscores their potential in diverse applications, particularly in the realms of sustainability and food preservation.

The promising results of this study are anticipated to be the benchmark for future investigations into the incorporation of antimicrobial agents, natural dyes, and other natural plasticizers into the BC/Sorbitol matrix. Additionally, scale-up processes and lifecycle analyses will be crucial for assessing the commercial viability and environmental impact of these biocomposite films. Exploring these avenues will significantly contribute to the advancement of sustainable food packaging technologies.

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References

1. Bharimalla, A.K., Deshmukh, S.P., Vigneshwaran, N., Patil, P.G. and Prasad, V. (2017). Nanocellulose-polymer composites for applications in food packaging: Current status, future prospects and challenges. *Polymer - Plastics Technology and Engineering*, 56: 805-23.
2. Moniri, M., Moghaddam, A.B., Azizi, S., Rahim, R.A., Ariff, A. Bin, Saad, W.Z. et al. (2017) Production and status of bacterial cellulose in biomedical engineering. *Nanomaterials*, 7: 1-26.
3. Nagtzaam, G., Geert, V.C., Steve, K. and Elena, K. (2023). Global plastic pollution and its regulation: History, trends, perspectives. Edward Elgar Publishing.
4. Gupta, I., Cherwoo, L., Bhatia, R. and Setia, H. (2022). Biopolymers: Implications and application in the food industry. *Biocatalysis and Agricultural Biotechnology*, 46: 102534.
5. Wang, H., Qian, J. and Ding, F. (2018). Emerging chitosan-based films for food packaging applications. *Journal Agriculture Food Chemistry,* 2018: 395-413.
6. Athirah Raihana Nor Aziz Hashim, N., Zakaria, J., Mohamad, S., Fathiyah Sy Mohamad, S. and Ab. Rahim, M.H. (2021) Effect of different treatment methods on the purification of bacterial cellulose produced from OPF juice by *Acetobacter xylinum*. *IOP Conference Series: Materials Science and Engineering*, 1092: 012058.
7. Kongruang, S. (2008) Bacterial cellulose production by *Acetobacter xylinum* strains from agricultural waste products. *Applied Biochemistry and Biotechnology*, 148: 245-56.
8. Amr, A. and Ibrahim, H. (2023). Bacterial cellulose: Biosynthesis and applications. *IntechOpen*, pp. 1-24.
9. Gan, M., Guo, C., Liao, W., Liu, X. and Wang, Q. (2023). Development and characterization of chitosan/bacterial cellulose/pullulan bilayer film with sustained release curcumin. *International Journal of Biological Macromolecules*, 34: 301-11.
10. Mishra, S., Singh, P.K., Pattnaik, R., Kumar, S., Ojha, S.K., Srichandan, H. (2022). Biochemistry, synthesis, and applications of bacterial cellulose: A review. *Frontier Bioengineering Biotechnology*, 2022: 780409
11. Vázquez, M., Velazquez, G. and Cazón, P. (2021). UV-shielding films of bacterial cellulose with glycerol and chitosan. Part 1: equilibrium moisture content and mechanical properties. *CYTA - Journal of Food*, 19: 105-114.
12. Rahman, M.A.A.A., Mohamad, S.F.S. and Mohamad, S. (2023). Development and characterization of bio-composite films made from bacterial cellulose derived from oil palm frond juice fermentation, chitosan and glycerol. *Trends in Sciences*, 20: 1-10.
13. Amorim, L.F.A., Mouro, C., Riool, M. and Gouveia, I.C. (2022). Antimicrobial food packaging based on prodigiosin-incorporated double-layered bacterial cellulose and chitosan composites. *Polymers*, 14: 20315.
14. Melro, E., Antunes, F.E., da Silva, G.J., Cruz, I., Ramos, P.E., Carvalho, F. et al. (2021). Chitosan films in food applications. Tuning film properties by changing acidic dissolution conditions. *Polymers*, 23: 1-12.
15. Mohammed, A.A.B.A., Hasan, Z., Omran, A.A.B., Elfaghi, A.M., Khattak, M.A., Ilyas, R.A. et al. (2023) Effect of various plasticizers in different concentrations on physical, thermal, mechanical, and structural properties of wheat starch-based films. *Polymers*, 15: 10063.
16. Maniglia, B.C., Tessaro, L., Ramos, A.P. and Tapia-Blácido, D.R. (2019). Which plasticizer is suitable for films based on babassu starch isolated by different methods? *Food Hydrocolloids*, 89:143-52.
17. Shahrampour, D. and Razavi, S.M.A. (2022). Physicochemical and structural properties of a bio-based antioxidant packaging film derived from *Eremurus luteus* root gum. *Materials Today Communications*, 33: 104756.
18. Indriyati, Dara, F., Primadona, I., Srikandace, Y. and Karina, M. (2021). Development of bacterial cellulose/chitosan films: structural, physicochemical and antimicrobial properties. *Journal of Polymer Research*, 28: 02328
19. Xu, Y., Liu, X., Jiang, Q., Yu, D., Xu, Y., Wang, B. et al. (2021). Development and properties of bacterial cellulose, curcumin, and chitosan composite biodegradable films for active packaging materials. *Carbohydrate Polymers*, 260: 117778.
20. Cazón, P., Velázquez, G. and Vázquez, M. (2019). Characterization of bacterial cellulose films combined with chitosan and polyvinyl alcohol:

Evaluation of mechanical and barrier properties. *Carbohydrate Polymers*, 216, 72-85.

21. Papadaki, A., Lappa, I.K., Manikas, A.C., Pastore Carbone, M.G., Natsia, A., Kachrimanidou, V. et al. (2024) Grafting bacterial cellulose nanowhiskers into whey protein/essential oil film composites: Effect on structure, essential oil release and antibacterial properties of films. *Food Hydrocolloids*, 147: 109374.
22. Nguyen, T.A. and Nguyen, X.C. (2022). Bacterial cellulose-based biofilm forming agent extracted from vietnamese nata-de-coco tree by ultrasonic vibration method: Structure and properties. *Journal of Chemistry*, 2022: 7502796.
23. Yanti, N.A., Ahmad, S.W., Ramadhan, L.O.A.N., Jamili, Muzuni, Walhidayah, T. et al. (2021). Properties and application of edible modified bacterial cellulose film based sago liquid waste as food packaging. *Polymers*, 13: 3570.
24. Azaza, Y. Ben, Hamdi, M., Charmette, C., van der lee, A., Jridi, M., Li, S. et al. (2023). Development and characterization of composite films based on chitosan and collagenous proteins from bluefin tuna: application for peeled shrimp preservation. *Cellulose*, 30: 373-395.
25. Lee, J.S., Lee, E. sil and Han, J. (2020). Enhancement of the water-resistance properties of an edible film prepared from mung bean starch via the incorporation of sunflower seed oil. *Scientific Reports*, 10: 1-15.
26. Mohamad, S., Abdullah, L.C., Jamari, S.S., Al Edrus, S.S.O., Aung, M.M. and Mohamad, S.F.S. (2022). Influence of drying method on the crystal structure and thermal property of oil palm frond juice-based bacterial cellulose. *Journal of Materials Science*, 57: 1462-1473.
27. Wang, X., Guo, C., Hao, W., Ullah, N., Chen, L., Li, Z. et al. (2018). Development and characterization of agar-based edible films reinforced with nano-bacterial cellulose. *International Journal of Biological Macromolecules*, 118: 722-730.
28. Rahmawati, M., Arief, M. and Satyantini, W.H. (2019). The effect of sorbitol addition on the characteristic of carrageenan edible film. *IOP Conference Series: Earth and Environmental Science*, 236: 12129.
29. Ghamari, M.A., Amiri, S., Rezazadeh-Bari, M. and Rezazad-Bari, L. (2022). Physical, mechanical, and antimicrobial properties of active edible film based on milk proteins incorporated with *Nigella sativa* essential oil. *Polymer Bulletin*, 79: 1097-1117.
30. Janik, W., Ledniowska, K., Nowotarski, M., Kudła, S., Knapczyk-Korczak, J., Stachewicz, U. et al. (2023). Chitosan-based films with alternative eco-friendly plasticizers: Preparation, physicochemical properties and stability. *Carbohydrate Polymers*, 301: 120277.
31. Pakoulev, A., Wang, Z. and Dlott, D.D. (2003). Vibrational relaxation and spectral evolution following ultrafast OH stretch excitation of water. *Chemical Physics Letters*, 371: 594-600.
32. Jia, Y., Wang, X., Huo, M., Zhai, X., Li, F. and Zhong, C. (2017). Preparation and characterization of a novel bacterial cellulose/chitosan bio-hydrogel. *Nanomaterials and Nanotechnology*, 7: 1-8.
33. Hasan, N.S.A., Mohamad, S., Mohamad, S.F.S., Arzmi, M.H. and Supian, N.N.I. (2023). Ex-situ development and characterization of composite film based on bacterial cellulose derived from oil palm frond juice and chitosan as food packaging. *Pertanika Journal of Science and Technology*, 31: 1173-1187.
34. Boey, J.Y., Lee, C.K. and Tay, G.S. (2022). Factors affecting mechanical properties of reinforced bioplastics: A review. *Polymers*, 14:183737.
35. Wang, B., Yang, D., Zhang, H.R., Huang, C., Xiong, L., Luo, J. et al. (2016). Preparation of esterified bacterial cellulose for improved mechanical properties and the microstructure of isotactic polypropylene/bacterial cellulose composites. *Polymers*, 8: 1-11.
36. Paudel, S., Regmi, S. and Janaswamy, S. (2023). Effect of glycerol and sorbitol on cellulose-based biodegradable films. *Food Packaging and Shelf Life*, 37: 101090.
37. Arief, M.D., Mubarak, A.S. and Pujiastuti, D.Y. (2021). The concentration of sorbitol on bioplastic cellulose based carrageenan waste on biodegradability and mechanical properties bioplastic. *IOP Conference Series: Earth and Environmental Science*, 679: 12013.