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Development and Characterization of Bio-Composite Films Made from Bacterial Cellulose Derived from Oil Palm Frond Juice Fermentation, Chitosan and Glycerol

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Abstract

This study reported for the first time, the combined effects of chitosan and glycerol addition on the properties of bacterial cellulose (BC) based films for food packaging applications. Films were prepared by solution casting method using BC derived from oil palm frond juice as the main material combined with different concentrations of chitosan (0.5 and 1 %w/v) and glycerol (0.5, 1.5 and 2.5 %v/v). Pure BC, chitosan-free and glycerol-free films were used as control. The effect of incorporating chitosan and glycerol on bacterial cellulose (BC) based films was evaluated based on the physical properties (thickness, moisture content, solubility), mechanical properties (tensile strength, modulus Young, elongation at break) and chemical structure by FTIR. Increased concentration of chitosan and glycerol affected the physical and mechanical properties. The combination of 1 %w/v chitosan and 0.5 %v/v glycerol had a strengthening effect on the BC-based films with maximum tensile strength of 15 MPa and Young's modulus of 772 MPa. Meanwhile, BC films incorporated with 1 %w/v chitosan and 2.5 %v/v glycerol demonstrated high plasticizing effect of 7 % elongation at break. The acquired FTIR spectrum of the bio-composite films suggested intermolecular interactions between BC, chitosan, and glycerol. Therefore, the BC-based bio-composite films incorporated with chitosan and glycerol have the potential to be used as food packaging materials.

Keywords: Bacterial cellulose, Bio-composite films, Mechanical properties, Chitosan, Glycerol, Food packaging

Abbreviations

BC = bacterial cellulose,
FTIR = Fourier-transform infrared,
MC = moisture content,
SW = solubility in water,
TS = tensile strength,
EAB = elongation at break,
YM = Young's modulus

Introduction

Petroleum-based plastic packaging materials (e.g., polyvinylchloride, polyethylene, and polypropylene) have been commonly used in the food industry due to their easy processability, low cost, and superior resistance to chemical and mechanical stress [1]. However, extensive usage of these plastic-based materials for food packaging has caused major concerns for the environmental pollution and ecological imbalances due to their poor recyclability, derivability from non-renewable resources, and non-biodegradability [2]. For that reason, an increased public awareness on the effect of pollution and disposal of conventional plastic materials on the environment and consumer demand for improved sustainability has triggered interest in developing alternative environmentally friendly food packaging materials using various natural biopolymers. Several biopolymers, such as chitosan [3], gelatin [4], alginate [5], starch [6] or bacterial cellulose (BC) [7], have been explored as potential matrices for food packaging applications owing to their excellent biodegradability, biocompatibility, nontoxicity, and abundant availability [8].

Among all, bacterial cellulose (BC) has been vastly utilized as a promising biopolymer for developing food packaging films for its exceptional intrinsic properties, including high purity, high surface area, and biodegradability [9]. BC is an extracellular polysaccharide synthesized mainly by *Acetobacter xylinum* and linearly composed of β-D-glucopyranose units ($C_6H_{11}O_5$) connected by β-1,4-glycosidic bonds. Over the years, BC have been researched a lot as the primary material to produce food packaging films [10], as a reinforcing agent [11], and also in the production of active and intelligent food packaging materials with the addition of bioactive compounds [12,13]. However, BC is insoluble in most solvents and is therefore not a filmogenic polymer without modification or functionalization [14]. BC-based films typically had poor elasticity and brittleness, which limits its application as food packaging materials [14]. Hence, BC is often blended with other biopolymers or plasticizers, such as chitosan and glycerol to improve the structure and characteristics of the BC-based composite films.

Chitosan is the second most abundant natural biopolymer, after cellulose, obtained mainly from the residues of the shellfish industry [15]. Chitosan has been demonstrated as a versatile biopolymer for a wide range of applications, including food packaging due to its non-toxicity, biodegradability, film-forming properties, solubility, and antimicrobial ability. In addition, BC and chitosan share similar chemical structures and mutually complementary qualities, resulting in reinforced molecular interactions among polysaccharide chains [16]. Blending chitosan into BC resulted in a better structural behavior of the films due to the film-forming ability of chitosan [17]. Compared with films of pure chitosan, BC/chitosan composite films exhibit more advantageous mechanical qualities, such as rational thermal stability and low oxygen permeability [18]. Thus, chitosan possesses great potential for being utilized as a filler in producing BC-based films, in terms of promising mechanical qualities and film-forming capability [19].

In spite of numerous reports in the literature on the properties and applications of BC/chitosan blends for various applications [20], Thakhiew *et al.* [21], revealed that pure chitosan and BC/chitosan films cannot be used directly as packaging materials due to the poor flexibility and ability to elongate caused by significant intermolecular pressures involving polymer chain-to-chain connections. Hence, the addition of plasticizers into the film formulation is required to improve the film's mechanical properties and suppress its brittleness. It is hypothesized that plasticizers can interfere with the chains' intra- and inter-molecular forces, while increasing the free volume of the chains, which could help improve the film's stretchability and flexibility [22]. Glycerol is one of the most common plasticizers used for improving the stretchability and flexibility of bio-composite films. Glycerol is water-soluble, non-volatile, and also regarded as a food additive by the Food and Drug Administration [23]. By adding glycerol, it might be possible to produce bio-composite films that have a higher percentage of elongation at break and are flexible enough to facilitate the processing and applications of polymers [24].

BC/chitosan composite films can be prepared either by directly adding chitosan into the culture medium during the fermentation of BC (*in-situ*), or by immersing the BC gels in chitosan solution (*ex-situ*), or solvent casting method. In this study, we reported a solvent casting method using disintegrated BC produced through static cultivation of *Acetobacter xylinum* in oil palm frond juice blended with chitosan and glycerol. To our knowledge, there is only one report available on the preparation of BC/chitosan composite films via solvent casting method in the literature [18]. However, the previous study only varied the different percentages of chitosan solution used and did not consider the combined effects of different concentrations chitosan and glycerol on the functional properties of bio-composite film produced. Therefore, the aim of this study was to evaluate the effects of incorporating different chitosan contents (0.5 and 1 % w/v) and glycerol (0.5, 1.5 and 2.5 % v/v) on BC/chitosan/glycerol bio-composite films prepared via the solvent casting method. The obtained BC/chitosan/glycerol films were evaluated for their properties in terms of film thickness, water solubility, moisture content, chemical structure, and mechanical performance.

Materials and methods

Materials

Oil palm frond juice was obtained by pressing fresh oil palm frond petioles collected from LKPP Oil Palm Plantation located in Pekan, Pahang, Malaysia with a hydraulic pressing machine (MATSUO Inc., Japan). Chitosan of medium molecular weight and sodium hydroxide were purchased from Sigma-Aldrich. Glycerol and acetic acid were purchased from R&M Chemicals. *Acetobacter xylinum* was obtained from the Malaysian Agriculture Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. All other chemicals used were of analytical grade.

Production and purification of bacterial cellulose

BC was produced following the method of Mohamad *et al.* [25] with slight modifications. In brief, 80 % v/v oil palm frond juice (adjusted to pH 4.5 using 50 %v/v acetic acid) was used as the culture medium for BC production. After sterilization at 121 °C (15 min), the medium was inoculated with 10 %v/v *Acetobacter xylinum* and statically incubated for 14 days at 30 °C. At the end of 14 days of growth, BC pellicles formed at the surface of the culture medium were harvested and repeatedly washed using distilled water. The BC pellicles were then purified by boiling them in 0.5 M NaOH for 2 h to ensure that the BC surface was free from any bacterial cell attachment. The pure BC pellicles were then treated with 5 % v/v NaOCl (12 h, room temperature) and then rinsed with distilled water several times until neutral pH was attained.

Preparation of BC slurry

The wet pure BC pellicles were blended using a domestic blender for 1 h (ProMix Handblender, Philips) until a thick and smooth BC slurry was obtained. The slurry obtained was collected and stored at room temperature for further use.

Preparation of BC-based film with chitosan and glycerol

The bio-composite films were developed using the BC slurry (70 mL), chitosan (0.5 and 1.0 %w/v) and glycerol (0.5, 1.5 and 2.5 %v/v) as the plasticizer according to the composition shown in **Table 1**. First, the 2 concentrations of the chitosan solutions were prepared by dissolving chitosan powders in 1 %v/v acetic acid using a magnetic stirrer at room temperature until the powder fully dissolved. All film formulations were magnetically stirred at 70 °C for 1 h until homogenous. The film solutions were then cast into a modified casting mould (8×12 cm^2) and dried at 70 °C for 24 h in an oven. After the solutions were fully evaporated, the film was peeled off from the modified casting mold and stored in zip lock bags for further analysis.

Table 1 Composition of BC, Chitosan and Glycerol in BC/Ch/G films.

Sample Name	Bacterial Cellulose (BC)	Chitosan (Ch)	Glycerol (G)
	mL	%w/v	%v/v
BC	70.00	0.00	0.00
BC/0.5G	70.00	0.00	0.50
BC/1.5G	70.00	0.00	1.50
BC/2.5G	70.00	0.00	2.50
BC/0.5Ch	70.00	0.50	0.00
BC/1.0Ch	70.00	0.50	0.50
BC/0.5G/0.5Ch	70.00	0.50	1.50
BC/1.5G/0.5Ch	70.00	0.50	2.50
BC/2.5G/0.5Ch	70.00	1.00	0.00
BC/0.5G/1.0Ch	70.00	1.00	0.50
BC/1.5G/1.0Ch	70.00	1.00	1.50
BC/2.5G/1.0Ch	70.00	1.00	2.50

Characterization of film samples at ambient temperature (± 25 °C)

Film thickness

A handheld micrometer (Mitutoyo, Japan) was used to measure the thickness of each composite film to the nearest 0.001 mm. Measurements were carried out at 10 different film locations, and the average thickness value was used to calculate the mechanical properties of the films.

Moisture content

The moisture content of the films (approximately 2×2 cm^2) was determined according to Eq. (1) by measuring the weight loss of films before and after drying in an oven at 105 °C until a constant dry weight was obtained. Three replications of each film treatment were used for calculating the moisture content.

$$\mathrm{MC}(\%) = \frac{\text{Initial sample weight - Dry sample weight}}{\text{Initial sample weight}} \times 100 \tag{1}$$

Film solubility in water

In this study, solubility in water was defined as the ratio of the water-soluble dry matter of film that is dissolved after immersion in distilled water [26]. The film samples were cut to size (approximately 2×2 cm^2) in triplicate, dried at 105 °C for 24 h in an oven and weighed to determine the initial dry weight. Dried film samples were then placed in 50 mL screw-capped centrifuge tubes containing 30 mL of distilled water. The tubes were placed in a water bath (Memmert, D-91126, Schwabach, Germany) under constant shaking (100 rpm) at 25 °C for 24 h. After that, undissolved films were removed by filtering through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) and dried at 105 °C until the weight is constant to determine the final dry weight. The water solubility (SW), expressed as a percentage was calculated using the following Eq. (2);

$$\mathrm{SW}(\%) = \frac{\text{Dry sample weight - Final dry weight}}{\text{Dry sample weight}} \times 100 \tag{2}$$

Mechanical properties

The mechanical properties of the films were measured using a universal testing machine (Shimadzu, AGS-X Series, Japan), according to the ASTM D822 method. The tests were run with an initial grip separation of 50 mm and a crosshead speed of 5.08 mm/min. For each film sample, the tensile strength (TS) and elongation at break (EAB) were defined using Eqs. (3) and (4), respectively.

$$\mathrm{TS} = \frac{\text{Force (F)}}{\text{Area (A)}} \tag{3}$$

$$\mathrm{EAB} = \frac{\Delta \mathrm{L}}{\mathrm{L}_0} \times 100 \tag{4}$$

In Eq. (3), F is the loading force obtained from the mechanical test (N) and A is the cross-section area measured as the width × thickness of the sample (mm^2). Meanwhile, in Eq. (4), ΔL has exerted the extension from the starting point and L_0 initial length of the sample (70 mm). The slope of the straight part of the tension/strain curve was used to define Young’s Modulus (YM).

FTIR spectrophotometer

The film samples were cut into small pieces (1×1 cm^2) and analyzed using an FTIR Spectrophotometer (Nicolet iS5, Thermo Fisher Scientific, USA). For each sample, a total of 32 scans with a resolution of 4 cm^{-1} were collected over the wavenumber ranging from 400 - 4,000 cm^{-1} in absorbance mode.

Results and discussion

Qualitative observations of BC/chitosan and BC/glycerol/chitosan films

Figure 1 presents the photographs of the BC-based films prepared with different concentrations of chitosan and glycerol. Visually, the appearance of the BC-based films showed plastic-like, semi-transparent thin sheet films. The BC film had a few air bubbles appearing in the film, probably attributed to the vigorous mixing of the film-forming solution. The BC film also had an uneven surface due to the appearance of air bubbles. The incorporation of 0.5 %v/v glycerol in BC resulted in a smoother surface film without any bubbles formed, with a white color and opaquer compared to other films. However, the presence of more than 2.5 %v/v glycerol resulted in an oily texture in the film. After fully dehydrating, the visual appearances of BC/Ch and BC/G/Ch films were more yellowish in color than pure BC film. As chitosan solution has a yellow color, increasing chitosan concentration used in the film-forming solution may lead to lightness loss in the film color, which results in a film with a darker shade of white or yellow.

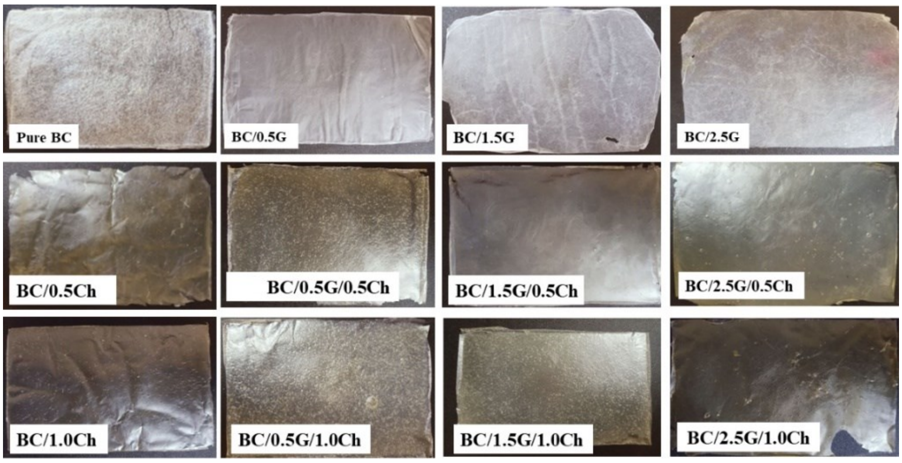

Figure 1 Physical appearances of BC/chitosan/glycerol films.

Film thickness, moisture content and solubility in water

Film thickness is an important characteristic that determines numerous physicochemical features of the films. As depicted in **Table 2**, the thickness of the films ranged from 0.0541 to 0.2507 mm, and the thickness increased with the increase in chitosan and glycerol concentration. The thickness of the BC film alone (without the addition of chitosan and glycerol) was greater that of other films produced with chitosan and glycerol, probably due to the presence of air bubbles in its film matrix. A similar trend was reported for edible films of BC from sago, although the thickness values of the films obtained in this study are much higher than those observed by Indriyati *et al.* [18], who reported a thickness value of 0.045 to 0.083 mm. This variation might be attributed to differences in the procedures used to create the films and the formulations of the film-forming solution. The increase in thickness indicates a reduction in film compactness and increased free volume in the films due to intermolecular interactions between glycerol and biopolymers (BC and chitosan) [11]. The incorporation of the highest concentration of glycerol (2.5 %v/v) produced the thickest films because glycerol dissolves rapidly in water, increases the viscosity of the film-forming solution, and binds water. As a result, the more glycerol added, the less water evaporates because part of the water in the film solution is held by the glycerol, affecting the thickness of the film produced [27].

Table 2 Thickness, moisture content (MC) and solubility in water (SW) of the BC/Chitosan/Glycerol films.

Sample name	Thickness (mm)	Moisture content (%)	Solubility in water (%)
BC	0.1800	12.46	9.53
BC/0.5G	0.0541	34.98	34.60
BC/1.5G	0.1653	56.08	33.77
BC/2.5G	0.1775	65.09	30.18
BC/0.5Ch	0.0652	12.98	8.72
BC/1.0Ch	0.0740	13.57	7.76
BC/0.5G/0.5Ch	0.1127	50.12	41.54
BC/1.5G/0.5Ch	0.1487	69.36	25.00
BC/2.5G/0.5Ch	0.2176	62.44	23.61
BC/0.5G/1.0Ch	0.1191	32.34	28.86
BC/1.5G/1.0Ch	0.1645	51.42	27.53
BC/2.5G/1.0Ch	0.2507	59.55	26.83

Determining the moisture content (MC) of the films was crucial because an increase in moisture content results in faster microbial growth and degradation [26]. The moisture could also work as a plasticizer and majorly influences the mechanical properties of the films. According to **Table 2**, the MC of the 3 BC-based films (without addition of glycerol) ranged between 12.46 and 13.57 %. Meanwhile, the MC of the film (which contains glycerol) increases dramatically as the amount of glycerol increases. The capacity of BC/Ch/G films to retain water is probably related to the hydrophilic nature of glycerol, which has a strong affinity for water. Ahmadi *et al.* [28] reported similar results in films prepared from *Psyllium* hydrocolloids (Plantago seeds of the plant), where increasing glycerol concentrations significantly enhanced the moisture content of the evaluated films.

The solubility of film in water (SW) is another important factor in determining the hydrophilicity of the films. **Table 2** shows that the SW value for BC film is only 9.53 %. As predicted, all the BC-based films containing glycerol has higher SW than the pure BC film. However, regardless of the chitosan concentration used, the SW decreased as the glycerol concentration increased. Glycerol is entirely miscible with water and absorbs moisture when exposed to damp air (hygroscopic). According to Indriyati *et al.* [18], increasing glycerol concentration lowered the SW of the BC from sago-based films. The SW of the BC/chitosan/glycerol films developed in this study ranges from 26 to 42 %, much lower compared to the starch-based films that span between 80 and 90 %. The low SW was probably due to the availability of hydrophilic cellulose, which is insoluble in common solvents due to the presence of abundant free hydroxyl (OH) groups that form strong intra-and intermolecular hydrogen bonds between the chains [18].

Mechanical properties

In terms of food packaging, the applicability of bio-composite films strongly depends on their mechanical properties. In general, bio-composite films should be strength and flexible enough to withstand various types of external stress and serve the containment function while maintaining their structural integrity. As shown in **Table 3**, combination of both chitosan and glycerol in BC-based films resulted in much higher TS, YM and EAB values than the pure BC film. Increased glycerol concentration in all BC/G and BC/G/0.5Ch film samples, on the other hand, resulted in a decrease in TS, YM, and EAB. The addition of a high concentration of glycerol in the film samples reduces the strength of hydrogen bonds between adjacent cellulose chains, thus, altering the tensile strength of the film membranes [29]. The high content of glycerol may cause the 'cross-linker' effect, which reduces the polymer's free volume and segmental mobility, which could mitigate mechanical strength and increase the flexibility of the films [18]. Multiple studies have found that an increase in plasticizers weakens the mechanical qualities of biodegradable film products [30,31].

In addition, for BC/G/1.0Ch films samples, increasing concentration of glycerol, which is a hydrophilic compound, leads to an increase in the percentage of EAB and makes it easier for biodegradable films to crack when they are subjected to stress. The increase in percent EAB occurs because the plasticizer can reduce the fragility and increase the flexibility of polymer films by disrupting hydrogen bonds between adjacent polymer molecules, thus decreasing the TS of the intermolecular in the polymer chain [32]. Meanwhile, increasing the chitosan concentration in the formulation of BC/Ch films (without glycerol) from 0.5 to 1.0 %w/v slightly reduced the TS and YM of the bio-composite films. This is due to decreased intermolecular contact between the hydroxyl groups of BC and chitosan, resulting in a reduction in mechanical characteristics [33]. These findings differed slightly from those found in the literature. Similar findings for bacterial cellulose-glycerol-chitosan composites discovered that chitosan improved the composites' mechanical qualities when compared to pure bacterial cellulose film [34].

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

Film	Tensile strength (MPa)	Young's Modulus (MPa)	Elongation at break (%)
BC	3.31	173.28	3.29
BC/0.5G	30.19	519.08	5.79
BC/1.5G	1.56	29.11	4.28
BC/2.5G	0.52	9.25	1.86
BC/0.5Ch	36.71	1,303.10	3.32
BC/1.0Ch	30.11	1,238.00	3.36
BC/0.5G/0.5Ch	9.42	370.18	5.22
BC/1.5G/0.5Ch	7.48	394.68	5.59
BC/2.5G/0.5Ch	4.14	213.72	7.06
BC/0.5G/1.0Ch	15.29	772.10	2.66
BC/1.5G/1.0Ch	12.34	436.71	3.44
BC/2.5G/1.0Ch	9.54	219.34	7.09

Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra of the BC-based composite films with and without the addition of either chitosan or glycerol in the wavenumber range of 400 - 4,000 cm^{-1} are shown in **Figures 2(a)** and **2(b)**. As can be seen, the broad peaks located at the wavenumber at 3,200 - 3,500 cm^{-1} were found in all film samples, attributed to the O–H stretching vibration formed by the hydroxyl group of BC and glycerol. The hydroxyl groups in BC help to form various types of inter-and intramolecular hydrogen bonds, and the formation of inter-and intramolecular hydrogen bonds in cellulose is essential for the physical properties of cellulosic materials [18]. Meanwhile, the peaks between 2,800 and 3,000 cm^{-1} have been attributed to C–H stretching vibration [35]. The peak around 1,650 cm^{-1} is assigned to the –CO group and the peaks at 1,410 and 1,370 cm^{-1} are assigned to the –CH asymmetric deformation and –CH bending vibration, the characteristic signature for samples with chitosan [20]. Another intense peak at 1,050 cm^{-1} was assigned to C–O–C pyranose ring stretching vibration. The shifting of bands in BC/chitosan/glycerol films as compared to BC film suggests that there might be a possible interaction between the hydroxyl groups of BC, glycerol and the amino groups of chitosan (**Figure 3**). But, as shown in **Figure 2(b)**, when both chitosan and glycerol were added to the films, there was no new peak in the spectrum. This means that there were no chemical interactions between BC, chitosan, and glycerol.

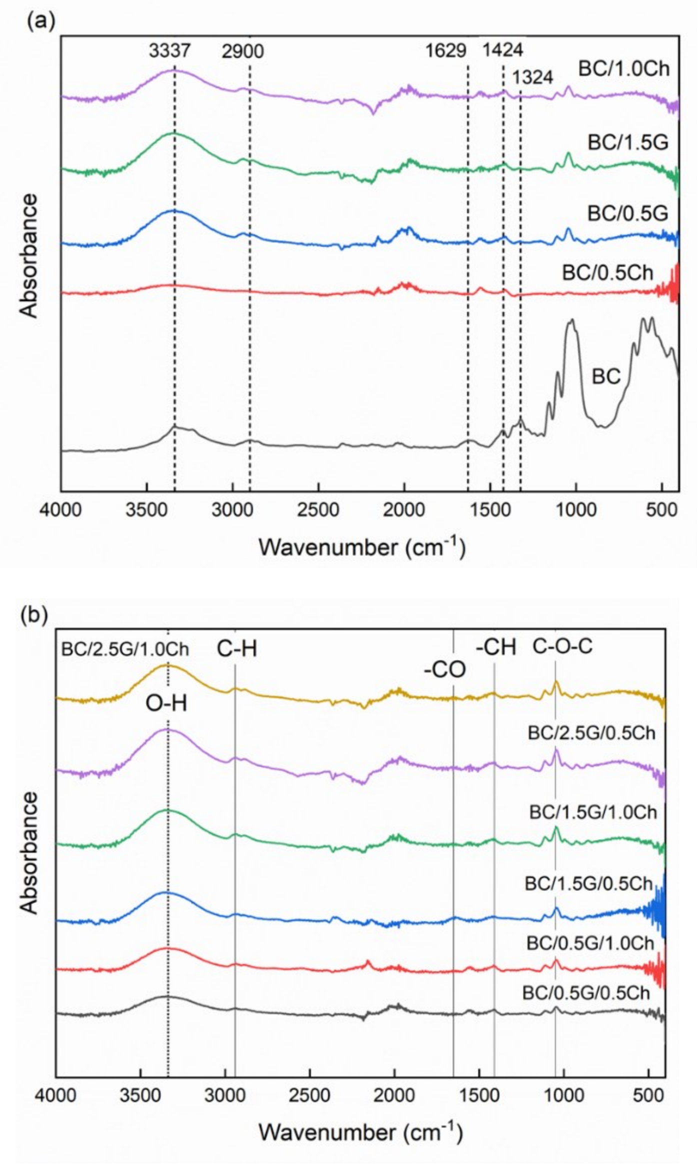

Figure 2 FTIR spectrums for BC/chitosan/glycerol films.

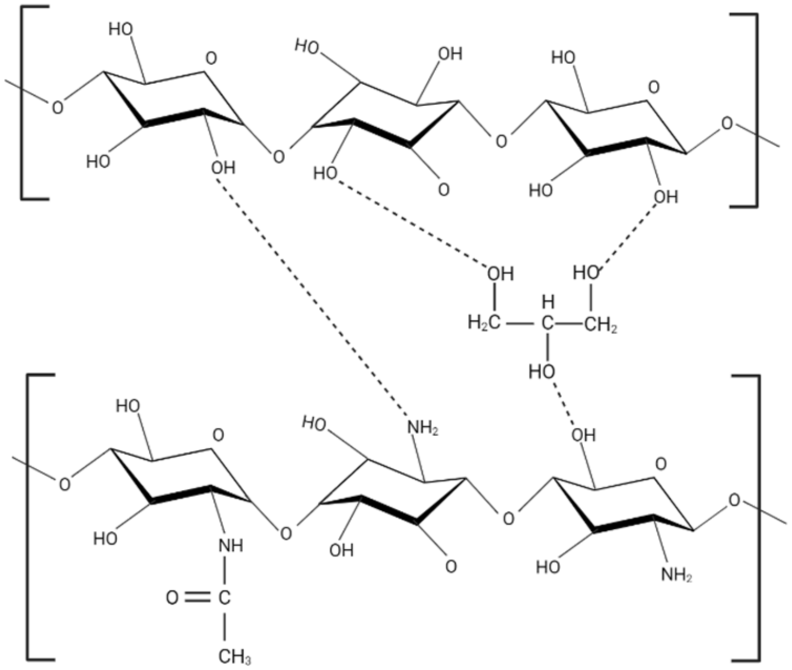

Figure 3 Interaction between bacterial cellulose, chitosan, and glycerol.

Conclusions

The developed BC/chitosan/glycerol bio-composite films demonstrated good film-forming characteristics as potential food packaging. The physical and mechanical properties of the film were affected by the increased concentration of chitosan and glycerol. The interaction of a mixture of BC, chitosan, and glycerol as the edible film increased the thickness and strength of the film, which significantly increased moisture content and decreased water solubility. The incorporation of chitosan and glycerol increased the flexibility of the BC/chitosan/glycerol films due to the increased interaction between the hydroxyl group of glycerol and the chitosan in the BC matrix. FTIR revealed that glycerol did not change the functional groups according to the film spectrum. All these findings provide a basis for developing edible food packaging materials for food storage purposes.

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