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Research Article

Research on Development and Characterization of Composite Membranes Based on Hybrid Bacterial Cellulose Combined with Glycerol and Vegetable Residues for the Preservation of Fresh Fruit and Food

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This study aims to develop a biocompatible and bioactive food-packaging composite film material. The material is based on bacterial cellulose (BC) microfibers from coconut jelly biomass combined with olive oil as a carrier of antibacterial properties. A composite membrane was fabricated with 20%, 30%, and 40 wt % glycerol and separately impregnated with 1%, 2%, and 3 wt % olive oil in the presence of BC. The results of SEM image structure and morphology show that the membrane was successfully fabricated with uniform distribution of BC, without losing its natural structure. The film was initially applied to preserve apples, and the research results showed that the mass index did not change significantly (ranging from 1.13 to 5.1 g); the hardness through the bearing test results showed a decrease. The preservation time of the composite membranes, which are based on bacterial cellulose combined with glycerol and vegetable residues, extends up to 20 days. The membrane with the highest concentration of vitamin C is soaked in BC/glycerol 30%/olive oil 2% membrane and the lowest is soaked in BC membrane.

1. Introduction

Research on the development and characterization of composite membranes based on hybrid bacterial cellulose combined with glycerol and vegetable residues for the preservation of fresh fruit and food is a topic of growing interest in the field of biomaterials and food packaging. Several studies have contributed valuable insights into various aspects of this research area, encompassing the utilization of bacterial cellulose and its composites, incorporation of bioactive compounds, and enhancement of barrier properties for food preservation. Choudhary et al. explored the preparation and characterization of bacterial cellulose-based composites [1]. Revin et al. provided a comprehensive review of bacterial cellulose-based polymer nanocomposites [2], while Mbituyimana et al. discussed research progress and existing products in bacterial cellulose-based composites for biomedical and cosmetic applications [3]. Noh et al. focused on the fabrication of bacterial cellulose-collagen composite scaffolds and their osteogenic effect [4]. Bodea et al. investigated the antimicrobial properties of bacterial cellulose films enriched with bioactive herbal extracts [5], while Lin et al. discussed the current research and future prospects of bacterial cellulose in the food industry [6]. Azeredo et al. highlighted the potential of bacterial cellulose as a raw material for food and food packaging applications [7].

Several studies explored the development of bacterial cellulose composites with other materials. Amjadi et al. prepared gelatin-based nanocomposites containing chitosan nanofiber and ZnO nanoparticles [8], while Ju et al. and Mei et al. characterized bacterial cellulose composite films incorporated with chitosan and chitosan nanoparticles [9, 10]. Roy et al. developed gelatin/cellulose nanofiber-based functional nanocomposite films incorporated with zinc oxide nanoparticles [11]. Furthermore, Xu et al. provided a review of nanocellulose composite films in food packaging materials [12], while Pa'e et al. investigated the thermal behavior of bacterial cellulose-based hydrogels with other composites [13]. Pandey et al. discussed bacterial cellulose as a smart biomaterial for biomedical applications [14], and Nunes et al. developed bacterial cellulose biocomposites combined with starch and collagen [15]. In addition to bacterial cellulose, other biomaterials and their composites were also explored for food packaging applications. Motelica et al. reviewed biodegradable antimicrobial food packaging trends and perspectives [16], while Velásquez-Riaño and Bojacá investigated the production of bacterial cellulose from alternative low-cost substrates [17]. Tharanathan and Kittur discussed chitin as a biomolecule of great potential [18]. The incorporation of bioactive compounds into food packaging materials was also studied. Santana-Gálvez et al. reviewed chlorogenic acid's dual role as a food additive and nutraceutical against metabolic syndrome [19], and Jimenez-Lopez et al. explored bioactive compounds and quality aspects of extra virgin olive oil [20].

Overall, the research landscape related to the development and characterization of composite membranes for food preservation is rich and diverse, encompassing various materials, fabrication techniques, and functional properties aimed at enhancing food quality and safety.

This research aims to investigate the properties and performance of these composite membranes, with a primary focus on their ability to extend the shelf life of fresh fruits and food products. The combination of bacterial cellulose, glycerol, and olive vegetable oil holds the promise of offering an environmentally friendly, effective, and sustainable solution to the challenge of food preservation. This research represents an essential step towards bridging the gap between the remarkable properties of these biopolymers and their practical applications in preserving fruits and fresh food.

2. Materials and Methods

2.1. Materials. Coconut jelly in the study was provided by Minh Tam Coconut Jelly manufacturer, Ben Tre Province, Vietnam (address: 287D Doan Hoang Minh, Binh Khoi Quarter, Ward 6, Ben Tre City, Vietnam). Glycerol provided by Merck Vietnam Company LTD (9th Floor, Center Point, 106 Nguyen Van Troi, Phu Nhuan District, Ho Chi Minh City, Vietnam). Olive oil (Olv) is a product produced at ACEITES YBARRA S.A factory, Spain.

2.2. Methods

(i) Method for determining total lipid content: Crush the apples, weigh 10 g of the crushed apple sample, put it in a sample tube, and put it into the extraction tower of the Soxhlet machine. After the extraction process is finished, wait for the system to cool, remove the sample tube, and then proceed to recover the solvent right in the flask using a rotary vacuum evaporator with the heating bath temperature maintained at 50°C and vacuum pressure not about 150–300 Pa. After completing the evaporation process to expel all the solvent, place the flask in the drying oven, dry at a temperature of 100–105°C, and dry to a constant volume.

(ii) Hardness measurement method: Use an original apple to remeasure its hardness and save the results of the original apple to compare with subsequent storage times. Apples, after undergoing the storage period corresponding to the time points, are cut to the correct size of 1 cm, 2 cm.

(iii) Method for determining vitamin C content: Weigh accurately 10 g of apples, put them in a ceramic mortar, and crush them in 2% (m/m) oxalic acid extraction solution, or metaphosphoric acid/acetic acid solution. Transfer the sample to a 100 ml volumetric flask. Shake well and continue adding extraction solution to rinse the mortar and make up to the mark. Filter with filter paper or cloth to collect the vitamin C filtrate for analysis (the amount of sample taken is such that the vitamin C concentration is within 0.5 mg/ml).

1. Erlenmeyer flask 1 (about 50 ml): use a pipette to take 10 ml of vitamin C filtrate for analysis.
2. Titrate rapidly with 2,6 dichlorophenolindophenol (DCIP) until a pink color appears that lasts for 1 minute. During this process, vitamin C (ascorbic acid) acts as a reducing agent, converting DCIP from its blue (enhanced) form to its pink (reduced) form V1 (ml) is the volume of standard solution that was used to reach the endpoint during the titration.
3. Repeat the experiment 3 times and average the results between 3 measurements.
4. Dye solution (2,6 dichlorophenolindophenol): Dissolve 50 mg of sodium salt of 2,6 dichlorophenolindophenol in 150 ml of hot water (50–600°C) containing 42 mg of sodium hydrogen carbonate in a 200 ml volumetric flask and add water to the mark and filter. Store this solution in a dark brown bottle and keep it in the refrigerator. Because this solution decomposes over time, a new solution must be prepared periodically.
5. Vitamin C filtrate: Accurately weigh 10 g of sample (error 0.1 g), put it in a ceramic mortar and crush it in 2% oxalic acid solution, then transfer the sample to a 100 ml volumetric flask, and make up the volume with oxalic acid solution to the mark. Filter with filter paper or vacuum to obtain vitamin C filtrate.

(iv) SEM (scanning electron microscopy, HITACHI, S4800-NIHE, 10 kV, National Institute of Hygiene and Epidemiology (NIHE), Hanoi, Vietnam): the adsorption material was assessed for its structural morphology using the SEM (scanning electron microscopy) method, capturing surface fracture features at various magnifications.

(v) Evaluation of the antibacterial ability method: the bacteriostatic activity was assessed using the agar diffusion plate method. The bacteriostatic activity test follows the method described by Hadacek et al. conducted at the Institute of Biotechnology, Vietnam Academy of Science and Technology. Bacterial strains tested: *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 12222; and *Moraxella catarrhalis* ATCC 49143.

2.2.1. Process for Manufacturing Composite Membranes from Coconut Jelly Biomass. Figure 1 shows the manufacturing process of BC and BC/olive oil/glycerol composite membranes. Coconut jelly, consisting of over 90% nanocellulose fibers formed by *Acetobacter xylinum* bacteria in coconut water, is known as Bacterial Cellulose (BC). When purchasing BC, it needs to undergo chemical processing to eliminate impurities, especially *Acetobacter xylinum* bacteria remains. The refinement process proceeds as follows: Initially, raw BC is rinsed with distilled water and then immersed in distilled water while boiling coconut jelly for about 90 minutes at temperatures ranging from 90°C to 95°C. Following boiling with distilled water, the coconut jelly is further boiled with a 0.01M NaOH solution for 90 minutes at temperatures of 90°C to 95°C. The coconut jelly is continuously rinsed with distilled water until the pH reaches 7 (see Figure 1).

After treatment with a 0.01M NaOH solution, BC is subjected to blending with water in a blender until homogeneity is achieved. The resulting BC composite membrane is then obtained through vacuum filtration and dried under ambient conditions. Subsequently, BC is mixed with a mixture of olive oil (2 wt.%) and glycerol (30 wt.%) before being mechanically stirred at 2000 rpm for 90 minutes. This mixture is used to coat fresh fruits and create a thin layer of film for preservation purposes (see Figure 1).

2.2.2. Packaging Fruits by Immersion Method. The process of packaging fruits using the immersion method with BC composite membrane is carried out through the following specific steps (see Figure 2):

(i) Preparing the fruits: Fruits, including apples, are sourced from a local supplier (Moc Chau, Son La). Before packaging, fruits are carefully selected to ensure they are commercially ripe and show no signs of any damage.

(ii) Fruit disinfection: Fruits are disinfected for 5 minutes at commercial ripeness using a 200 ppm NaClO solution to eliminate harmful bacteria and microorganisms. They are then air-dried to remove excess water before proceeding with the packaging process.

(iii) Immersion in BC composite solution: Fruits are immersed in a BC/olive oil/glycerol solution for 10 minutes. This immersion process allows the BC composite membrane to adhere tightly to the surface of the fruits, forming a thin protective membrane layer without altering the sensory properties of the fruits.

(iv) Repeat the process: Each fruit sample is used three times for each packaging process to ensure consistency and reliability of results. In the experimental process, fruits without membrane coating are used as controls for each treatment.

(v) Evaluation and storage: During storage after packaging, the freshness of the fruits is evaluated based on sensory characteristics such as odor, color, dryness, and contamination. This ensures the quality and food safety of the fruits after packaging.

3. Results and Discussion

3.1. Characteristics of Composite Film (BC)

3.1.1. Structural Morphology of the Composite Film (BC). Based on the SEM images of the BC structure, an organized arrangement of nanocellulose fibers ranging from 30 to 60 nanometers is observed, exhibiting clean and smooth surfaces. This structural configuration forms a natural entangled layer, with cellulose fibers naturally intertwining and bonding with each other. This unique architecture endows the BC membrane with excellent capability in preserving fresh fruits. Recent research has shed light on the multifaceted roles of glycerol and its derivatives in various biological processes, as well as their significance in the prebiotic origins and evolution of life. Gull and Pasek explored this topic comprehensively, elucidating the biochemical importance of glycerol and its derivatives in living organisms [21]. Their study delved into the molecular mechanisms underlying the involvement of glycerol in essential biological functions, highlighting its relevance in cellular metabolism, membrane structure, and energy storage. Furthermore, the authors investigated the prebiotic origin of glycerol and its derivatives, proposing their potential role in the emergence and evolution of life on Earth. In a related vein, Yang et al. examined the delivery of probiotics using cellulose-based films and their applications in the food industry [22]. Their study focused on utilizing cellulose as a carrier for probiotics, offering insights into novel approaches for enhancing the stability and viability of probiotic cultures in food products. This research underscores the importance of innovative packaging materials in maintaining the efficacy of probiotics and promoting their beneficial effects on human health. Additionally, Patricia and Manuel reviewed the use of bacterial cellulose as a biodegradable material for food packaging [23]. Their study highlighted the potential of bacterial cellulose as an eco-friendly alternative to conventional packaging materials, emphasizing its biocompatibility, barrier properties, and sustainability. By leveraging bacterial cellulose, researchers aim to develop packaging solutions that not only preserve food quality but also minimize environmental impact. Collectively, these studies contribute to our understanding of the diverse applications of glycerol, cellulose-based films, and bacterial cellulose in the realms of biochemistry, food technology, and sustainable packaging. By elucidating the roles and potentials of these materials, researchers strive to innovate and advance in various fields, from enhancing

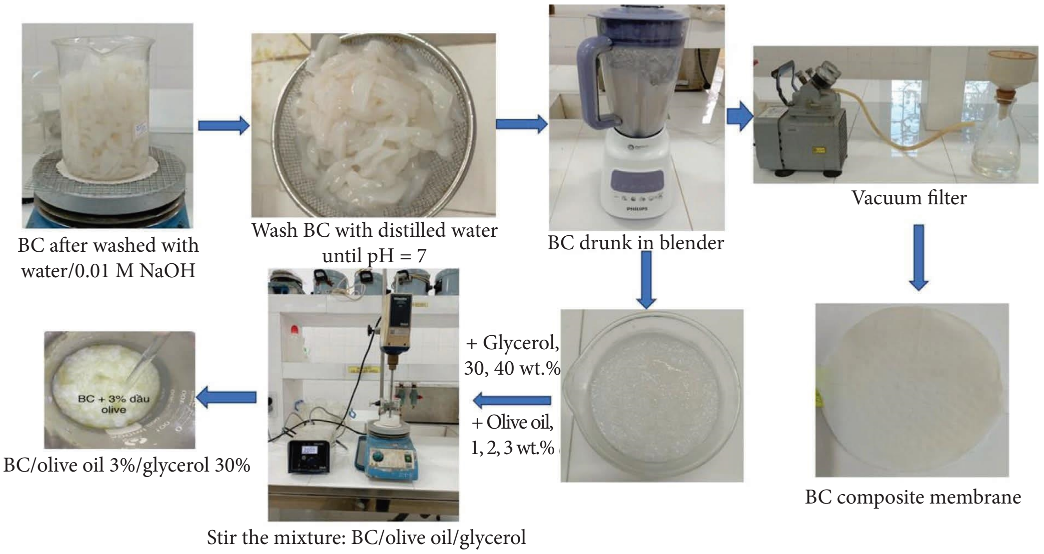


FIGURE 1: Process for manufacturing composite membranes from coconut jelly biomass.

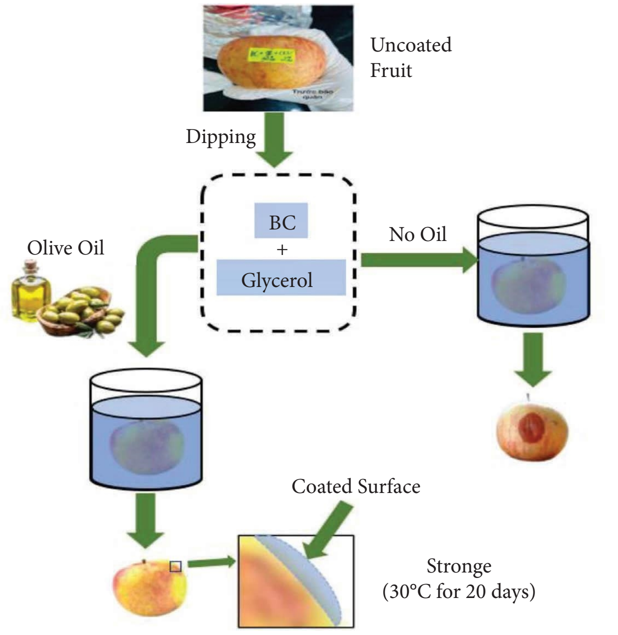


FIGURE 2: The process of packaging fruits using the immersion method.

cellular processes to improving food preservation and packaging practices (see Figure 3).

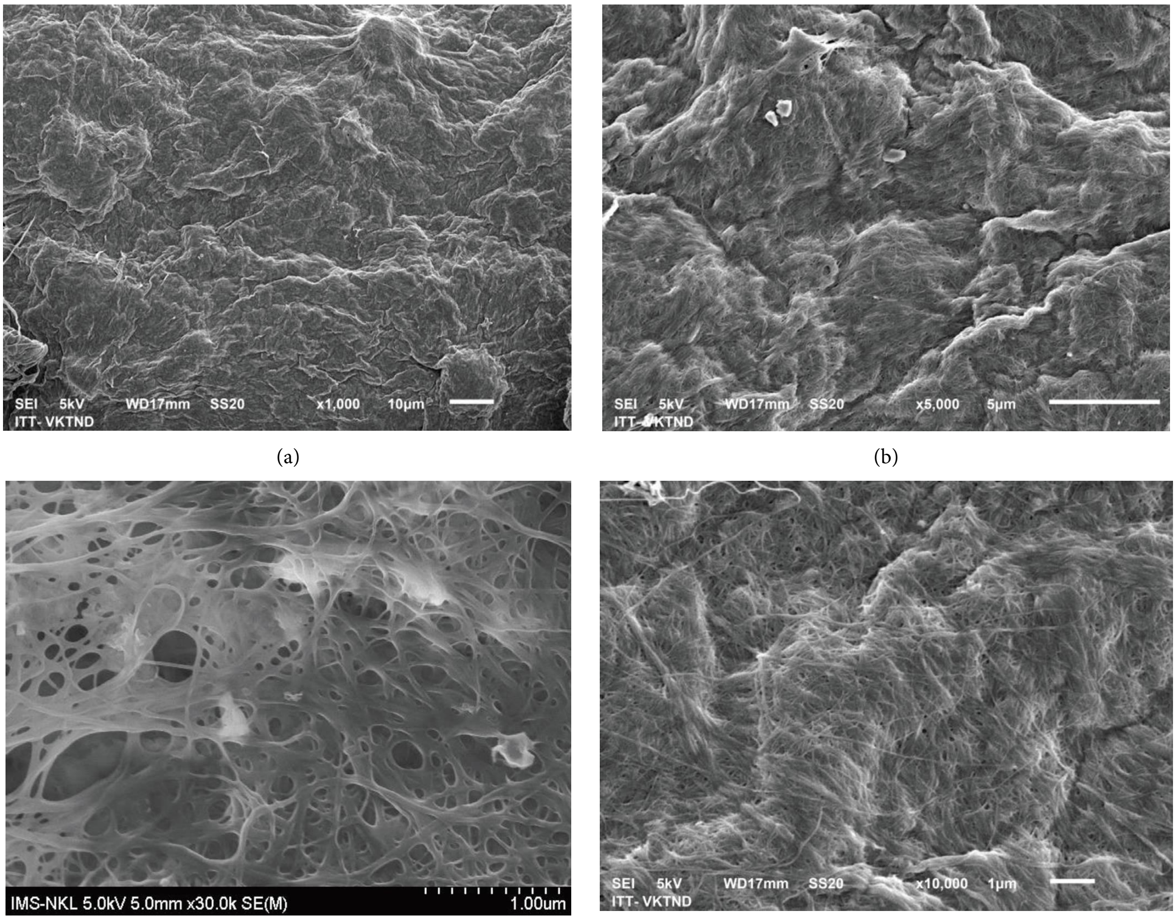
3.1.2. Infrared (IR) Spectral Characteristics of BC Composite Membranes. The Fourier Transform Infrared (FTIR) spectroscopy analysis of pristine bacterial cellulose (BC) provides valuable insights into its structural composition and purity. The presence of characteristic peaks associated with cellulose confirms the authenticity of BC synthesized by *Gluconacetobacter xylinum* and underscores the efficiency of postsynthesis refinement techniques, such as treatment with 0.01 M NaOH and repeated rinsing with distilled water (see Figure 4).

In particular, the FTIR spectrum of pristine BC reveals distinct peaks at specific wavenumbers, each corresponding to unique molecular vibrations and functional groups within the cellulose matrix. At 3344 cm^{-1}, a prominent peak signifies the stretching vibration of hydroxyl groups (O-H), a fundamental characteristic of cellulose polymers. This peak intensity reflects the abundance of hydroxyl groups present in the BC structure, crucial for its hydrophilic nature and interactions with water molecules. Additionally, peaks observed at 2895 cm^{-1} correspond to the stretching vibrations of carbon-hydrogen (C-H) bonds, indicative of the aliphatic hydrocarbon chains within the cellulose backbone. The presence of these C-H bonds contributes to the structural integrity and stability of the BC material, reinforcing its mechanical properties. Furthermore, the FTIR spectrum exhibits peaks at 1489 cm^{-1} and 1316 cm^{-1}, attributed to the symmetric stretching of methyl (CH) groups and the deformation of methylene (CH_2) groups, respectively. These peaks underscore the presence of alkyl side chains in the cellulose structure, which play a crucial role in enhancing the flexibility and tensile strength of BC.

Overall, the comprehensive analysis of the FTIR spectrum confirms the purity and structural integrity of pristine BC, highlighting its suitability for various applications ranging from biomedical engineering to environmental remediation. The precise characterization provided by FTIR spectroscopy serves as a valuable tool for researchers in elucidating the intricate molecular architecture of BC and advancing its multifaceted applications in diverse fields.

3.1.3. Enhancing Antibacterial Performance of Bacterial Cellulose Composite Membranes. The antibacterial activity of composite membranes based on BC/olive oil/glycerol was evaluated by the agar disk diffusion method against the following strains: *E. coli*, *M. catarrhalis*, and *S. aureus*.

From the results shown in Figure 5, it is evident that the BC membrane does not exhibit antibacterial properties against the



(a)

(b)

(c)

(d)

Figure 3: Images of the structure of the BC composite layer at various resolutions. (a) ×1.000. (b) ×5.000. (c) ×30.000. (d) ×10.000.

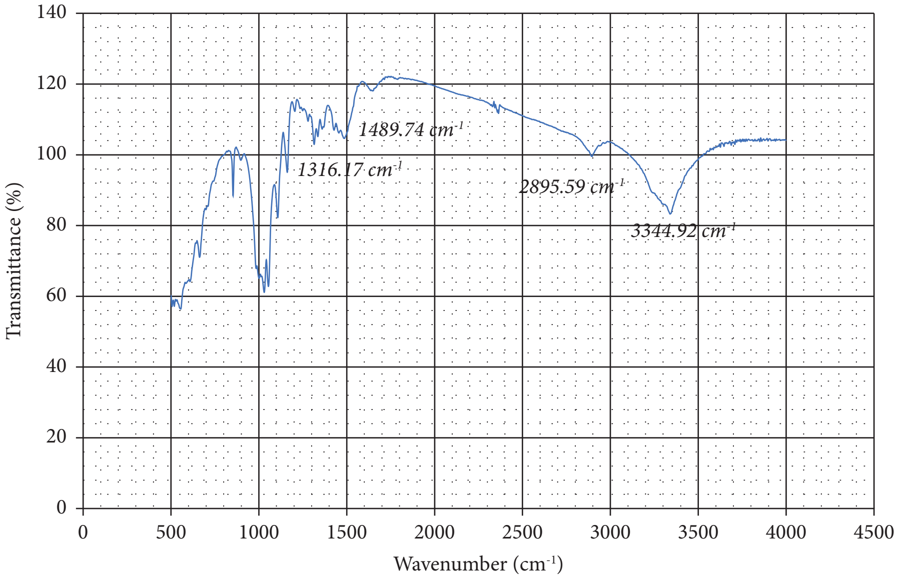


Figure 4: Infrared (IR) spectral characteristics of BC composite membranes.

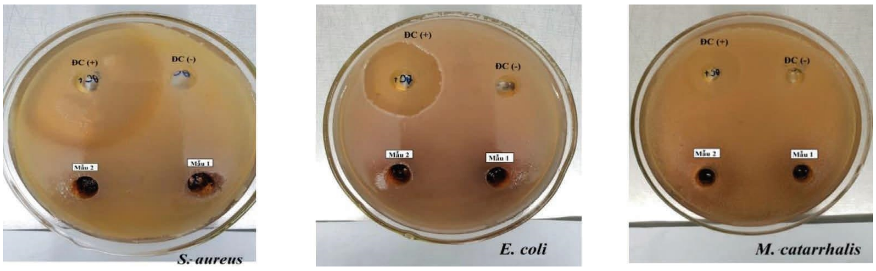


Figure 5: Results of testing the antibacterial properties of BC membrane against *E. coli*, *M. catarrhalis*, and *S. aureus* strains.

three strains: *E. coli*, *M. catarrhalis*, and *S. aureus*. This finding suggests a discrepancy with the intended purpose of preserving fresh fruits using BC composite membranes, as the absence of antibacterial activity may pose challenges in preventing microbial contamination and extending the shelf life of fruits. Although BC membrane lacks antibacterial capability against strains of *E. coli*, *M. catarrhalis*, and *S. aureus* as indicated in Figure 5, the use of BC can still be acceptable for fruit preservation. BC is a safe, flexible, and water-resistant biodegradable material, which helps protect fruits from spoilage. Despite its lack of antibacterial properties, the combination of BC with other measures such as temperature and humidity control can still effectively preserve fruits during storage.

3.2. Morphology of Bacterial Cellulose (BC)/Olive Oil (2%)/Glycerol (30%) Composite Films. A bacterial cellulose- (BC-) based composite film is one of the significant innovations in the field of apple and food preservation. The operational mechanism of BC film makes it a promising solution for apple preservation. Firstly, the mechanical properties of BC make the film robust and elastic. This means that BC film has the capability to shield apples from external physical factors such as impact and compression. This plays a crucial role in maintaining the structure and texture of apples, helping them retain their firmness and freshness over an extended period. Secondly, BC also exhibits excellent water absorption abilities, protecting apples from moisture loss. This aids in maintaining the necessary moisture content for apples, preventing them from drying out and losing their freshness.

Thirdly, BC can create an ideal physical environment for apples. BC film can regulate gas exchange, helping apples maintain their natural color and flavor. Lastly, BC has the capacity to interact with antibacterial and antioxidant compounds, safeguarding apples against bacterial growth and oxidation. This enhances the preservation duration of apples and maintains their quality. In summary, the mechanism of apple preservation using a BC-based composite film combines mechanical properties, water absorption, environmental control, and interaction with antibacterial and antioxidant agents. All these features culminate in an effective preservation solution for apples, ensuring they maintain their freshness and quality for an extended period.

The pristine BC exhibits a characteristic 3D network structure with randomly dispersed fibers (Figure 3). Conversely, SEM micrographs of the BC/olive oil (2%)/glycerol (30%) composite membrane reveal a higher packing density of cellulose fibers, depicting a more solid, smooth, and uniform fiber surface (Figures 6 and 7). This indicates that the addition of glycerol has increased the smoothness of the composite cellulose fibers. Recent advancements in food packaging have seen the development of strong and high-barrier films utilizing bacterial cellulose modified with cyclic anhydrides. Jiang et al. explored this innovation, focusing on the enhancement of food packaging films' mechanical strength and barrier properties [24]. By modifying bacterial cellulose with cyclic anhydrides, researchers aimed to improve its performance as a packaging material, offering increased strength and better resistance to gas and moisture permeation. This development holds promise for the production of more robust and effective food packaging solutions, contributing to the preservation and shelf-life extension of perishable food products. In a related study, Ullah et al. investigated the structural and physico-mechanical characteristics of bio-cellulose produced by a cell-free system [25]. Their research delved into the properties of bio-cellulose, emphasizing its potential as a sustainable and eco-friendly alternative to conventional packaging materials. By understanding the structural and mechanical aspects of bio-cellulose, researchers aim to optimize its production and tailor its properties for various applications, including food packaging. These studies collectively contribute to the ongoing efforts to develop innovative and sustainable food packaging solutions. By leveraging bacterial cellulose and its derivatives, researchers aim to address the challenges associated with conventional packaging materials, such as environmental pollution and limited barrier properties. Through continuous research and development, the field of food packaging is evolving towards more efficient, eco-friendly, and versatile solutions, ensuring the safety and quality of food products while minimizing environmental impact. The SEM results align with previous studies, demonstrating the microstructural surface of synthetic materials based on BC with glycerol, BC [26, 27], PVA [28], cellulose [29], chitosan [30], and other materials, revealing compact and rugged surface morphologies.

The enhanced smoothness and uniformity of the cellulose fiber surface in the BC/olive oil/glycerol composite membrane can contribute to better preservation outcomes for fruits or other perishable goods. A smoother and more uniform surface can provide a more effective barrier against external contaminants, moisture loss, and microbial ingress, thus helping to prolong the shelf life and quality of the preserved products. Additionally, the higher packing density

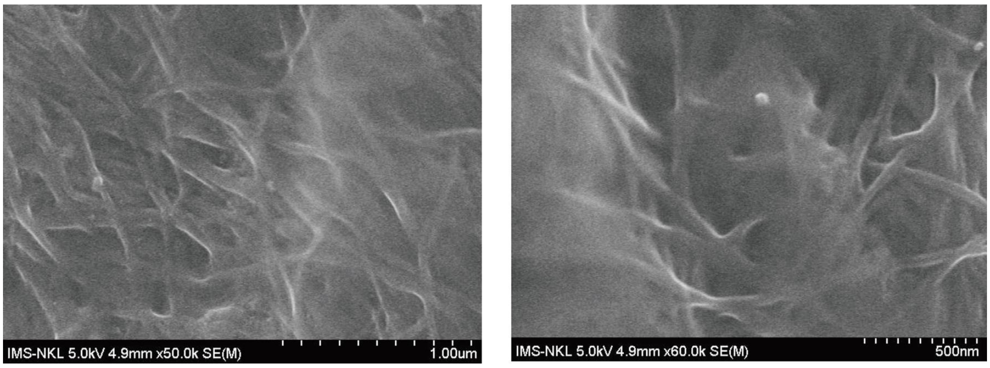


Figure 6: Morphology of bacterial cellulose (BC)/olive oil (2%)/glycerol (30%) composite films.

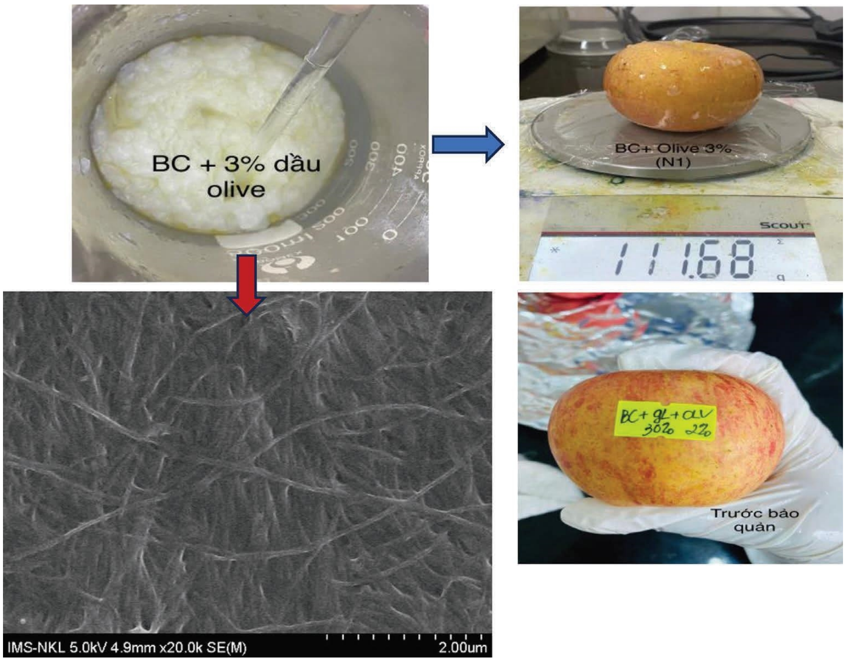


Figure 7: Morphological image of the structure of BC material and apple after being covered with a layer of composite film.

of cellulose fibers in the composite membrane may result in improved mechanical strength and structural integrity, further enhancing its ability to protect the preserved items during storage or transportation. Therefore, the observed structural characteristics of the composite membrane are beneficial for preservation purposes.

3.3. Antibacterial Properties of Bacterial Cellulose (BC)/Olive Oil (2%)/Glycerol (30%) Composite Films. The results regarding the antibacterial properties of bacterial cellulose (BC)/olive oil (2%)/glycerol (30%) composite films are presented in Figure 8. From the findings in Figure 8, it is evident that the composite membranes exhibit antibacterial activity against all three strains: *E. coli*, *M. catarrhalis*, and *S. aureus*. This underscores the practical significance of preserving fresh fruits using BC/olive oil/glycerol composite membranes. Based on the referenced literature, it can be affirmed that the use of a material system consisting of bacterial cellulose (BC), olive oil, and glycerol with antibacterial properties is a suitable option for packaging fresh fruits. Firstly, studies such as those of Ahmed et al. have demonstrated that the use of films and coatings made from bacterial cellulose derived from bacteria can exhibit antibacterial properties when combined with clove extract [30]. It can also be observed that utilizing materials derived from nature, such as bacterial cellulose and plant extracts with antibacterial properties, could be a safe and effective solution for preserving fresh fruits without the need for synthetic chemicals. Furthermore, research by Paul-Alexandru Popescu et al. has also shown that using coatings made from chitosan combined with natural oils with antibacterial properties can enhance the preservation efficacy of organic strawberries and apples during cold storage [31]. This indicates that the combination of natural materials like chitosan and natural oils can create a favorable environment for food preservation that is beneficial for health and environmentally friendly. Lastly, the study

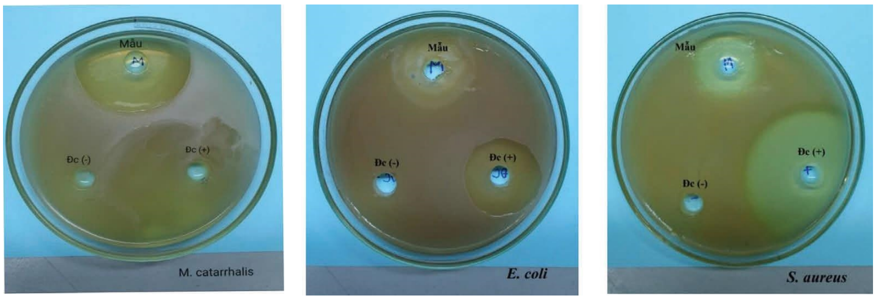


Figure 8: Antibacterial properties of bacterial cellulose (BC)/olive oil (2%)/glycerol (30%) composite films.

by Xing et al. provides further evidence of the effectiveness of using chitosan combined with antimicrobial agents in preserving fruits and vegetables [32]. By combining chitosan with antimicrobial agents, this material system not only inhibits the growth of harmful bacteria but also creates a safe environment for food. In conclusion, the referenced studies provide scientific evidence for the use of a material system comprising bacterial cellulose, olive oil, and glycerol in the preservation of fresh fruits. The combination of these natural components not only enhances the antibacterial properties of the packaging but also maintains the safety and quality of the food, while minimizing environmental impact.

The composite membrane BC/olive oil/glycerol mainly exhibits antibacterial properties due to the combination of its key components.

BC is a natural biopolymer with a nano-fibrous structure that forms a 3D network. This creates unfavorable conditions for bacterial growth as they struggle to penetrate and thrive in such a tightly structured environment. The dispersed and intertwined cellulose fibers can also impede the provision of nutrients to bacteria, reducing their survival rate.

Olive oil contains compounds like polyphenols, terpenes, and tocopherols with antibacterial properties. These compounds can either directly kill bacteria or inhibit their growth by interfering with enzymatic activity and metabolic processes. Therefore, olive oil enhances the antibacterial capability of the membrane.

Glycerol also possesses antibacterial properties and acts as a humectant, helping to maintain moisture within the membrane and preventing bacterial growth in dry environments. Additionally, its antibacterial properties contribute significantly to the membrane's overall antibacterial effectiveness.

In summary, the combination of components in the composite membrane BC/olive oil/glycerol creates an inhospitable environment for bacterial growth through the structured network of BC and the antibacterial properties of olive oil and glycerol. This enhances the membrane's ability to inhibit bacterial growth effectively.

3.4. Studying the Hardness of Apples before and after Storage.

The preservation of fruits and vegetables is a critical challenge in the food industry. Apples, a popular and widely consumed fruit, are no exception to this challenge, given their susceptibility to various deteriorative processes during storage, such as moisture loss, enzymatic browning, and microbial growth. Finding innovative and sustainable methods for extending the shelf life of apples while maintaining their quality is of paramount importance. This study delves into the promising avenue of using a three-component-based film, comprised of bacterial cellulose, glycerol, and olive oil, to enhance apple preservation. The research spans a 20-day storage period, and its primary objective is to evaluate the influence of film thickness on the hardness of the coated apples.

Bacterial cellulose, a biopolymer produced by acetic acid bacteria, has recently attracted significant attention for its remarkable mechanical properties and high water-holding capacity. These properties make it a viable candidate for food preservation applications, as it can effectively prevent moisture loss and maintain the texture of fruits. Glycerol, commonly employed as a plasticizer in the production of biodegradable films, can enhance the flexibility and tensile properties of the films. Olive oil, rich in antioxidants and antimicrobial compounds, has been studied for its potential to serve as a coating material that inhibits oxidation and microbial growth in fruits and vegetables. The combination of these three components in a film matrix presents a holistic approach to apple preservation.

Film Preparation. The three-component-based film was prepared by thoroughly mixing bacterial cellulose, glycerol, and olive oil in a specific ratio. This mixture was then uniformly spread onto the surface of fresh apples, creating a protective film. Two different film thicknesses were investigated: a 1 cm-thick film and a 2 cm-thick film. *Hardness Testing*. The hardness of the coated apples was assessed using a mechanical testing machine. Each apple underwent a compressive force test, and the peak force required to penetrate the fruit's surface was recorded. Three replicates were tested for each film thickness at various time intervals during the 20-day storage period. *Hardness Assessment*. The results of the hardness assessment for apples coated with the three-component film are presented in Figure 1. Apples coated with a 1 cm-thick film displayed a remarkable hardness of 89.78 N, while those coated with a 2 cm-thick film exhibited a slightly lower hardness of 88.177 N. In comparison, the hardness of the control group, consisting of uncoated apples, significantly decreased over the same period, reaching 60.35 N (see Figure 9).

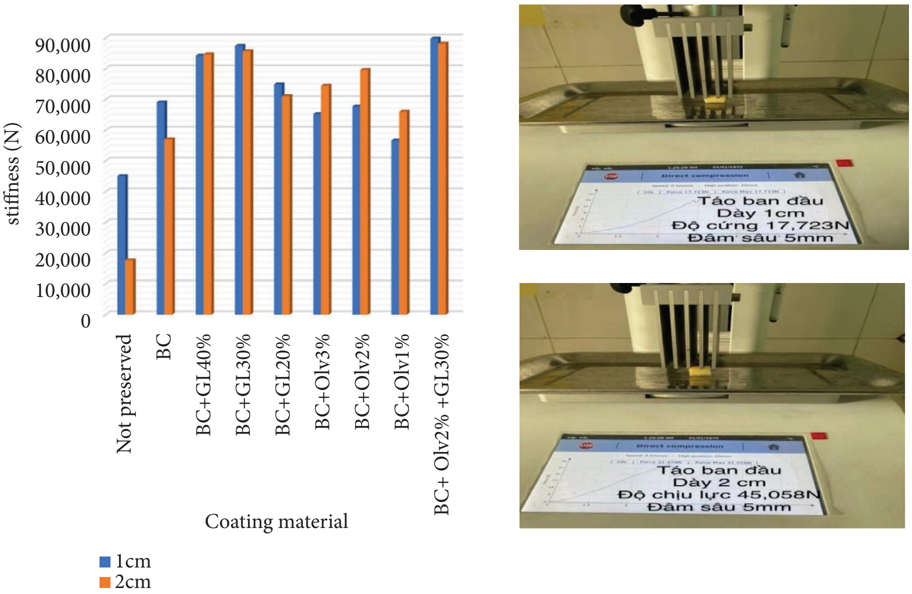


Figure 9: Results of measuring apple hardness after 10 days of storage.

In conclusion, this study provides compelling evidence of the potential of the three-component-based film, composed of bacterial cellulose, glycerol, and olive oil, in enhancing the preservation of apples. Over a 20-day storage period, the film-coated apples exhibited higher hardness values compared to uncoated apples. This suggests that the film effectively reduced moisture loss and prevented the softening of the apples, thereby maintaining their quality and firmness. These findings offer promising insights into the development of sustainable and effective fruit preservation methods.

3.5. Research on Determining the Vitamin C Content in Apples. Formula for determining total vitamin C content: $V_{VTMC} = (V_0 - V_1).m_1/m_0 .100$ (ml), where V_0: volume of 2.6 DCIP blank titration solution (ml); V_1: volume of 2.6 DCIP solution titration of vitamin C filtrate sample (ml); m_0: mass of test sample in aliquot for titration (g); and m_1: mass of ascorbic acid equivalent to 1.0 ml dye solution (mg) (see Table 1).

The preservation of fruits and vegetables is a critical concern within the food industry, driven by the need to extend shelf life and maintain nutritional quality. Apples, a highly consumed fruit worldwide, are particularly susceptible to various deteriorative processes, including vitamin C degradation. Vitamin C, known for its antioxidant properties and essential role in human health, is sensitive to environmental factors and can significantly decrease during storage. This study explores the use of a three-component-based film composed of bacterial cellulose (BC), 30% glycerol, and 2% olive oil to enhance apple preservation, with a specific focus on preserving vitamin C content.

The research spans a 20-day storage period, and its primary objective is to investigate the impact of the film on preserving vitamin C levels in apples. Bacterial cellulose (BC) is a biopolymer produced by acetic acid bacteria that exhibits remarkable mechanical properties and high water-holding capacity. Glycerol, a common plasticizer, is used to enhance the flexibility and tensile properties of biodegradable films. Olive oil is renowned for its rich content of antioxidants and antimicrobial compounds, making it a potential coating material to inhibit oxidation and microbial growth in fruits and vegetables [31–33].

The combination of these three components presents a holistic approach to apple preservation. *Film Preparation*. The three-component-based film was prepared by mixing bacterial cellulose, 30% glycerol, and 2% olive oil in a specific ratio. The mixture was then evenly spread onto the surface of fresh apples to create a protective film. The vitamin C content in the apples was analyzed using a high-performance liquid chromatography (HPLC) method. Samples were taken from both the coated apples and control group (uncoated apples) at various time intervals over the 20-day storage period. *Vitamin C Preservation*. The results of vitamin C content in the coated and uncoated apples are presented in Table 1. After 20 days of storage, the coated apples maintained a vitamin C content comparable to their initial levels, with a negligible decrease observed. In contrast, the uncoated apples exhibited a significant decline in vitamin C content over the same period. The findings of this study emphasize the potential of the three-component-based film in preserving vitamin C in apples. Vitamin C is highly sensitive to factors such as oxygen, temperature, and light, all of which can accelerate its degradation. The film effectively acted as a barrier, protecting the apples from these adverse environmental conditions. This is evident in the minimal change in vitamin C content observed in coated apples compared to the uncoated ones. Furthermore, the concentration of glycerol and olive oil in the film matrix likely contributed to the preservation of vitamin C. Glycerol enhances film flexibility and provides a protective layer, while

Table 1: Results of total vitamin C concentration after 10 days and 20 days.

No	Total vitamin C concentration, after 10 days (V_{VTMC})		Total vitamin C concentration, after 20 days (V_{VTMC})		
	V_1	V_{VTMC}	V_1	V_0	V_{VTMC}
Not yet preserved	0.23	0.00115	0.25	0.3	0.001035
BC	0.25	0.00069	0.275	0.3	0.000575
BC + olive oil (Olv) 3%	0.26	0.00046	0.268	0.3	0.00023
BC + Olv 2%	0.268	0.000276	0.28	0.3	0.00023
BC + Olv 1%	0.26	0.00046	0.285	0.3	0.000345
BC + glycerol 40%	0.23	0.00115	0.26	0.3	0.00092
BC + glycerol 20%	0.23	0.00115	0.27	0.3	0.00069
BC + glycerol 30% + 2% Olv	0.18	0.0023	0.25	0.3	0.00115
BC + glycerol 30%	0.26	0.00092	0.28	0.3	0.00046

olive oil, with its antioxidant properties, may have aided in maintaining the vitamin C levels. The balanced ratio of these components appears to be crucial in achieving effective preservation.

In conclusion, this study provides compelling evidence of the potential of a three-component-based film, composed of bacterial cellulose, 30% glycerol, and 2% olive oil, in preserving vitamin C in apples. Over a 20-day storage period, the film-coated apples exhibited a negligible decrease in vitamin C content compared to the significant decline observed in uncoated apples. This suggests that the film effectively protected the apples from vitamin C degradation, offering the potential for extended shelf life and maintaining nutritional quality.

3.6. Research Identifies the Antioxidant Capacity in Apples.

Use the meter to measure UV-vis at 517 nm measured with DPPH (2,2-diphenyl-1-picrylhydrazyl). The results of research on the antioxidant capacity of apples after being preserved with a 3-component film using a UV-vis machine using the DPPH method are presented in Table 2.

From the results of Table 2, it shows that the antioxidant capacity of apples increased compared to other times, not exceptionally high but still increasing quite steadily at each time point: initial, 10 days, 20 days, and 30 days. This proves that in addition to dehydration, the nutrients in apples are almost preserved compared to the original. Apples at day 30 have the highest antioxidant capacity.

The apple encapsulating film, composed of three main components: bacterial cellulose (BC), olive oil (2%), and glycerol (30%), not only exhibits the capability to preserve vitamin C, as discussed in the previous article, but also demonstrates remarkable antioxidant properties. To evaluate this capability, the UV-vis method, measuring at 517 nm using DPPH (2,2-diphenyl-1-picrylhydrazyl), is a common technique in the study of the antioxidant capacity of substances.

Table 2: Results of determining antioxidant capacity using DPPH method.

Sample	IC50 (μg/ml)
Standard (vitamin C)	0.0420
Original apple model	0.0593
Apple model day 10	0.0575
Apple model day 20	0.0541
Apple model day 30	0.0531

When an antioxidant compound interacts with DPPH, it causes DPPH to lose its color and undergo a color change from purple to blue or yellow, depending on the degree of interaction. The results measured at 517 nm reflect the ability to reduce the DPPH concentration in the sample, indicating the capability to eliminate oxidative free radicals. The apple encapsulating film used in this study has shown significant antioxidant potential when measured at 517 nm using the DPPH method. The effectiveness in reducing oxidative free radicals can be observed through a significant decrease in DPPH concentration, highlighting the important role of the film's components, including BC, olive oil (2%), and glycerol (30%), in protecting apples from the process of oxidation. A robust antioxidant apple encapsulating film not only shields apples from degradation due to the impact of free radicals but also has the potential to offer health benefits to consumers when consuming apples. This is a significant discovery in the development of safe and effective food preservation methods, while also providing optimal nutritional value.

The research findings indicate that utilizing a bacterial cellulose- (BC-) based coating material combined with 2% olive oil and 30% glycerol has proven effective in preserving fresh fruits for up to 20 days at room temperature. Research in the field of food preservation has seen significant advancements with the development of edible coatings and films containing antimicrobial agents derived from natural sources. Ahmed et al. explored the use of edible microbial cellulose-based coatings and films incorporated with clove extract, showcasing their potential as antimicrobial barriers for food products [30]. Similarly, Camelia Ungureanu et al. investigated biocoatings aimed at preserving fresh fruits and vegetables, highlighting the importance of natural coatings in extending the shelf life of perishable produce [34]. Moreover, Paul-Alexandru Popescu et al. focused on chitosan-based edible coatings enriched with essential oils to maintain the quality and prolong the shelf life of organic strawberries and apples during cold storage [31]. Research into the preservation of fruits and vegetables has been greatly influenced by the development of chitosan-based coatings containing antimicrobial agents. In a study by Xing et al., a chitosan-based coating with antimicrobial properties was investigated comprehensively [32]. The study delved into the preparation, properties, mechanisms, and application effectiveness of such coatings on fruits and vegetables. Chitosan, derived from chitin, a natural polymer found in the shells of crustaceans, possesses inherent antimicrobial properties, making it an attractive material for food preservation. By incorporating antimicrobial agents into chitosan-based coatings, researchers aimed to enhance their effectiveness in inhibiting microbial growth and extending the shelf life of fresh produce. The study highlighted the importance of understanding the properties and mechanisms underlying the action of chitosan-based coatings to optimize their application in food preservation. Furthermore, it explored the practical effectiveness of these coatings on fruits and vegetables, offering insights into their potential role in improving food safety and quality. Overall, the research underscores the significance of chitosan-based coatings as a promising approach for enhancing the postharvest preservation of fruits and vegetables, thereby contributing to the development of sustainable and effective food preservation methods. These studies underscore the growing interest in utilizing natural compounds and biomaterials for the development of sustainable and effective food preservation strategies. Edible coatings offer a promising approach to minimize food spoilage, reduce the need for synthetic preservatives, and enhance the safety and quality of fresh produce for consumers. In addition to the current findings, future directions could involve further optimizing the composition of the bacterial cellulose- (BC-) based coating material to enhance its effectiveness in fruit preservation. This optimization process could include exploring different ratios of olive oil and glycerol, as well as incorporating other natural additives or antimicrobial agents known for their preservative properties. Furthermore, future research could focus on evaluating the sensory attributes of fruits preserved using the BC-based coating, such as taste, aroma, and texture, to ensure that the coating does not negatively impact the overall quality of the fruit.

Additionally, studying the feasibility of scaling up the production of the BC-based coating for commercial applications would be valuable. This could involve investigating cost-effective methods for mass production and assessing the coating's performance on a larger scale. Moreover, considering the environmental sustainability aspect, future studies could explore the biodegradability and eco-friendliness of the BC-based coating compared to conventional synthetic coatings, aiming to develop more sustainable packaging solutions for fruits and other perishable foods. Overall, these future directions aim to further improve the efficacy, practicality, and sustainability of BC-based coatings for fruit preservation, ultimately benefiting both consumers and the environment.

4. Conclusion

In conclusion, the research on apple preservation using a three-component-based film consisting of bacterial cellulose (BC), olive oil (2%), and glycerol (30%) has yielded noteworthy results. After a 20-day storage period, the film demonstrated a considerable but not substantial decrease in its antioxidant capacity, as indicated by the IC50 value (μg/ml) of 0.0541. Moreover, the vitamin C content exhibited a reduction but remained within the specified acceptable range. These findings suggest that the three-component film has the potential to serve as a valuable method for preserving apples, offering extended shelf life and retaining nutritional quality. Further studies and optimizations may enhance the effectiveness of this preservation technique, paving the way for practical applications in the food industry, ultimately benefiting both producers and consumers [7, 35–38].

Data Availability

The data are available upon request to the authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Nguyen Tuan Anh was responsible for conceptualization, methodology, investigation, data analysis, original draft preparation, review and editing, and supervision. Xuan Huy Nguyen was responsible for methodology, investigation, and data analysis. Thuy Van Ngo was responsible for review and editing and data analysis. All authors have reviewed and approved the final version of the manuscript for submission. We have acknowledged any other contributions appropriately within the manuscript, and all contributors have given their permission to be acknowledged. All authors meet the criteria set forth by the International Committee of Medical Journal Editors (ICMJE) and have made significant scientific contributions to the research in the manuscript. We have ensured that anyone else who contributed has been acknowledged with their permission.

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