CHARACTERIZATION AND ANTIBACTERIAL EFFECT OF *EX-SITU* CURCUMIN-LOADED BC FILMS AS A FOOD PACKAGING MATERIAL

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This study aimed to produce a food packaging material designed to protect food from pathogens by loading bacterial cellulose (BC) films with curcumin, an important antimicrobial agent. It was observed that BC treated with 0.4 mg/mL of curcumin resulted in bacterial reductions of 57.74%, 64.33%, and 72.67% against *E. coli*, *Salmonella* sp., and *S. aureus*, respectively. An increase in curcumin concentration was found to enhance the inhibitory effect on bacteria, with a 1% increase in curcumin concentration leading to reductions of over 99% in *Salmonella* sp. and *S. aureus*. This obtained bio-coating material was determined to extend the shelf life of tomatoes and strawberries. FT-IR analysis confirmed the presence of characteristic peaks of curcumin, and TGA revealed high thermal stability. FIB-SEM imaging showed that curcumin covered the surface of the porous, 3D nanofibrillar BC film. XRD analysis indicated that the obtained biomaterial had a crystallinity index of 59.95%, a crystalline size of 3.42 nm and exhibits type 1 cellulose. Its potential for industrial production and widespread use is supported by its eco-friendliness, sustainability, high thermal stability, and biodegradability, making it a viable option for extending the shelf life of foods, suggesting that it can be used as a food packaging material.

Keywords: bacterial cellulose, curcumin, antibacterial, food packaging, characterization

INTRODUCTION

Cellulose, a natural polysaccharide produced in quantities of almost 180 billion tons per year, is widely available all over the world.[1] Bacterial cellulose (BC) eliminates the use of toxic chemicals necessary for extraction and purification processes, and is thus preferable for industrial use to plant cellulose.[2] Furthermore, its low density, high purity and water absorption ability, as well as its biocompatibility and biodegradability, are sufficient for BC recommend it for many application areas.[3] Among BC producers that can be isolated from sources with pH and temperature diversity (such as fermented foods or beverages like Kombucha, fruits, rotten fruits, coconut milk and vinegar), *Komagataeibacter*, formerly known as *Gluconacetobacter*, is considered to be a successful organism in BC synthesis, which can synthesize cellulose by using monosaccharides, disaccharides, oligosaccharides and even sugar alcohols as carbon sources.[4,5] Therefore, cellulose produced by *Komagataeibacter* may be a preferable option for industrial use.[6]

BC nanofibers can produce a 3D nanoporous network, with superior properties, that can be used in different areas; these are formed by the interaction between the hydroxyl groups of glucose monomers in the polymer chains during the synthesis stage.[7] Various polymers, such as carbon-based nanomaterials and metal nanoparticles (NPs), have been incorporated with *ex-situ* and *in-situ* modifications, creating hybrid materials with improved functional properties.[8] By combining BC with different agents, composite materials are obtained, exhibiting enhanced properties, although with some limitations.[2]

The *ex-situ* impregnation technique, which consists in physical absorption, in which a substance or another material is impregnated into pure BC fibers without modification, is the most widely used method in BC modification. An alternative method that can be used is *in-situ* modification, where the material is initially added to the culture medium, and an integrated process is carried out during BC fibril network synthesis. Both modification methods are suitable to change the physical, chemical, mechanical and morphological aspects of the resulting BC composite.[9]

Microbial outbreaks that affect the quality, freshness and safety of food products and develop resistance to traditional antimicrobial products have led to new searches to prevent microbial growth.[10] It is estimated that food packages, which constitute a large portion of package consumption, causing significant waste production, were used 1130 billion times in the European Union in 2018.[2] BC has high mechanical properties, is biodegradable and thus not harmful to the environment, and it can replace synthetic plastics widely used in the food and non-food packaging industry today as a supportive and smart packaging material.[11,12]

Curcumin, which contains demethoxycurcumin (curcumin II), bisdemethoxycurcumin (curcumin III) and the recently uncovered cyclocurcumin, is extracted from rhizomes of turmeric (*Curcuma longa* L.). It is a natural polyphenol, it has anti-inflammatory and antibacterial properties and pH-dependent solubility.[13] Recent reports point out the incorporation of curcumin into polymers to produce functional polymer composites for diverse applications. In the literature, cellulose/curcumin films were produced by using 1-allyl-3-methylimidazolium chloride (AMIMCl) by dissolving cellulose and curcumin.[14] Due to its hydrophobicity, curcumin can protect foods from moisture-related deterioration by reducing the water vapor permeability (WVP) of packaging films.[15]

In this context, curcumin, noted for its antimicrobial properties and natural origin, was utilized in this study by loading it onto BC films, using an *ex-situ* method, in order to develop antimicrobial films for food packaging applications. The BC films produced with *K. maltaceti* strains in Hestrin Schramm medium were purified, and the water between the fibrils was removed using freeze-drying method. The antimicrobial effect of BC films treated with varying concentrations of curcumin was investigated against *E. coli*, *S. aureus*, and *Salmonella* sp. species. Strawberries and tomatoes were coated with BC films loaded with curcumin at selected concentrations to evaluate the films' effectiveness in food preservation. Additionally, the obtained cellulose films were characterized by focused ion beam scanning electron microscopy (FIB-SEM), Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) and thermogravimetric analysis (TGA-DSC). This study presents the production of a new generation of biopolymer-based packaging materials as an alternative to petrochemical-derived plastics.

EXPERIMENTAL

BC production and purification

K. maltaceti, used as a BC producer, was inoculated into Hestrin-Schramm broth (containing, in g/L: 20 glucose, 5 peptone, 5 yeast extract, 2.7 Na_2HPO_4, 1.15 citric acid; pH=6.0), with an inoculum amount of 10% (OD_{600} = 0.20–0.25). Incubation was performed at 30 °C for 10 days (MCI 120; Mipro, Turkey). Following the incubation period, the obtained BC film was purified as described by Avcioglu.[16]

Curcumin loading

Purified BC films were freeze-dried at -80 °C with a Telstar LyoQuest freeze-drier (Telstar Technologies, Barcelona, Spain) to remove the water content of the BC films. Lyophilized films were immersed in variable concentrations (0.2-1.0 mg/mL) of curcumin (Sigma-Aldrich, USA) dissolved in absolute ethanol (99%) for curcumin loading and mixed for 24 h at room temperature in a rotator (Multi Bio RS-24, Biosan, Riga, Latvia). Following this, the Cur-loaded BC (Cur-BC) films were kept at -80 °C overnight.[17]

Antimicrobial activity of Cur-BC films

To determine the antibacterial effect of Cur-BC films against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. species, bacteria were inoculated on nutrient agar (Sigma-Aldrich, USA) and incubation was performed at 37 °C for 24 h (MCI 120; Mipro, Turkey). Then, a colony of each bacterium was selected and inoculated in brain heart infusion broth (BHI) (Sigma-Aldrich, USA) and incubated at 37 °C and at 150 rpm overnight (IKA KS 4000i Control, Staufen, Germany). The obtained bacterial cultures were washed with sterile phosphate buffer saline (PBS) (PBS; Sigma-Aldrich, USA) for 3 times at 4000 rpm (Eppendorf Centrifuge 5810 R, Hamburg, Germany), and the supernatant was discharged after each cycle to purify the bacterial culture from the cultivation medium. The obtained pellet was adjusted to 0.08-0.1 (OD_{600nm}) (UV-Visible Spectrophotometer; Shimadzu) according to McFarland turbidity standard.[18] Each bacterium was inoculated in brain heart infusion broth (BHI) containing Cur-BC films with variable concentrations. Incubation was performed at 37 °C, at 150 rpm for 24 h (IKA KS 4000i Control, Staufen, Germany). The obtained bacterial cultures were diluted with PBS and each dilution was inoculated onto nutrient agar plates to assess the antimicrobial effect of the Cur-BC films with the plate counting assay. Each plate was replicated three times. Following the incubation period, bacterial colonies were counted, and bacterial growth was determined in CFU/mL.[19]

The antibacterial effect of Cur-BC films was described as bacterial reduction (%R) by the modified procedure described by Amorim *et al*. and bacteriocidal activity was calculated according to Tangsatianpan *et al.* as follows:[17,20]

$$(\%R) = \frac{C-S}{C} x100 \quad (1)$$

Bacteriocidal activity = (log CFU T_o control) – (log CFU T_{24} composite) (2)

Cur-BC film characterization

Morphological characterization of freeze-dried films was performed with a GAIA3 FIB-SEM, Tescan. The obtained BC films were cut and gold coated (Leica ACE 600) and scanned at 9.34, 50.0 and 66.6 kx magnifications at 10 kV.

The functional groups in BC and Cur-BC films were analyzed using a Vertex 70 FT-IR instrument, Bruker (Germany). FT-IR spectra were collected over the range of 4000 to 600 cm^{-1} with a resolution of 1 cm^{-1} and 20 scans in the specified region.

Thermogravimetric analysis of Cur-BC films was performed with a TA Instruments Q600 SDT system under an inert gas environment (N_2), and in the temperature range between 0 and 700 °C with approx. 10 mg of sample.

XRD patterns of Cur-BC films were analyzed with a Rigaku D/Max 2200 diffractometer using CuK_α radiation wavelength (λ = 1.54 Å). Samples were scanned from 0°-60° in a 2θ angle with an increasing size of 0.02°.

Food packaging

Cur-loaded BC films were dried at room temperature to be used in food packaging. Tomato and strawberries were obtained from a local market and covered with the modified film (Cur-BC) and stored at +4 °C. The tomatoes/strawberries were checked each day from day 1 to day 16. Uncovered tomatoes/strawberries were used as negative controls.

RESULTS AND DISCUSSION

Antimicrobial activity of Cur-BC films

BC, which stands out with its extensive surface area, microfibrillar structure and high porosity, can serve as a matrix for loading antimicrobial agents.[21] Lacking antimicrobial activity of its own, researchers have added various antimicrobial agents to BC, such as antimicrobial peptides, synthetic and biological polymers, antibiotics, antiseptics and inorganic elements.[22] BC films with incorporated antimicrobial agents, like chitosan, have been put forth for use in wound healing applications. It has also been found that BC films containing silver nanoparticles have antibacterial activity against *C. albicans*, *S. aureus*, *K. pneumoniae*, and *E. coli*.[23,24] BC acquires new properties when modified by the addition of other active compounds, thus expanding its application to various fields, including the pharmaceutical, medical, and food industries. Accordingly, it is widely investigated for environmentally friendly antimicrobial and antioxidant food packaging, texture and bioavailability-enhancing compounds, paper industry, face masks, blood tubes, heart valves, and drug delivery systems, and other applications.[25]

For food packaging applications, BC has been modified with various agents, such as antimicrobial compounds, antioxidants, aromatic compounds, and nanoparticles. Among them, antimicrobial agents, which help extend the shelf life of food products, have been incorporated into BC coatings.[26] Whereas antioxidants are crucial agents used to prevent oxidative deterioration in foods, aromatic compounds can also be incorporated into BC coatings to impart specific taste and odor properties to food products.[26-28] Nanoparticles are also among the modification agents used in BC coatings, enhancing coating properties or providing additional functionalities. These modifications have expanded the use of BC in food coating applications, offering more diverse functionalities.[29-31]

Curcumin has been demonstrated to possess a wide range of beneficial properties, including wound healing, anti-inflammatory, antioxidant, antibacterial, and anticancer effects, as evidenced by *in vitro* and *in vivo* studies. Additionally, recent studies have focused on producing functional composites by incorporating curcumin into various polymers.[14] Studies have observed that the efficiency of polymer films is enhanced by curcumin adsorption into the unique 3D network structure of BC, as well as by the regulation of curcumin release.[32] Particularly, the incorporation of antimicrobial agents into the structure of BC is a highly significant feature. Under 400–500 nm blue light exposure, curcumin becomes highly photosensitized and can effectively destroy food-borne bacteria, such as *Salmonella typhimurium*, *Aeromonas hydrophila*, *S. aureus* and *E. coli*. Additionally, as a biosensor, curcumin is sensitive to pH changes, making it an excellent material for measuring food quality and degradation. Consequently, curcumin may be valued as a multifunctional dye in food packaging that is both proactive and sensitive.[15,33] In recent years, as the demand for high-quality food has increased, the concepts of active and intelligent food packaging have emerged to meet the needs of food safety.[34] As a food packaging material, BC can be an excellent alternative to other packaging materials for storing foods and extending their shelf life, due to its porous and fine network structure, which can effectively filter dust, microorganisms, and fungi from the air.[35] BC, which can be modified with other ingredients in a variety of ways, is suitable for most food and food packaging applications and processes.[36]

BC is a natural nanomaterial that has emerged as a viable substitute for various environmentally harmful materials. Its biocompatibility, adaptability, robustness, and barrier qualities make it an attractive option.[26] In this study, curcumin was considered for loading into BC films, due to its anticancer, antioxidant, anti-inflammatory, and antibacterial properties. The immobilization technique we chose not only ensures uniform distribution of curcumin within the BC matrix, but also provides sustained release of the bioactive compound, thereby extending its functionality throughout the shelf life of the packaged food products.[37-41]

Foodborne infections result from the consumption of foods contaminated with microbiological agents.[42] Among them, *Salmonella* sp. holds a significant position resulting in diarrhea, vomiting and fever.[43] *Escherichia coli*, a member of the normal or commensal gut flora in mammals, is also reported to be a causative agent of various infections, including enteric diseases, urinary tract infections, sepsis, and other extra-intestinal infections.[44,45] *Klebsiella pneumoniae*, defined as an opportunistic pathogen, is not only a causative agent of nosocomial infections, but also a significant pathogen that can cause infections through food.[46] Over time, the increase in the incidence of multidrug-resistant (MDR) strains of *Klebsiella pneumoniae* infections has led to a focus on efforts to reduce the sources of transmission of this pathogen.[47] Therefore, preventing the contact of these pathogens with food or using coating materials on foods to inhibit microbial growth and prolong the food's shelf life is of paramount importance.

In this study, the antimicrobial effect of Cur-BC films, produced as a food coating material, was investigated against the foodborne pathogens *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* sp. BC films coated with varying concentrations of curcumin were observed to cause bacterial reduction even at a concentration of 0.4 mg/mL. Specifically, reductions of 57.74% in *E. coli* (with 0.37 bactericidal activity), 64.33% in *Salmonella* (with 0.44 bactericidal activity), and 72.67% in *S. aureus* (with 0.56 bactericidal activity) were recorded. It was found that increasing the amount of curcumin resulted in an inhibitory effect on the bacteria, with a 1% curcumin concentration leading to a reduction of over 99% in both *Salmonella* and *S. aureus* (Figs. 1 and 2).

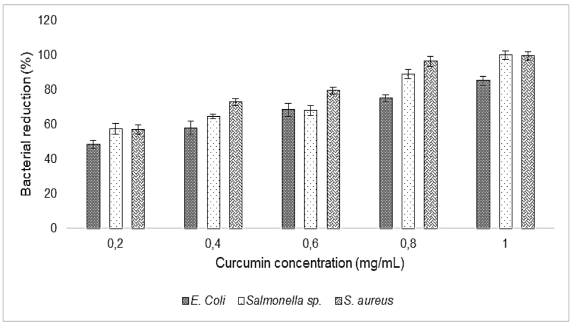


Figure 1: Effects of curcumin on bacterial reduction

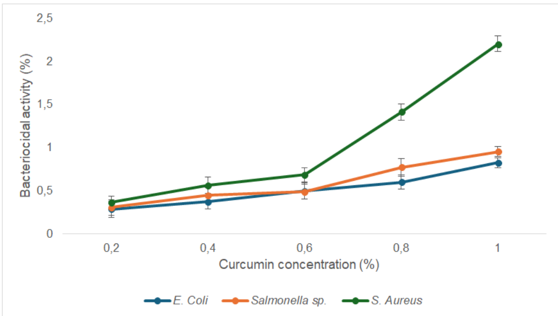


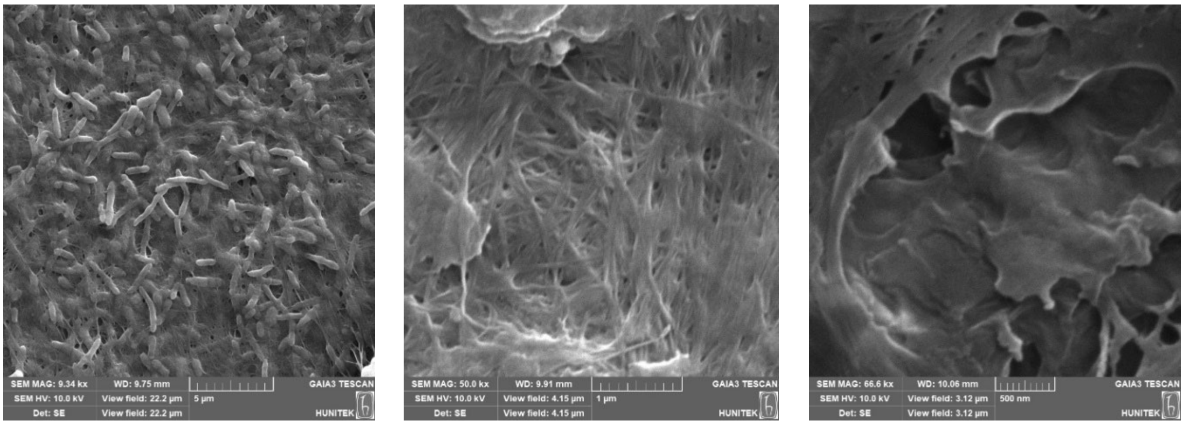
Figure 2: Bactericidal activity of curcumin against bacterial strains

Similarly to our study, Tangsatianpan *et al.*[17] found that the highest concentration of curcumin of 1.0 mg/mL resulted in a 98% reduction in viable *S. aureus* cells after 24 hours of exposure. In another study, the addition of 1 wt% curcumin was found to improve the mechanical and water vapor barrier properties of gelatin/curcumin composites, while also demonstrating antimicrobial effects against the foodborne pathogens *E. coli* and *L. monocytogenes*.[41] Similarly, PLA/curcumin composite films were reported to be usable as food coatings, with an increase in curcumin concentration leading to enhanced antimicrobial activity against both Gram-positive and Gram-negative bacteria.[27] A PLA matrix containing curcumin and fenugreek essential oil (FEO) was found to exhibit antimicrobial and antioxidant effects against *S. aureus* and *E. coli*.[48] These studies also indicated that the addition of curcumin enhances the thermal stability and mechanical properties of the modified films and reduces oxygen, carbon dioxide, and water vapor permeability.[49] In this context, our study shows that the use of curcumin as an antimicrobial agent is widely recognized in the literature for loading into various matrices in food coating materials. The combination of BC and curcumin is significant to produce biomaterial-based packaging materials as an alternative to petrochemical-based coatings.

Characterization of food packaging materials

Focused ion beam scanning electron microscopy (FIB-SEM)

Figure 3 shows the morphological characteristics of unpurified and purified BC, and of Cur-BC films. It was observed that the BC film has a 3D nanofibrillar structure, with curcumin covering the surface of the fibrils and filling the pores between the fibrils throughout the membrane.



(a) (b) (c)

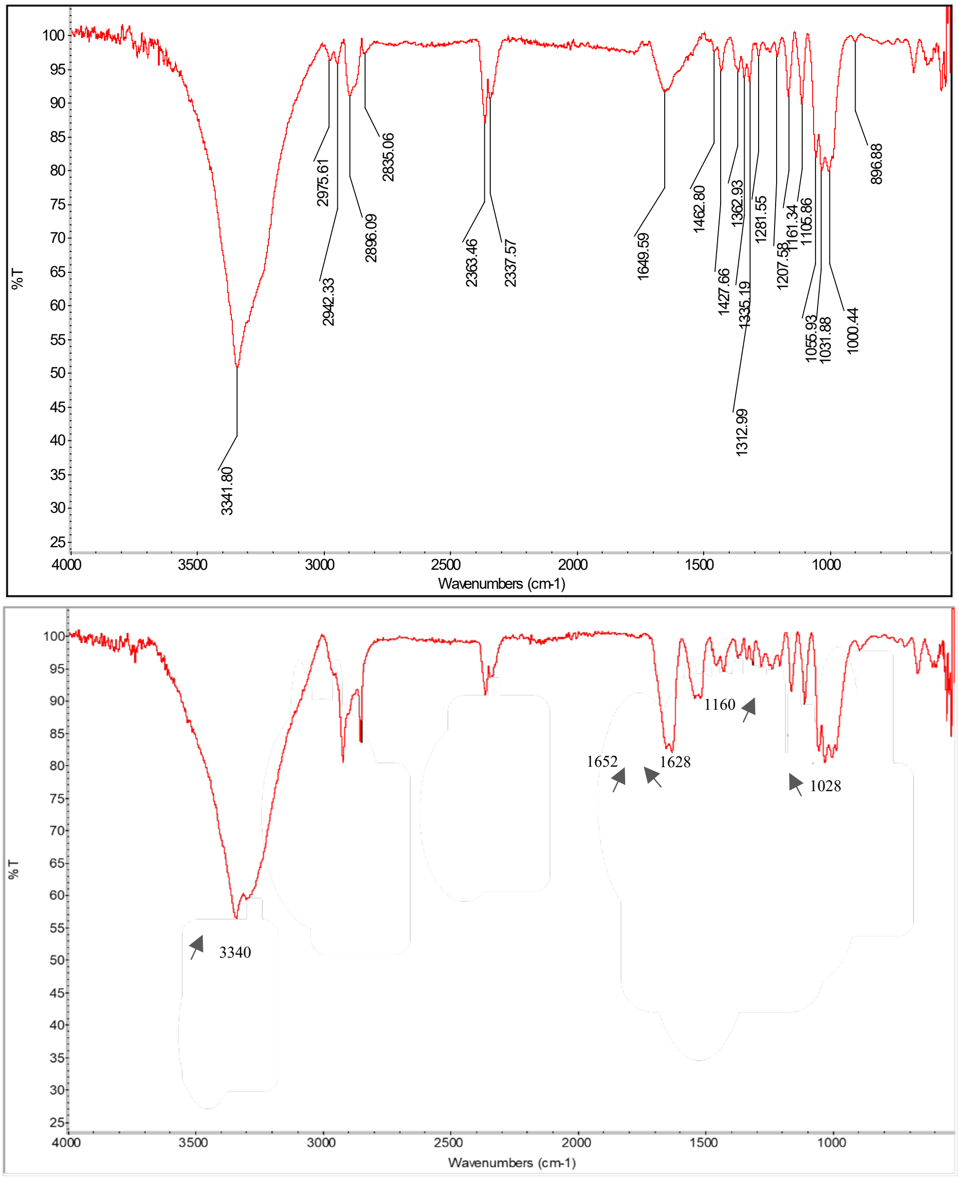
Figure 3: FIB-SEM images of BC films, (a) unpurified, (b) purified and (c) Cur-BC

Fourier transform infrared (FT-IR) spectroscopy

Figure 4 presents the FT-IR spectra of unloaded and loaded BC films. Accordingly, the peaks around ~3340 cm^{-1} are assigned to -OH groups associated with the stretching of intra-chain and inter-chain hydrogen bonded OH groups, that at ~2870-2960 cm^{-1} corresponds to C-H stretching and that at ~1647 cm^{-1} is described as the CO stretching band. The peak at ~1160 cm^{-1} indicates asymmetric stretching of β–glycosidic linkage of C-O-C and that at 1030 cm^{-1} indicates the C-O-C ring skeletal vibration.[3,37,50-52] The bands at 1652 cm^{-1} and 1628 cm^{-1} correspond to the characteristic interaction signal of curcumin with the BC film (Fig. 4b). The peaks at 1512-1518 cm^{-1} are attributed to the aromatic skeletal vibration of the benzene ring in Cur-BC.[13,14,27,33] The peaks obtained from the FT-IR analysis of BC and Cur-BC films were found to be consistent with the literature.

Thermogravimetric analysis (TGA-DSC)

The thermal stability of the Cur-BC film was determined using TGA analysis. Accordingly, the Cur-BC film revealed three phases of degradation (Fig. 5). The initial weight loss occurred up to ~115 °C (7.5%), corresponding to the evaporation of the adsorbed and interlayer water molecules.[22,27] The second stage occurred between ~115 °C and 375 °C (62.5%), and this weight loss is mainly attributed to the decomposition of glucose units by means of depolymerization and dehydration.[53,54] The third weight loss occurred between ~375-575 °C (7.5%), corresponding to the formation of charred residue.[55] The total weight loss of the Cur-BC film was found as 77.5% (Fig. 5). According to the thermogram results, the Cur-BC film exhibited high thermal stability, leading to the conclusion that it can be used not only as a coating material for fruits and vegetables, but also for baked foods.



a)
b)

Figure 4: FT-IR spectra of (a) unloaded BC film and (b) Cur-BC

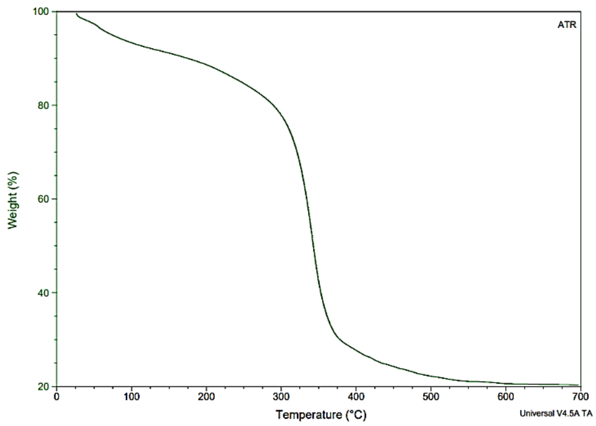


Figure 5: Thermogravimetric curve of Cur-BC film

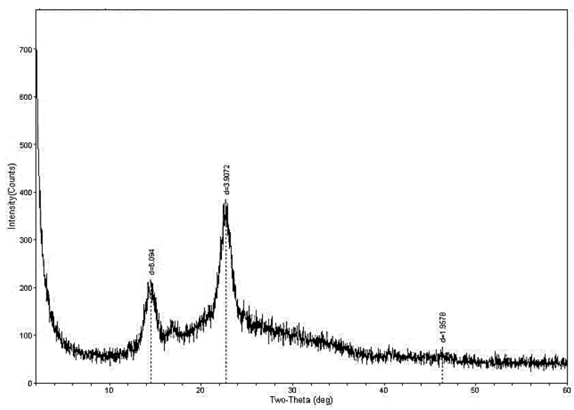


Figure 6: XRD pattern of Cur-BC film

X-ray diffraction (XRD)

Figure 6 presents the diffractogram of Cur-BC. It shows three visible peaks assigned to the (100), (010) and (110) crystallographic planes, corresponding to the diffraction angles of 2Θ = 14.48°, 17.32° and 22.8°, respectively.[16,51,54] The d-spacings, crystallinity index and crystalline size were determined according to Bragg's law,[56] Segal's method[57] and Scherer's formula[58], respectively. The allomorphism of cellulose fibrils (I_β and I_α) was also determined by using the Z value[59] and is shown in Table 1. According to the obtained data, the Cur-BC film exhibits typical cellulose Iα structure.[16,33,54] In previous studies, it was observed that the crystallinity index of BC was higher, and the addition of curcumin had a decreasing effect on the degree of crystallinity.[16]

Food packaging with Cur-BC films

The use of biodegradable plastics has begun to spread due to the known environmental hazards associated with conventional plastics. The inability of petroleum-based packaging widely used in clinical, food, and pharmaceutical industries to naturally and biologically degrade through microorganisms poses a significant environmental threat.[39,60,61] Therefore, biodegradable natural packaging materials based on biopolymer, obtained from natural renewable materials, such as proteins and carbohydrate-based polymers, are of great importance.[20] Specifically, biopolymers, whose properties can be improved through modification agents, are very important in packaging.[61-64] Biocomposites prepared with BC are suitable as food packaging materials because they are biodegradable, bioactive, and non-toxic.[10] Also, BC allows integrating antimicrobial agents, thus materials that can prevent food degradation and are environmentally friendly can be obtained.[65]

In this context, to use Cur-BC packaging materials for the preservation of food products was investigated. It was observed that tomatoes began to show physical degradation on the 12th day in the control group, while those coated with Cur-BC films retained their freshness until the 16th day, thereby the antimicrobial coating extended their shelf life. For strawberries, compared to the control group where degradation started on the 6th day, the Cur-loaded coating delayed the onset of physical degradation until the 8th day (Fig. 7).

Table 1
XRD data of Cur-BC films

d_1-spacing	d_2-spacing	Z value	Crystallinity index (CrI%)	Crystalline size (CrS) (nm)
0.611	0.389	Z>0	59.95	3.43

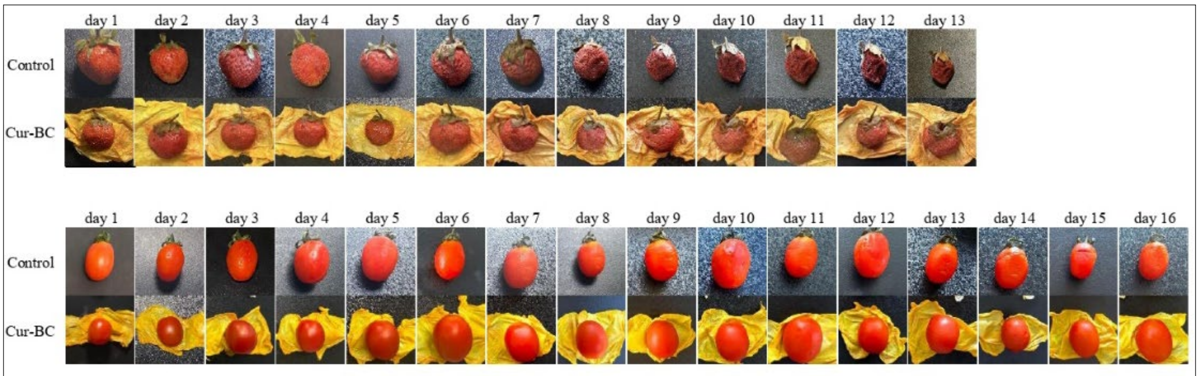


Figure 7: Effects of Cur-BC films on the shelf life of strawberries and tomatoes

In the literature, it is stated that CS-OBC-Cur films are effective in protecting strawberries due to the presence of antibacterial and antioxidant substances,[32] and the use of PLA/FEO/Cur film as a food coating material is based on the phenolics contained in curcumin and the linoleic bioactive properties contained in FEO. It has been stated that these compounds increase the effectiveness of the resulting film by affecting the CFU value.[48] It was also found that the mechanical, optical and thermal properties of curcumin loaded corn starch (CS)-based composite films were improved, while the 11% curcumin addition showed a high antioxidant effect, and the resulting coating material was effective in extending the package life of blueberries.[66] In another study, it has been stated that chitosan/*Tenebrio molitor* larvae protein films loaded with curcumin reduced the mass loss rate of blueberries.[67] Also, crosslinked chitosan/cellulose nanofiber-based films, used as a food coating material, had a shelf life-extending effect on banana, tomato and apple slices by reducing microbial invasion, oxidation and water loss of the fruit.[68]

In this study, it was determined that the addition of curcumin to the BC films, even at low concentration, has bactericidal activity, causing bacterial reduction. As a result, it has a prolonging effect on the shelf life of the coated fruits and vegetables (Figs. 1, 2 and 7). In this context, the Cur-BC films obtained in this study can be considered as environmentally friendly packaging materials.

CONCLUSION

Increasing environmental awareness has turned much research attention towards biodegradable packaging materials in recent years. In this study, a food coating material was produced by combining curcumin, an effective antimicrobial agent, with bacterial cellulose, which has a unique 3-dimensional structure and biodegradable feature. Although *ex-situ* modification was employed in the preparation of this biomaterial, no crosslinking agent was required for this, contributing to the cost-effectiveness of the resulting coating. It has been determined that the obtained biopackaging material is effective against *S. aureus*, *Salmonella* sp. and *E. coli*, and can extend the shelf life of tomatoes and strawberries. The loading of curcumin onto bacterial cellulose was confirmed by FIB-SEM and FT-IR analysis, and the crystallographic properties were determined by XRD analysis. In addition, it was observed that the coating material had high thermal resistance, enhancing its applicability not only in the preservation of fruits and vegetables, but also in the storage of baked goods. Consequently, the resulting material can be used as protective antimicrobial packaging, being a biodegradable and environmentally sustainable alternative to polluting plastics.

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