Development of antibacterial biodegradable materials based on starch–nanocellulose–lemongrass essential oil

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Abstract. In this study, biofilms based on starch, nanocellulose (NC) obtained from durian peel and lemongrass essential oil (LEO) at concentrations ranging from 1% to 10% were developed. Nanocellulose obtained from agricultural by-product durian peel was analyzed by FTIR and optical imaging. The obtained biofilms with different LEO contents were examined morphologically by optical microscopy and evaluated for film-forming ability, color, biodegradability, antibacterial activity, water absorption capacity and solubility. The results showed that the addition of LEO at a concentration of ≤2.5% would help maintain the brightness and enhance the antibacterial activity of the film, especially against *E. coli* strains. However, higher concentrations of LEO (5% and above) will cause the stability of the obtained film to decrease, the color to become darker, the hydrophilicity to decrease and the biodegradation to be slower. In addition, the membranes containing NC and LEO exhibited lower solubility than the original membrane. These results suggest that the combination of starch-NC and LEO could form a potential and environmentally friendly packaging material.

1 Introduction

Nowadays, to reduce the negative environmental impacts of plastic packaging and the depletion of petroleum resources, biodegradable packaging derived from natural sources is emerging and attracting significant attention from materials scientists. Among the natural polysaccharides with film-forming ability, starch is particularly noteworthy. It is a promising material for the fabrication of biodegradable membranes due to its availability, abundance, good biodegradability, and low cost [1, 2]. However, the use of natural starches, such as corn starch, in biofilm production is limited due to their hydrophilic nature. To overcome this drawback, various materials, such as glycerol, cellulose, carboxymethyl cellulose (CMC), and chitosan, have been used to enhance the water-repellent properties of starch-based films [1].

Agricultural waste and by-products, such as tree trunks, leaves, and fruit peels, generate large amounts of solid waste that enter the environment. These types of solid waste typically

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take up a significant amount of landfill space. Meanwhile, these by-products contain valuable components such as cellulose and cellulose products, lignin, pectin, etc [3]. Therefore, utilizing these by-products to obtain valuable products is being supported by many scientists. Following this trend, corn starch films containing NC obtained from durian peel were developed and oriented as preservative films [4]. The interaction between WATER and starch improved the solubility and antibacterial properties of the original starch film. However, the antibacterial activity of starch-NC materials is still low, which will pose difficulties for preservation applications. Therefore, improving the antibacterial properties of NC amyloid films is necessary to expand the applications of food preservation and packaging.

The use of antibacterial substances directly in food is raising concerns about residues and their effects on human health. In addition, the addition of antibacterial substances to food ingredients will also affect the sensory properties of food [5]. Therefore, the use of antibacterial substances in packaging will be a more feasible and suitable solution. Essential oils are known to have relatively strong antibacterial properties. In addition, the oils have antioxidant properties that can help limit food spoilage and extend product shelf life. In addition, essential oils, including lemongrass essential oil, are generally considered safe and non-toxic to human health. Among them, lemongrass essential oil has been shown to have relatively strong antibacterial activity. This essential oil is relatively easy to produce due to the extremely rich and abundant raw material sources [6]. Besides, essential oils in general and lemongrass essential oil in particular are hydrophobic compounds; therefore, adding lemongrass essential oil to the biofilm will help enhance the antibacterial and antioxidant properties and improve the hydrophobicity of the film. Such active coating films can replace conventional preservatives currently used on products, extend shelf life, and enhance product value. Many studies have focused on incorporating essential oils into biodegradable films. However, no prior research has investigated the development of starch–NC films combined with lemongrass essential oil (LEO).

Therefore, in this study, bio-based coating films composed of starch, NC, and LEO were developed and evaluated. Various concentrations of LEO were added to the starch-NC-LEO film formulation. The resulting composite biofilms were assessed for external appearance, color properties, biodegradability, water absorption capacity, and antibacterial activity.

2 Material and methods

2.1 Material

The chemicals used in this study included: starch (VinaChem, Vietnam), glycerol (Xilong Scientific, China), PDA nutrient medium (Merck, Germany), sodium hydroxide (Guangzhou Chemical Reagent Factory, China), H_2SO_4 (Xilong Scientific, China), and H_2O_2 (VinaChem, Vietnam). The microbial strains used were *E. coli* and *St. aureus*, provided by the Vietnam Institute of Biotechnology.

2.2 Methods

2.2.1 Extraction of NC from durian peel

NC was extracted from durian peel following the method described in a previous study by Trang et al. [7]. Specifically, the raw material was ground into powder and treated with 15% sodium hydroxide at 100 °C for 2 hours at a ratio of 1:20 g/mL. The mixture was then filtered to obtain crude cellulose. This crude cellulose was bleached using 15% hydrogen peroxide for one day at a ratio of 1:15 g/mL. After bleaching, the material underwent hydrolysis using

64% sulfuric acid at 45 °C for 3 hours with a ratio of 1:20 g/mL. Following hydrolysis, the mixture was neutralized using a NaOH and H_2O_2 solution, then ground and centrifuged multiple times before undergoing ultrasonication to obtain the nanocellulose (NC) suspension.

2.2.2 Fourier transform infrared (FTIR)

FTIR spectroscopy was performed using an IR5 instrument (Edinburgh Instrument, UK) with a wavelength range from 400 to 4000 cm^{-1} and a wavelengthof 4 cm^{-1}.

2.2.3 Preparation of starch-NC-LEO films

10 grams of corn starch were added in 200 mL of water and stirred at 60 °C for 15 minutes. Then, 0.5 g of nanocellulose was added to the mixture, followed by 3 g of glycerol. The mixture was stirred at 600 rpm for 2 hours at 80 °C. After that, it was cooled using cold water, and essential oil was added at concentrations of 1%, 2.5%, 5%, and 10%, respectively. The mixture was continuously stirred at 600 rpm for 15 minutes at room temperature and then subjected to ultrasonication for 10–15 minutes. Approximately 30 g of the mixture was poured into molds to form films, which were then dried at 40 °C.

2.2.4 Optical image

The optical image of NC and the films were examined using a trinocular biological microscope equipped with LED illumination and a magnification range of 40X to 100X (AS ONE, Japan).

2.2.5 Color differences

The samples were prepared and analyzed for color using a handheld colorimeter NR60CP (China) under standard illuminant D65 and a 10° observer angle. The instrument was calibrated before each measurement using a standard white calibration plate provided by the manufacturer with $L^* = 98.5$, $a^* = -0.4$, $b^* = 0.5$.

2.2.6 Biodegradation

The material was weighed and buried in soil with a controlled moisture content. After a specified burial period, the sample was removed, cleaned, and weighed. The biodegradability was calculated based on the weight loss of the sample after burial compared to its initial weight before burial.

2.2.7 Water uptake

The sample was weighed and immersed in water for 5 to 90 minutes. After immersion, the sample was removed and weighed again. The water uptake of the film was calculated using the following formula [8]:

$$H = \frac{m_2 - m_1}{m_1}.100 \quad (1)$$

where: m_1 – initial weight of the film (g); m_2 – weight of the film after immersion (g); H – water uptake of the film (%).

2.2.8 Solubility

The solubility of the film samples was determined by weighing each sample and immersing it in water for 24 hours. After soaking, the sample was take out and weighed again. The solubility was calculated as the percentage of the sample mass dissolved after 24 hours in relation to the initial dry weight of the sample [8].

2.2.9 Antibacterial activity

Bacterial suspensions of *E. coli* and *St. aureus* were cultured in PCA medium and incubated at 37 °C for 24 hours to obtain a suspension with a concentration equivalent to 10^6 CFU/mL. Petri dishes containing the bacteria were prepared 24 hours in advance. The film samples were then placed on the agar plates and incubated at 37 °C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones formed around the film samples. Each test was performed in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD).

3 Results and discussion

3.1 Extraction of nanocellulose from durian peel

Nanocellulose was extracted from durian peel and analyzed using Fourier Transform Infrared (FT-IR) spectroscopy. The results, presented in Figure 1, showed characteristic absorption peaks indicating the functional groups present in the nanocellulose structure. The stretching vibration of the intermolecular and intramolecular –OH bonds is characterized by the adsorption peak at 3380.38 cm^{-1} [9]. Meanwhile, the C–H stretching vibration from CH and CH_2 groups in cellulose molecule is shown at the peak 2823.80 cm^{-1} [10]. The absorption at 1670 cm^{-1} is thought to represent the stretching vibrations of H–O–H bonds from water molecules [11]. The peak observed at 1454.24 cm^{-1} indicates intermolecular hydrogen bonding associated with the C6 group in the cellulose structure.

The FT-IR results of the nanocellulose obtained in this study are consistent with previous reports on nanocellulose extracted from waste chilli stems [11] and from Ri6 durian peel [7].

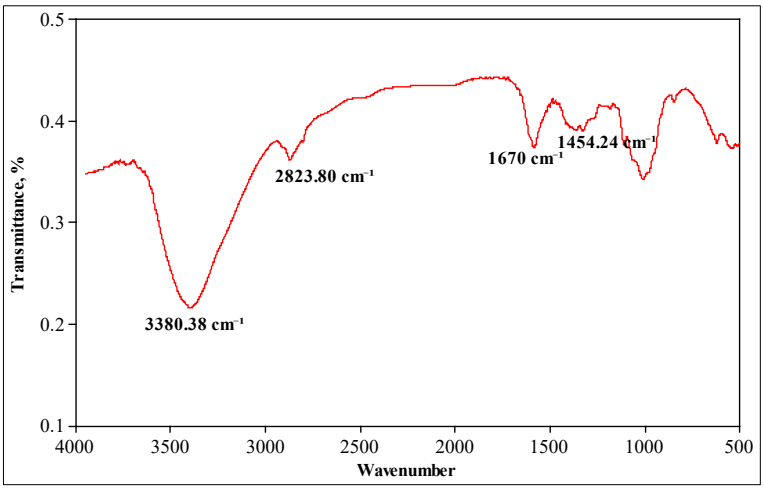


Fig. 1. FT-IR results of nanocellulose (NC) derived from durian peel.

Nanoparticles are typically defined as having a size below 100 nm. As shown in Figure 2, measurements taken at three different positions confirm that the nanocellulose (NC) particles in this study all have sizes smaller than 100 nm. The average particle size of the NC derived from durian peel was approximately 68.94 nm, indicating successful production of nanoscale cellulose.

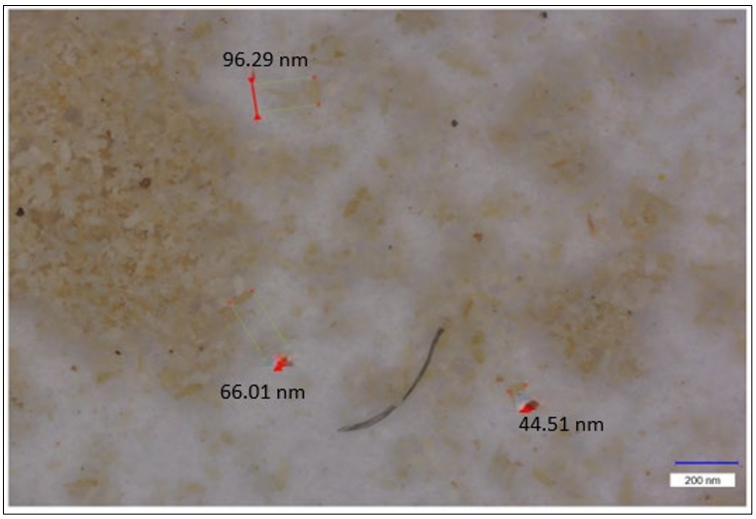
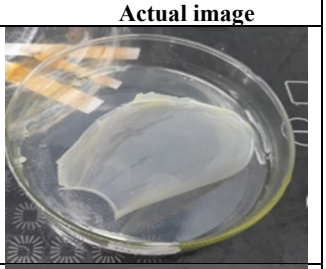
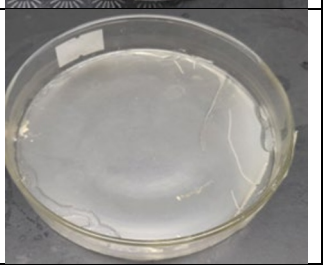


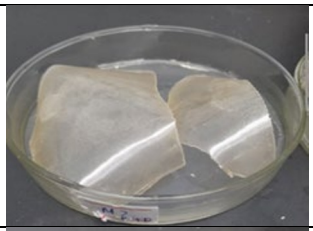
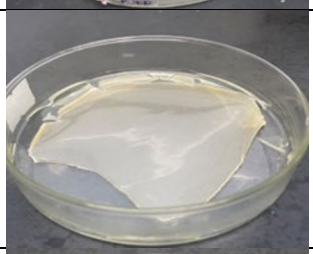
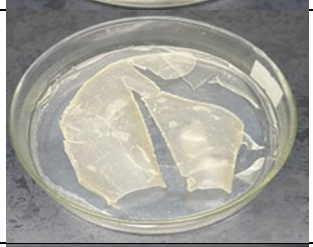
Fig. 2. Particle size analysis of nanocellulose powder derived from durian peel (magnification x100).

3.2 Preparation of biodegradable materials based on starch–NC–LEO

The composite film formulations were prepared by incorporating varying levels of LEO into the starch-NC mixture. The physical states of the material samples and the actual images obtained are presented in Table 1.

Table 1. Starch–NC-based composite biofilms with varying LEO concentrations.

Sample	Description of physical state	Actual image
Starch film (S)	Translucent white film, intact, slightly brittle.	
Starch–NC film (SN)	The film turns to a light-yellow color, easily removed from the mold, flexible and tough.	

Starch – NC – 1 % LEO film (SN1)	Light brownish-yellow film, intact, good gloss, flexible and tough.	
Starch – NC – 2.5 % LEO film (SN2.5)	Light brownish-yellow film, not intact after removal from the mold, flexible but easily torn.	
Starch – NC – 5.0 % LEO film (SN5)	Clearly brownish-yellow film, not intact after removal from the mold, easily torn.	
Starch – NC – 10 % LEO film (SN10)	Light brownish-yellow film, not intact after removal from the mold, breaks into small pieces.	

Visual images of biofilms and physical state observations of starch-NC films containing LEO at different concentrations showed that LEO addition significantly affected the morphology and mechanical properties of the resulting films. Starch films without NC and LEO were clear white, brittle, and fragile when dried, while films containing NC were light yellow, more flexible, and elastic. This demonstrates that adding NC to the starch film enhances the polymer network structure, resulting in increased film elasticity compared to the original starch film.

For the LEO-added films, 1% LEO addition made the films easier to peel from the mold after drying, preserved their integrity, and improved their gloss and flexibility compared to the starch film and the starch-NC film. However, when the LEO content exceeds 2.5%, the films become less stable, are difficult to remove from the mold after drying, and are prone to breakage and fragmentation. This may be because, at high concentrations of essential oils, interactions in the polymer network are disrupted, leading to the localization of the raw material components and thereby reducing the mechanical properties of the obtained film.

Thus, it can be seen that the LEO content added to the film formulation significantly affects the composite film's properties. In which the film containing 1% LEO is considered to be in the most intact state.

3.3 Optical images

Optical images of the studied films with varying LEO contents were examined using optical microscopy. Figure 3 shows that the incorporation of NC resulted in the clear appearance of NC particles on the film surface. When essential oil was added to the film formulation, it primarily surrounded the NC particles. The morphology observed under the microscope clearly displayed yellowish streaks or fibers encased in a light yellow ring of essential oil. The data also indicated that these oil rings around the NC fibers became more pronounced as the LEO content increased.

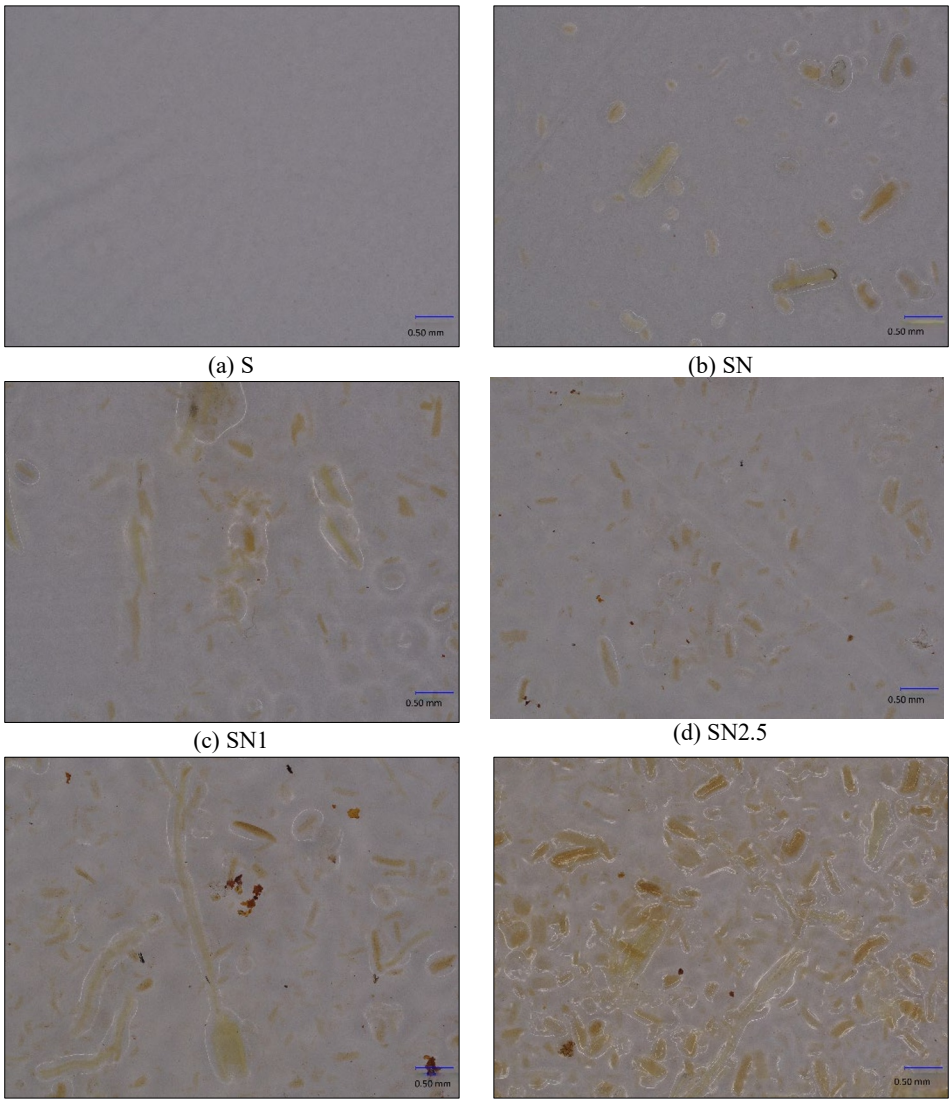


Fig. 3. Optical images of starch–NC–LEO films (magnification x100).

3.4 Color differences

The color differences of the studied films compared to the standard white are showed in Table 2. Brightness (ΔL^*): When NC was added to the starch, the film's brightness increased slightly. However, when LEO essential oil was added to the starch-NC film, its brightness decreased. Increasing the LEO content in film formulation reduces the brightness of the resulting films. The decrease in brightness upon LEO addition indicates that the films are darker and opaquer. This is explained by the fact that Leo has a characteristic lemon-yellow color. When added to the film-forming formula, the essential oil surrounds the NC molecules, forming a film layer. Such a distribution will cause light obstruction, making the film darker and opaquer than a film without essential oils.

Table 2. Color comparison results of the studied films.

Sample	Brightness (ΔL^*)	Red/Green component (Δa^*)	Yellow/Blue component (Δb^*)	Overall color difference (ΔE)
S	31.25	-3.23	5.18	31.84
SN	31.91	-1.40	6.79	32.65
SN1	31.79	-0.26	8.32	31.89
SN2.5	31.13	-1.41	8.53	31.46
SN5	29.01	-2.33	8.42	29.76
SN10	28.92	-2.84	9.01	29.17

The Δa^* value represents the red/green pigmentation. Table 2 shows that all Δa^* values are negative, indicating that the films in this study have a predominant green color rather than a red one. The blank starch film had Δa^* value of -3.23, showing the strongest green color trend. When NC and LEO were added to the starch film, the green color of the film decreased slightly and gradually turned neutral. However, if the LEO content exceeds 2.5%, the resulting films tend to turn green again. This trend is explained by the fact that the LEO (lemon yellow) color affects the Δa^* value of the resulting films.

Similarly, the Δb^* (yellow/blue) index for the studied membranes was > 0, indicating that the membranes tended to show a more dominant yellow color than blue. Among them, white starch films had the lowest yellow index, while films supplemented with NC and LEO increased the yellow index of the original film. This is explained by the yellow color of the nanocellulose and LEO components, increasing the yellow index of the studied membranes.

Evaluation of the overall color difference through ΔE values showed that the addition of NC to the starch films made the obtained composite films more distinct from the original starch films. However, simultaneous addition of NC and LEO to the film formulation resulted in films with a lower overall color difference than the blank starch film.

The above findings showed that the addition of LEO significantly affected the color indices of the films obtained. In particular, adding LEO at 2.5% or more tends to make the film turn more yellow, increase the overall color difference, and reduce brightness. Meanwhile, the 1% LEO-supplemented film exhibited color indices similar to those of the original starch film.

3.5 Water uptake

Figure 4 shows the water absorption capacity over time of the studied membranes. The obtained data showed that the native starch films had lower water absorption capacity than

the other films. When NC was added to starch, the resulting complex film exhibited a significantly increased water-absorption capacity. This is explained by the nanoscale size of NC, which results in a polymer network with more functional groups capable of forming hydrogen bonds with water, thereby increasing the water absorption of the starch-NC film. When LEO was added to the NC-amyloid membrane at low concentrations, although LEO was hydrophobic, the low concentration was insufficient to cover the water-absorbing functional groups in the polymer network. Therefore, the starch-NC film containing 1% LEO has the same water absorption capacity as the SN film. Continuing to increase the LEO content from 2.5% or more, the water absorption of the obtained films tended to decrease, because the LEO essential oil surrounding the NC particles, as observed under a spectroscopic microscope, prevented the hydroxyl groups in NC as well as starch from interacting with water. Thus, it reduces the film's water-absorption capacity.

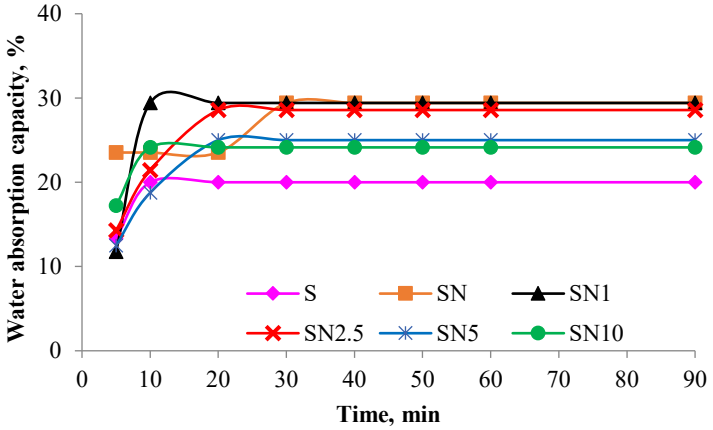


Fig. 4. Water uptake of the studied films.

Thus, the LEO content significantly influences the water absorption of the obtained material. Films containing 1% LEO have a higher water-absorption capacity and are more suitable for fresh-fruit or meat preservation applications, helping create a humid environment to keep these foods fresh. Films containing 2.5% or more LEO have a lower water absorption capacity and are better suited to applications with lower moisture content. However, in general, the addition of LEO decreased water absorption compared to the starch-NC film.

3.6 Biodegradation

The results of biodegradability studies of the studied membranes are presented in Table 3. The obtained data showed that the addition of LEO to film formulation significantly affected the degradation rate and extent of film degradation. Compared with blank starch films, NC-containing films have faster biodegradability and higher biodegradability than conventional starch films. When LEO was added at a concentration of 1%, the biodegradability of the film increased, but the degradation rate was slower than that of the starch film containing only NC. Increasing the LEO content to 2.5%, the obtained films had a significant decrease in the degradation level and rate. This could be explained by the antibacterial activity of LEO, which hinders the degradation of films with high LEO content.

However, the membrane samples containing LEO all showed biodegradation at levels of 82% or more after 20 days of burial in soil. This indicates that adding LEO to the starch–NC film-forming formulation did not significantly affect the biodegradation of the resulting films.

The results of this study showed that the degradation rate of starch-NC-LEO films was higher than that of chitosan films containing aloe extract [5] but slower than that of starch films supplemented with NC [3]. However, the biodegradation level of 80% or more after 20 days for the starch-NC-LEO film can still be considered good, contributing to reducing waste in the environment.

Table 3. Biodegradation rate of the prepared films.

Sample	After 1 day	After 2 days	After 5 days	After 10 days	After 20 days
S	18.50	23.12	38.73	53.18	84.97
SN	22.16	34.09	90.00	100.00	100.00
SN1	25.73	28.07	43.27	63.16	93.57
SN2.5	16.26	19.51	34.96	37.40	87.80
SN5	17.83	24.84	37.58	54.78	85.35
SN10	14.08	45.07	46.35	47.42	82.63

3.7 Solubility

The results of the water solubility evaluation of the studied films, shown in Table 4, indicated that the addition of NC and LEO to the starch films reduced the solubility of the original starch films. As the LEO content added to the film formulation increases, the solubility of the films tends to decrease. The addition of NC to starch creates a multidimensional polymer network between the NC and starch structures, thereby reducing the film's solubility. Besides, NC, although small in size, is insoluble in water, which will help the starch matrix remain less soluble in water when evenly distributed on the starch polymer matrix. For LEO with hydrophobic properties, films containing LEO will have improved solubility compared to original starch films. In addition, as shown in Figure 3, LEO surrounds NC molecules and starch granules, forming a barrier layer on the membrane surface and preventing the membrane from contacting water molecules. Therefore, membranes containing LEO become less soluble in water.

The reduction in solubility of the studied membranes will facilitate more straightforward application, especially in high-humidity ambient conditions, such as the preservation of fresh foods, including fruits, meat, and fish.

3.8 Antibacterial activity

The antibacterial activity data for the studied films in Table 4 showed that the addition of NC and LEO to the starch films significantly enhanced their antibacterial activity compared to the original starch films. As shown in Table 4, the starch films without NC and LEO exhibit low resistance to *St. aureus* and *E. coli*. When NC was added to the film formulation, the materials' resistance to both *St. aureus* and *E. coli* increased significantly. Adding 1% LEO essential oil to the film formulation increased resistance to *E. coli*, but resistance to *St. aureus* did not differ from that of the starch-NC film. When the LEO amount was 2.5% or more, resistance to both strains increased significantly. However, if Leo was added at a dose of 10%, the inhibition of *St.aureus* and *E.coli* did not increase further.

Table 4. Solubility and antibacterial activity against *St. aureus* and *E. coli* of the prepared films.

Sample	Solubility, %	*St. aureus*, cm	*E. Coli*, cm
S	15.38	0.20±0.06	0.20±0.09
SN	15.20	0.30±0.18	0.30±0.12
SN1	13.33	0.30±0.05	0.40±0.12
SN2.5	14.17	0.35±0.11	0.50±0.08
SN5	14.08	0.40±0.06	0.45±0.19
SN10	13.64	0.40±0.07	0.45±0.09

Thus, it can be seen that starch films–NC– LEO are potential antibacterial materials. Due to the material's antibacterial properties, it is suitable for applications that preserve food and prolong shelf life, especially in starch-NC films containing 2.5% or more LEO essential oil.

4 Conclusion

In this study, nanocellulose was extracted from durian peel, with an average particle size of 68.94 nm, and confirmed by infrared spectroscopy. NC obtained from durian peel was added to starch films with varying amounts of lemongrass essential oil (LEO) at concentrations of 1% to 10%. The results showed that at a 1% LEO concentration, the film quality after drying was significantly improved: it retained its intact structure, and the color was similar to that of the original film. The addition of LEO to the film formulation results in a film with a stronger tendency to yellow, reduced hydrophilicity, and reduced biodegradability compared to the original film. In addition, adding LEO to the starch-NC films reduced solubility and increased antibacterial activity compared with the original film, especially against *E. coli* strains. These results highlight the potential of nanocellulose-reinforced starch-based biodegradable films derived from durian peel and LEO for eco-friendly packaging applications.

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