Antibacterial activity of bacterial cellulose-based edible film incorporated with *Citrus* spp essential oil

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Abstract. Bacterial cellulose-based edible films have been known as natural material for packaging containing carboxymethyl cellulose (CMC) and glycerol. Edible film is also known as an external food protection since it contains antibacterial substance. Incorporating antimicrobial compounds with essential oil (EO) from *Citrus* spp such as lime, lemon and sweet orange into edible films provides a novel approach to improve the safety, shelf-life of foods or fruits as well as physical properties of the edible film. The present study was aimed to investigate the antibacterial activity of edible film incorporated with EO. Antibacterial activity performance was carried out using broth and dilution agar method to determine EO role to inhibit the growth of pathogen bacteria such as *Escherichia coli* and *Staphylococcus aureus*. The results showed that edible film containing 2% lime, lemon and sweet orange performed moderate antibacterial activity with minimum inhibition concentrations (MICs) of about 100, 250 and 225 mg/mL, respectively.

1. Introduction

Biocellulose or microbial cellulose is one of natural and eco-friendly fibers and have important economic globally. Bacterial cellulose fibers are very pure, very stiff and similar to plant cellulose. Commonly, biocellulose is produced by *Acetobacter* species, displays unique properties including high tensile strength, high water absorption capacity, high crystallinity and an ultra-fine as well as highly pure fiber nanofibrillar structure [1,2]. Biocellulose has a diameter of 20-50 nanometers—1000 times thinner than the average human hair. The fiber length are interconnected and well-arranged, resulting in forming a network structure [2,3]. Nowadays, bacterial cellulose as a biological nonwoven fabric with special emphasis to provide the application of natural and biodegradable material package in order to reduce the use of synthetic materials that contribute to environmental contamination has been studied by many researches [4]. Biocellulose is now used for beauty treatments as well, since biocellulose composite has been introduced to enhance biocellulose properties through addition of reinforcement materials [3].

Biocellulose is grown in laboratory by tiny bacteria Acetobacter that convert glucose into cellulose and need oxygen to survive which resulted in forming the fibers. *Acetobacter xylinum* is a gram negative rod-shaped microorganism which is also known as *Gluconacetobacter xylinus*. The usage of glucose as carbon source during biocellulose production is associated with the formation of gluconic acid in the culture medium that affect the quantity of biocellulose production [2,3]. Edible film from nata de coco is one of the products generated from *Acetobacter* spp.
Edible films have been used for a long time for packing food. Currently, they are undergoing an advanced development [5,6]. Film or coating is a thin layer of material used for wrapping or coating layers among the foods. Currently, materials of film manufacturing are bio-based, naturally biodegradable and environmental friendly. These newer film materials have replaced conventional plastic films because of their harm to the environment [1]. The natural biodegradable films are normally applied in food products in order to prevent their spoilage and extend their shelf life [7].

Edible films also have been used as carriers of foods additives such as antioxidant or antimicrobial agent to improve the characteristic of the edible film and to reduce the materials that contribute to environmental contamination [4]. One of antimicrobial agents are essential oils (EOs) which demonstrated their ability in inhibiting microbial population in order to help food products with higher quality and safety [5,8]. Initial experiments using plate overlay assays demonstrated that 2% oregano EO was active against *Staphylococcus aureus* and *Salmonella*. Two percent rosemary EO was active against *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* [9]. The study of the antimicrobial effect of edible films containing 1% of oregano oil and cinnamaldehyde was evaluated in strawberry and spinach during cold storage. These results indicated that edible films containing EOs and their constituents can be used as release system for volatile antimicrobial agents to improve the safety and shelf life of fresh-cut fruits and vegetables [6]. In recent study, EOs from *Citrus* spp (*Rutaceae*) are considered as semi-sweet taste material [10] and possesses as an antimicrobial [4]. Studies of incorporation of EOs into edible films to improve the microbiological safety of foods are still limited. This study aimed to determine the antibacterial activity of edible film incorporated with lime, lemon and sweet orange EO. The effects of EOs addition to the growth of pathogen bacteria according to the minimum inhibition concentration (MIC) were evaluated.

2. Methods

2.1. Materials and preparation of bacterial cellulose-based edible film

Lime, lemon and sweet orange oils were purchased from Lansida Group. Nata de coco gels were purchased from local industry in Cianjur, West Java Province, Indonesia. The washing, purification, and preparation of bacterial cellulose (BC) slurry were conducted as previously reported [10].

2.2. Preparation of bacterial cellulose composites

An amount of 200 mL of BC slurry was composted of 30% w/w glycerol (Merck), 30% w/w CMC (Himedia), and 1-2% essential oil based on the gross weight of BC slurry. Each of additives was mixed with BC slurry in a beaker glass under magnetic stirring condition at 60°C. Subsequently, the solutions were degassed with vacuum bell jar to remove the bubbles, then poured on the tray. The trays were held overnight at 45°C in oven blower, then cooled at room temperature before peeling the films off the trays and stored in plastic bags until used [10].

2.3. Antibacterial activity of essential oils and edible films

The antibacterial activity of essential oils and films incorporated with EOs against the bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) were tested and determined by the disc diffusion assay. Film samples were cut into discs with 6 mm in diameter using a sterile punch and placed on plates containing Mueller–Hinton agar (MHA) (Himedia) which had been previously seeded with 100 μL of an overnight broth culture containing approximately 10^5 CFU/mL of the test bacteria. The plates were incubated at 37°C for 24 h and the clear zone formed around the film disc on the media was recorded [6].

The minimum inhibition concentrations (MIC) of active edible films containing EOs were also determined. The MIC value was evaluated as the lowest concentration of samples. Three replicates were conducted for each microorganism. The plates were incubated under anaerobic conditions for 24 h in an oven maintained at 36 ± 1 °C and subjected to visual inspection. The presence of microbial
growth on the medium indicated that the essential oil possessed bacteriostatic activity, while the absence of the growth implied bactericidal activity of the oil sample [11,12].

3. Result and Discussion

3.1. Antibacterial activity of essential oils

The growth inhibition zones of EOs were measured using agar disc diffusion assay. The antibacterial activity of essential oils as antimicrobial agents are shown in Figure 1.

![Figure 1](image1)

**Figure 1.** The inhibition zones of essential oils against pathogen bacteria *E. coli* (left) and *S. aureus* (right). A=Lime, B=Lemon and C=Sweet orange.

Figure 1 shows that the strong antibacterial activity for both bacteria was from lemon and sweet orange with inhibition diameter about 22 and 25 mm, respectively. Lime performed clear zone about 14 mm for both bacteria. This result reveals that both lemon and sweet indicates strong activities whereas lime as moderate. The current EO antimicrobial activity was suggested based on previous study of which bergamot EOs was characterized against *Listeria monocytogenes* [13]. In addition, it was also confirmed that sweet orange and lemon showed good antibacterial activity against both gram-negative and gram-positive bacteria. The MICs for selected EOs ranged 15–250 µg/mL [14].

The different ability as the antibacterial properties might be due to the chemical contents in EOs. Some studies showed that limonene and other metabolites which is belong to alkaloids, flavonoids, steroids, terpenoids, saponins, cardiac glycosides as active compound of *Citrus* spp could inhibit the growth of *E. coli*, *S. aureus*, *Salmonella typhimurium* and *Bacillus cereus* cells [4,16,17]. Another study showed that the essential oils of cinnamon, oregano, thyme, and clove attribute to the existence of cinnamaldehyde, carvacrol, thymol, and eugenol, which is possess similar activities against *L. monocytogenes*, *Salmonella typhimurium*, *E. coli*, *Brochothrix thermosphacta*, and *Pseudomonas fluorescens* [18]. It indicated that bioactive compounds of essential oils possessed antibacterial properties against several pathogen bacteria.

![Figure 2](image2)

**Figure 2.** The antibacterial activity of edible films each containing 2% lime, lemon and sweet orange.
3.2. Antibacterial activity of edible films

Edible films that were incorporated with 1-3% EOs also showed antibacterial activity (Figure 2). Addition of 1-3% EOs in edible films show the same clear inhibition zone. It might be due to the high saturation stage of EOs in BC slurry when it was gear oven-dried.

Figure 2 shows that the area of clear zones of edible films with EOs decreased compared with clear zones of disc containing EOs only (Figure 1). Zones of inhibitions for E. coli of the edible films containing lime, lemon and sweet orange were 18, 0.9, and 17 mm, respectively. On the other hand, diameter of inhibition for S. aureus of the edible film incorporated with lime, lemon and sweet orange were 0, 13, and 13 mm, respectively. Edible films containing EOs with clear zone of 0 = 13, 17, and 18 mm show moderate activity, θ = 0.9 m was weak activity, θ = 0 mm was none activity.

The edible film with 3% orange oil against E. coli and S. enterica showed no inhibition area [5]. A study showed that addition EOs into film might reduce the interaction between protein molecules and contribute to the formation of discontinuous and uncompact texture with pores structure, resulting in film thickness increased [19]. Therefore, the ability of EOs gradually faded. The decrease of inhibition area of bacteria from edible film might be due to the polarity EOs to be bounded with BC slurry which contained high polarity.

The minimum inhibition concentration (MIC) of 2% EOs in edible films inhibited the cells of bacteria are shown in Figure 3. The MIC of each edible films containing the same EOs revealed the same concentration for both E. coli and S. aureus.

![Figure 3. Minimum inhibitory concentration (MIC) from edible films containing 2% EOs against E. coli and S. aureus.](Image 303x405 to 373x476)

Figure 3 shows the MICs of lime, lemon and sweet orange for E. coli and S. aureus are about 100, 250 and 225 mg/mL, respectively. Compared with previous study, of which 15.7 mg/mL oregano was effective against E. coli, S. aureus, and L. monocytogenes [20], current study required higher EOs concentration. This might be due to the carvacrol as the main compounds of oregano binds well lemon peel. Other results showed that 23 different citrus EOs were more effective against S. Aureus, L. monocytogenes, and Salmonella enterica as gram positive and gram bacteria, respectively [4].

The antibacterial potential for any edible films containing EOs depends on the physical, mechanical and chemical of material’s characteristics. The present study showed that EOs from Citrus spp added to edible film could carry antibacterial agents in order to increase the value of edible film.

4. Conclusion

Based on the current study it is concluded that lime and sweet orange EOs were effective against E. coli and S. aureus with moderate activity whereas lemon was effective to inhibit S. aureus.

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