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Potential Inhibitory Power of Edible Starch Phosphate Film of Yellow Ivory Coconut Shoots (*Cocos nucifera var. eburnea*) Incorporated with Chitosan-Nisin on The Growth of Patogenic Bacteria

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A B S T R A C T

Edible film can be used to package food well and improve the condition of the food, but damage to food ingredients can also occur due to bacteria, so chitosan-nisin is added as an antibacterial agent. In this research, Edible Film was made by adding 1,2,3,4 g of nisin to a 1% (w/v) chitosan solution to obtain four antibacterial agent solutions, namely 1.0% chitosan and chitosan-nisin: 0.1; 0.2; 0.3 and 0.4% (w/v), then mixed with distilled water, phosphate starch of yellow ivory coconut shoots, and sorbitol, then stirred with a magnetic stirrer, molded and dried in an oven and then tested for the antibacterial properties of the Edible film. The research results showed that Edible Film containing chitosan-nisin solution was the most potent antibacterial in inhibiting 1% chitosan solution with 0.4% nisin with the inhibition zone for each bacterium ranging between 29.9 mm, 30.6 mm. and 28.2 mm. Antibacterial activity increased with increasing concentration of chitosan-nisin solution.

keyword: *edible film, potential inhibitory power, antibacterial agent, patogenic bacteria.*

I N F O A R T I K E L

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INTRODUCTION

Gading coconut (C. Nucifera var eburnea) is a dwarf coconut variety that has high productivity with round fruit and ivory yellow skin. Due to its current low economic value, this plant should be used more, especially in the food industry (Gadizza Perdani et al., 2017).

Coconut stems do not have food reserves but the tip of the stem tastes sweet because of the sugar content. This part is called the tumbut which is where the leaves

and flower clusters grow (Arimbawa, 2016). This tuber is usually eaten as a coconut root vegetable (Fauzana et al., 2021) and not used for other purposes and left alone. Therefore, further action is needed to utilize coconut shoots to become a renewable innovation.

The sugar substance that makes the tip of the coconut stem taste sweet indicates the presence of sucrose (Sutowo et al., 2010). Sucrose or sugar with the formula $C_{12}H_{22}O_{11}$ is chemically a carbohydrate (Anwar, 2019). Carbohydrates are one of the hydrocolloids besides protein that are used to make edible films (Ismaya et al., 2021).

Food packing may be done with edible film. Foods are kept in the ideal atmosphere thanks to packaging. Packaging is often used to keep food ingredients from coming into touch with the air and to postpone spoiling for a certain amount of time. On the other hand, danage can happen on its own and is typically brought on by outside factors (Marpongahtun, 2016).

The mostdeadly kind of damage is microbiological. Bacterial damage is the primary cause of food deterioration. (Fretes et al., 2018). Microbiological contamination can put consumers at risk and is one of the reasons why food quality has declined (Raningsih et al., 2021).

According to the explanation above, antimicrobial incorporation is one way to preserve the stability of edible film (Amaliya & Putri, 2014). Antimicrobial materials may be used in the creation of edible film. The practice of adding antimicrobial components to edible film is widely utilized.

Antimicrobial packaging is one of the most important active packaging systems to maintain food quality and safety against microbes (Rüegg et al., 2022). The main goal of antimicrobial packaging systems is to ensure food safety, maintain food quality and extend shelf life (Kumar et al., 2020) and prevent the spread of unwanted pathogenic microorganisms on food surfaces (Gonçalves, 2017).

One of the natural ingredients that contains antimicrobial compounds is chitosan. Chitosan is a polysaccharide produced from deacetylation of chitin (Rochima, 2014). Chitosan and its derivatives are biodegradable, non-toxic and biocompatible and have antimicrobial and antifungal properties (Flórez et al., 2022). Chitosan also has good mechanical properties and the capacity to form films, so it can make transparent films that can meet various packaging needs (Singh et al., 2015)

Studies show that chitosan has antibacterial ability only on gram-negative bacteria and is not effective on gram-positive bacteria. As a result, changes need to be made to improve the antibacterial properties of chitosan films. It is known that the addition of bacteriocins increases the coating ability of chitosan. Bacteriocins are safe, heat stable, and easily broken down by digestive proteolytic enzymes. Nisin is one bacteriocin that can be used for this purpose. Nisin is made by the bacteria Lactococcus lactis and consists of 34 types of amino acids. The active groups on nisin, such as -NH_2, -NH, -C=O, and -OH, can interact with the active groups on chitosan hydrogenally or covalently. This causes nisin to bind to polymers made from chitosan. Nisin acts as an antibacterial on gram-positive bacteria better than gram-negative bacteria at a concentration of 0.6% (w/v). If nisin is added to chitosan, the active groups in the coating will increase, which means more types of

bacteria can be removed (Kusumaningsih et al., 2019)

Based on this, the researchers aim to create the latest innovation in edible film which is antibacterial using phosphate cross-linked starch from the stem shoots of Gading coconut, with chitosan-nisin and STMP, a non-toxic cross-linking agent.

METHODS

Materials

This research used aquadest, chitosan, nisin, starch phosphate yellow ivory coconut stem shoots *(Cocos nucifera var. eburnea*), petri dish, porcelain cup, cork borer, funnel, desiccator, beaker, measuring cup, hotplate stirrer, universal bunsen burner, filter paper, uv lamp, magnetic bar, oven blower, acrylic plate, tweezers, statives and clamps, furnace, and thermometer.

Preparation of Chitosan – Nisin Antibacterial Agent

The antibacterial agent Chitosan-Nisin was made using the procedure of Kusumaningsih et al (2019) with modifications. Chitosan weighing 0.1 g was dissolved in 10 ml of 1% (v/v) acetic acid to obtain a chitosan solution of 1% (w/v) concentration (labeled as CS). Four other types of 1% chitosan solutions were prepared in the same way. A total of 1, 2, 3 and 4 g of nisin were added to four types of 1% chitosan solutions, to obtain chitosan-nisin solutions with variations in nisin concentration, namely 1% chitosan solution with 0.1% nisin (w/v) (CS- N1), 1% chitosan solution with 0.2% nisin (w/v) (CS-N2), 1% chitosan solution with 0.3% nisin (w/v) (CS-N3) and 1% chitosan solution with 0.4% nisin (w/v) (CS-N4).

Making Edible Starch Phosphate Film from Coconut Stem Shoots Using the Antibacterial Chitosan-Nisin

Making coconut Stem Shoots phosphate starch edible film follows the method of Maharani, Yulia (2017) with modifications. A total of 5.5 g of coconut Stem Shoots phosphate starch was put into a beaker containing 100 mL of distilled water and 1.5 mL of sorbitol was added. Then heated for 10 minutes at 85^{o}C while stirring while adding the antibacterial agent chitosan-nisin according to the last 5 minutes of treatment. Next, the solution was cooled to a temperature of 50^{o}C.

Next, it was vacuumed for 30 minutes to remove dissolved air in the film solution. The resulting solution is poured into a Teflon mold and leveled. Dried in the oven at 50^{o}C for 24 hours. Once dry, remove from the oven and cool for 15 minutes so that the edible film can be easily removed from the mold. Then the edible film was analyzed for antibacterial test. The same thing was done to make coconut Stem Shoots starch phosphate edible film without adding the antibacterial chitosan-nisin as a control.

Antibacterial Test

Edible coconut stem shoots film with antibacterial chitosan-nisin was tested following the procedure Ramos et al (2012) with modifications. The film was cut to a

diameter of 0.6 mm using a cork borer and then sterilized using UV for 10 minutes. A total of 10 ml of Muller-Hinton Agar (MHA) media was poured into a sterile petri dish and allowed to solidify. A sterile cotton swab was dipped in the culture suspension with a cell density of 107 cfu, and gently rubbed evenly over the surface of the medium, then allowed to dry at room temperature for several minutes. Using sterile tweezers, coconut Stem Shoots phosphate starch edible film with antibacterial chitosan-nisin with different concentrations was placed regularly on the surface of the test medium. The culture is incubated at the optimum growth temperature of 37-38ºC for the test bacteria for 24 hours. After the incubation period, the diameter of the inhibition zone (clear area) around the film was measured using a caliper. The activity of a film worth eating can be seen by the presence of an inhibition zone around the film. Research conducted by Ramos et al (2012) looked at differences in the sensitivity of the diameter of the inhibition zone, grouped into: not sensitive, sensitive, very sensitive and extreme sensitive. If the inhibition zone is less than 8 mm, it means it is not sensitive, between 9 and 14 mm means it is sensitive, between 15 and 19 mm means it is very sensitive and more than 20 mm is extreme sensitive.

RESULTS AND DISCUSSION

Edible starch phosphate film of yellow ivory coconut buds and several edible films of phosphate starch phosphate of ivory coconut buds incorporated with chitosan-nisin as an antibacterial that have been obtained can be seen in the figure 1.

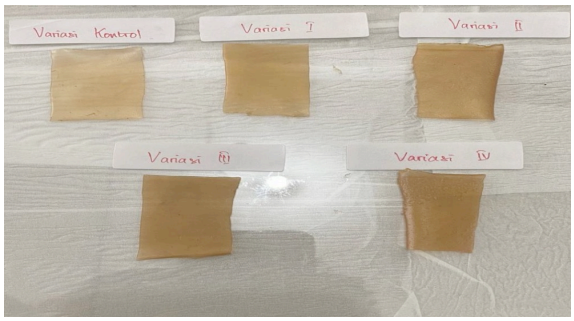


Figure 1. Edible Film, Phosphate Starch, Yellow Ivory Coconut Shoots and Edible Film Incorporated with Chitosan-Nisin as An Antibacterial

The agar diffusion method (Kirby-Bauer Diffusion) was used to test antimicrobial activity. This method was chosen because it can see the sensitivity of various types of microbes to antimicrobials at certain concentrations (Ratu et al., 2019).

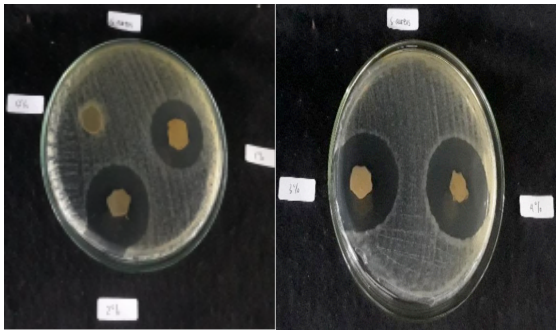
Based on the research carried out, the results obtained were the average inhibition zone of edible starch phosphate film at the tip of the stem of yellow Ivory coconut which was incorporated with chitosan-nisin solution as an antibacterial against pathogenic bacteria which can be seen in table 1.

Tabel 1. Inhibitory Zone of Edible Film Starch Phosphate Stem Shoots of Yellow Ivory Coconut Incorporated with Chitosan-Nisin Solution as An Antibacterial Against Pathogenic Bacteria

Bacteria	Consentrations (%) Kitosan-Nisin	Zone of Inhibition (mm)
S. aureus	0%-0% (Control)	7.2
	1%-0.1%	18.9
	1%-0.2%	23.1
	1%-0.3%	28.7
	1%-0.4%	29.9
Salmonella spp	0%-0% (Control)	7.9
	1%-0.1%	20.1
	1%-0.2%	28.2
	1%-0.3%	29.1
	1%-0.4%	30.6
E. coli	0%-0% (Control)	7.3
	1%-0.1%	19.2
	1%-0.2%	24.1
	1%-0.3%	27.6
	1%-0.4%	28.2

Table 1 shows that edible film starch phosphate yellow coconut stem shoots (control) without the addition of chitosan-nisin solution has resistant antibacterial properties because the resulting inhibition zone diameter is an average of 7.5 mm (inhibition zone is less than equal to 8 mm). This is in accordance with a study conducted by Addo-Mensah & Holland (2022) showing that bacterial sensitivity can be measured by measuring the diameter of the inhibition zone within millimeters. Bacteria that are resistant (less than 9 mm), moderately sensitive (10-11 mm), or sensitive (more than 12 mm) to antibiotics.

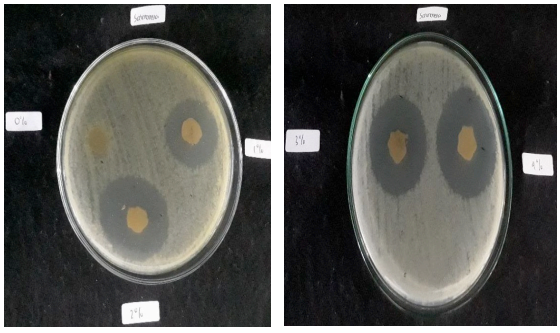
Based on the inhibition value (clear zone diameter) shown in Table 1 it can be seen that the inhibition of *S. aureus* bacteria is 18.9; 23.1; 28.7; 29.9 mm. The most likely inhibition zone for *S. aureus* is located at a chitosan concentration of 1% - 0.4% nisin, and the inhibition zone for *S. aureus* at a chitosan concentration of 1% - 0.1% nisin increases significantly. This may be due to the different growth phase of the *S. aureus* bacteria when the test was performed. The growth of *S. aureus* bacteria was slow at chitosan concentrations of 1% to 0.4% nisin. Antibacterial test on *S. aureus* can be seen in figure 2.



(a) (b)

Figure 2. Antibacterial test on S. Aureus bacteria. (a) variation control, variation 1 and variation 2; (b) variation 3 and variation 4.

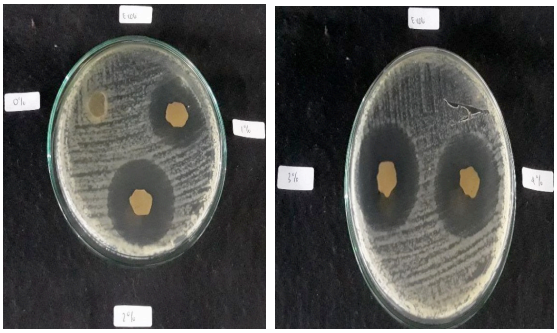
For *Salmonella spp* inhibitory zone value is 20.1; 28.2; 29.1; 30.6 mm. The most likely inhibition zone for *Salmonella spp* is located at a chitosan concentration of 1% - 0.4% nisin. Antibacterial test on *Salmonella spp* can be seen in figure 3.



(a) (b)

Figure 3. Antibacterial test on *Salmonella spp* bacteria. (a) variation control, variation 1 and variation 2; (b) variation 3 and variation 4.

And for *E. coli* inhibitory zone value is 19.2; 24.1; 27.6; 28.2 mm. The most potential inhibition lies in the chitosan concentration of 1% - 0.4% nisin. The inhibition zone of *E. coli* at a chitosan concentration of 1% - 0.4% nisin was 28.2 mm. Antibacterial test on *E. coli* can be seen in figure 4.



(a) (b)

Figure 4. Antibacterial test on *E. coli* bacteria. (a) variation control, variation 1 and variation 2; (b) variation 3 and variation 4.

This shows that the bacteria *Salmonella spp* and *E. coli* (gram negative) are more sensitive to the edible film starch phosphate of coconut palm bud which is incorporated with chitosan-nisin solution as an antibacterial compared to *S. aureus* bacteria (gram positive). The extraordinary antibacterial properties of chitosan and nisin have been emphasized in several studies. Nisin binds lipid II to inhibit bacterial cell wall formation. Additionally, it increases the permeability of cell membranes, which results in more pores. To explain the antimicrobial effects of chitosan, two main mechanisms have been proposed. Chitosan ultimately causes electrostatic accumulation on the surface of bacterial cells due to its polycationic nature. As a result, bacterial metabolism is disrupted. In addition, chitosan functions to bind DNA molecules, which stops transcription (Mirhosseini et al., 2023)

CONCLUSION

Edible film containing chitosan-nisin solution as an antibacterial was successful in inhibiting *S. aureus, Salmonella spp, E. coli* bacteria in a 1% chitosan solution with 0.1% nisin. Edible film containing chitosan-nisin solution as the most potential antibacterial in inhibiting pathogenic bacteria in a 1% chitosan solution with 0.4% nisin. Antibacterial activity increased with increasing concentration of chitosan-nisin solution.

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