Effect of natural ingredients addition as antimicrobial agents in *Dioscoreahispida* Dennst starch-based biofilm

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**Abstract.** The addition of antimicrobial agents in bioplastic food packaging aims to extend the food storage period and prevent the growth of pathogenic microbes. The use of antimicrobial agents from natural ingredient can make the biofilms safer to use. In this study, biofilms were made by adding natural antimicrobial agents from turmeric extract with the composition of starch 1.2% v/v total weight, 0.4% chitosan and variation of turmeric extract concentrations. 1.5% glycerol were added as a plasticizing. The best biofilms obtained by adding 0.375% of the extract with tensile strength and elongation values were 18.1 kgf/mm² and 30.24%. The edible films can inhibit the growth of *Escherichia coli* bacteria which can be seen from the formation of clear zones around edible films on antimicrobial activity assay by agar diffusion method. Application of the edible film as food coating delayed the decay of coated food.

1. **Introduction**

Janeng (*Dioscorea hispida* Dennst) is one of the potential sources for staple foods that support food diversification, due to its rich content of carbohydrates, protein, vitamin C, vitamin B, and other minerals [1]. However, its use is very limited due to its high toxicity [2]. Starch from janeng has been utilized to prepare biofilms. Nevertheless, high carbohydrate content in janeng is potential to be used as a source of natural polymer in biofilms preparation, especially as edible food packaging [3].

Food packaging is used to protect the foods from environmental factors such as microorganisms, dust, odor, vibration and compressive power [4]. Food packaging can extend the shelf life of the food, increase and maintain the content of the foods such as, meat, fruit, vegetable and ready-to-eat products which is susceptible to damage and also prevent the possibility of growth of food-borne pathogenic bacteria during preparation and storage of the food products. The use of edible films which are added by antimicrobial agents as packaging or food coatings is one of the alternative of an environmentally friendly technology to increase the shelf life of food products [5].

Natural ingredients, such as plant extracts, can be added to a biofilm as antimicrobial agents. Many studies have reported the use of plant extract as natural food preservatives due to its antimicrobial activities [6,7]. Addition of green tea and basil extracts improves the antioxidant activity and physicochemical properties of the cassava starch-based biofilm. Moreover, the film was degraded in soil for under two weeks [8]. The similar result is also reported that the addition of rosemary extract gives a high antioxidant activity to the cassava starch-based film [9]. The antioxidant and antimicrobial activities also shown by the combination of peanut skin extract and pink pepper residue in chitosan film, applied on restructured chicken products [10]. Edible film added with *S. aromaticum*
and *C. cassia* extracts is applied to chicken meat, extending the shelf life of chicken meat by inhibiting the microbial growth [11]. The addition of extruded white ginseng extract gives a potential antioxidant activity to alginate-based film for various foods coating. As an addition, ginseng extract is reported to give an increase in film elongation [12].

In this study, turmeric extract was used as an additional preservative in the biofilm made of janeng starch-chitosan composite. Turmeric is one of the known spices, where its extract can be used as a preservative. The active ingredients of curcuminoid from turmeric extract has been reported to have antimicrobial, anti-inflammatory, antioxidant and anti-cancer activities [13]. Antimicrobial activity of turmeric extract has been utilized in the food industry, due to its ability to prevent the growth of *Escherichia coli* and *Salmonella Typhi* [14]. The use of natural ingredients as an antimicrobial agent in janeng starch-based biofilm aims to make food packaging which can extend the shelf life of the food.

2. Materials and methods

2.1. Materials

Janeng tuber is obtained from the Aceh Besar region, turmeric rhizomes is bought from traditional markets, beef meatballs are home-made food without preservatives, glycerol, sodium bisulfite, AgNO$_3$, Hanus reagents, KI solution, starch indicator, iodine solution, nutrient agar (NA) medium, sabouraud dextrosa agar (SDA) medium, chitosan and acetic acid are pro-analysis chemicals obtained from Merck and Sigma Aldric. Chitosan solution is made by dissolving 2% chitosan in 100 mL of 1% acetic acid.

2.2. Preparation of edible films

Janeng tuber (6 kg) is peeled, cleaned, and then cut into small pieces and rinsed with distilled water. The tuber pieces are mashed with a blender in a sodium bisulfite solution (1.12 g/L) for 5 min. The homogenate sample is rinsed with distilled water and squeezed using a soft porous fabric. The squeezing is repeated several times until the juice is clear. The juice is left in a place for 24 h. Obtained precipitate is washed with distilled water and filtered with a vacuum buchner filter. This washing stage is repeated until no more HCN content in washwater. The qualitative test is done by using of 0.5 M AgNO$_3$ solution. The precipitate dried at 60°C for 24 h. The dry precipitate is crushed and sieved with a 100 mesh sieve, and the starch is used for edible film preparation.

Preparation of edible film is perfomed with casting method. Starch paste (6%) made from 6 grams of starch is dissolved in 100 mL distilled water, stirred and heated at 75-80°C for 15 min until gelatinized, and 0.75 mL glycerol is added. The janeng starch paste was cooled to a temperature of 27-30°C. Biofilm composite is prepared by mixing 8 mL of gelatinized starch, added with 8 mL of 2% chitosan solution in 1% acetic acid with various volumes of 10% turmeric extract in order to make final concentration of the extract to be 0.25, 0.375, 0.5, 0.625 and 0.75% in the 40 mL mixture solution. The mixture is stirred using a magnetic stirrer for 15 min and poured on a glass mold. The biofilm is dried for 48 h at room temperature.

2.3. Edible film properties assay

Mechanical properties of the edible films are evaluated by determining the tensile strength, percent elongation, using the Shimadzu Autograph 1000 model with a static load of 1 KN based on ASTM D638. Mean while, the surface morphology of the edible films are observed with scanning electron microscopy (SEM) type JSM-6510.

Antimicrobial activity of the biofilms is determined using agar diffusion method by looking at the formation of clear zones around the pieces of edible film on the media that has been inoculated with *E.coli* after 12 h incubation. Biofilms without the addition of active ingredients are used as control.

Edible films are applied as food coatings with dipping method. Beef meatballs are dipped into the edible film solution and allowed to dry at room temperature. Physical changes of the meatballs are
observed every day for 3 d. For the comparison purpose, the uncoated beef meatballs, coated meatballs with edible film without turmeric extract and wrapped meatball with sintetic plasticare prepared as well.

3. Result and discussion

The edible films are prepared by mixing janeng starch along with chitosan, glycerol and preservatives solution to produce casting solution and poured on a glass plate. The film will be formed after a solidification process with the evaporation of the solvent. Composite biofilms of janeng-chitosan starch with the addition of turmeric extract are obtained in the form of thin sheets that are transparent, smooth, elastic and homogeneous as shown as Figure 1. The elasticity and the smoothness of the films are ascribed to the addition of glycerol as the plasticizer [15]. The visible homogeneity is attributed to well-mixed components due to high temperature used in gelatinization that breaks the intra molecular cross bonds in starch [16].

![Figure 1](image1.png)

**Figure 1.** Profile of edible films with addition of various concentration of turmeric, (a) type A (0.25%), (b) type B (0.375%), (c) type C (0.5%), (d) type D (0.625%) and (e) type E (0.75%).

The addition of turmeric extract to the edible film gives a change to the color. The higher concentration the turmeric extract, the thicker the yellow color of the edible films. SEM photography is conducted to determine the homogeneity level of the film surface. Even though, the edible films appear to be homogenous (Figure 1.), the SEM photograph tell the otherwise. Figure 2. shows that films containing turmeric extract (B) have inhomogenous surfaces compared to films without the extracts (A). On the surface of the film with turmeric extract there are visible grains that do not blend with the film which are assumed to be incomplete blended extracts.

![Figure 2](image2.png)

**Figure 2.** SEM photographs of the surface of the starch-chitosan (a), and starch-chitosan-turmeric extract (b) edible films at 5000x magnification.

Physically, the edible films containing 0.625% (type D) and 0.75% (type E) of turmeric extract are thicker and more difficult to be torn. However, different concentration of the extract affects the tensile strength and percent elongation of the films. As shown in Table 1, type B composition gives the highest tensile strength value (18.1 kgf/mm²) with considerable percent elongation of 30.24%. Therefore, it is selected for further steps of analysis.
Antimicrobial activity of the edible films was observed again *Escherichia coli* (*E.coli*), one of food pathology bacteria. The effectiveness of antimicrobials activity on edible films were shown by the formation of clear zones around edible film on the surface of the media that has been inoculated with *E. coli* bacteria. Edible films with the addition of turmeric extract give a clear zones with a diameter of 7 mm, larger than inhibition zone of the starch-chitosan edible film without turmeric extract (6 mm). This results showed that the addition of turmeric extract increases the activity of the edible film. In other hand, chitosan provides antimicrobial activity, the film made from 2% chitosan gives a 9 mm of diameter clear zone, while film made from janeng starch without other components does not show any clear zone. This can be a scribed to the chitosan ability to inhibit the growth of bacteria due to its positive charged amine group [17]. The presence of charged groups in chitosan causes ionic interactions with bacteria wall constituents which lead to peptidolic hydrolysis of microorganism wall and trigger electrolyte leakage [18]. Incorporating chitosan with turmeric extract increases the antimicrobial effectiveness of edible film. Other studies report that addition of antimicrobial agents such as garlic oil in chitosan films increases the antimicrobial activity of the films produced [19].

Antimicrobial active compounds in edible film can reduce, inhibit and stop the growth of pathogenic bacteria originating from food or other microorganisms that are on the surface of food [20]. Turmeric (*C. Longa*) has active compound curcumin which helps the inhibition of bacterial growth. Sesquiterpenes and curcuminoids compounds from *C. longa* rhizome are reported to have biological activities, such as wound healing and antibacterial activities [21]. The turmeric extract has been used in the food industry. Adding turmeric water extracts to potatoes based food, bonelles fried chicken and masala is reported to be able to inhibit the growth of *E. coli* and *S. Typhi* room temperature storage [14]. Hydrogel edible film made from cassava starch, gelatin and turmeric residues in combination with UV-A light irradiation, applied as edible coating on cold sausage is reported to give an antimicrobial activity [22]. The chitosan film with addition of turmeric extract was reported to have high antimicrobial activity and increase the tensile strength of the film for food packaging applications [23].

A solution with the same composition as edible film type B, applied as a coating material on beef meatballs samples. Dipping method is used to coat beef meatballs, where the meatballs are dipped in an edible mixture, and dried at room temperature. The physical form of the meatballs was observed for 3 days of storage at room temperatures. Figure 3. shows the changes in the appearance of coated and non-coated meatballs during 3 days of storage.

Meatball is wet foods and without preservatives, it can easily rot. Coating the meatball with edible film has shown different physical change after 3 days of storage compared to the non-coated meatballs. Visually, there is no significant difference between all the meatball samples until the second day of storage. On the third day of storage, the non-coated meatballs are covered with mold and becoming softer, while the meatball that coated with biofilms containing turmeric extract and chitosan remain the same. This show that coating meatballs with edible coating from janeng-chitosan starch combined with natural turmeric preservatives can inhibit fungal growth.
Sensory analysis was performed based on the assessment of 30 panelists on all meatball samples (Figure 3). Although statistically, the results of the panelists' assessment did not show any significant differences for all treatments ($P > 0.05$), the coated samples (D and E) were preferred in terms of flavor, texture, and color almost the same as the meatballs wrapped in wrapped plastic (B). This stated that coating food with an edible film containing natural preservatives can maintain food conditions better than uncoated food. The edible coating with antimicrobial agents can extend the shelf-life of fresh-cut fruit [24] and commercially has been used to reduce moisture loss, prevent physical damage, enhance product appearance and carry food ingredients including anti-browning agent, colorants, flavors, nutrients, spices and antimicrobial [25]. The functionality of edible coating can be expended by incorporating antimicrobials to protect food products from microbial spoilage, extend their shelf-life and enhance their safety [24].

4. Conclusion
Addition of turmeric extract into the composite film of Janeng-chitosan starch affects its physical properties, that include color, tensile strength, and elongation. Turmeric extract in the edible film can inhibit microbial growth and extend the shelf life of food that coated with the edible film.

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