Antibacterial Active Packaging Edible Film Formulation with Addition Teak (*Tectona grandis*) Leaf Extract

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Abstract—The objective of this research is to investigate the effect of teak leaf extract as antibacterial to the quality and antibacterial activity of active packaging edible film. This research is arranged used Randomized Block Design consists of 2 factor combinations. First factor is carrageenan concentration consist of 3, 5 and 7% of tapioca’s weight. Second factor is teak leaf extract’s concentrations consist of 10, 15 and 20% of edible film’s solution’s volume and repeated 3 times. The data were analyzed with ANOVA and continued with BNT or DMRT α=5%. The best treatment was determined using Multiple Attribute Method. The result showed that the addition of 7% carrageenan and 20% teak leaf extract was the best treatment. The edible film produced had 0.183 mm thickness, 11.635 gram/m², 24-hour water vapor transmission, 54.334 % elongation, 1,034 N/cm² tensile strength and 0.600 cm and 0.650 cm antibacterial activity toward *E. coli* and *S. aureus*, respectively.

Index Terms—antibacterial, caragenan, edible film, tectona grandis leaf extract

I. INTRODUCTION

Seventy percent of food poisoning cases in Indonesia, caused by the bacterium *Staphylococcus aureus, Escheria coli* and *Clostridium* because of unhygiene packaging. So it requires packaging of food products that capable of avoiding the risk of product contamination by pathogenic and spoilage bacteria. To date some techniques have been developed in order to produce active packaging which is environmentally friendly and naturally active. Packaging usually contain antibacterial, absorbent material O₂, CO₂ absorbent or enhancer [1].

Widely used packaging which can be applied as an active packaging is edible films. Edible film is made of a thin layer of edible material, formed on top of the food component as a barrier to mass transfer, as a carrier of food and improves food handling [2]. The material for edible film can use materials such as tapioca, carrageenan [3], [4] and glycerol. Antibacteria are compounds that inhibit the activity of bacteria pathogen. Antibacteria have been widely used as bioactive compounds for edible film. One of Antibacterial compound is phenol. Phenol is one of compound found in the leaves of teak [5]. Average production of PT. Perhutani of 800 thousand m³ per year, however the utilization of teak leaves is still limited [6]. Addition of teak leaf extract as antibacterial material to edible made from carrageenan and starch should capable of extending the shelf life of the edible film and minimize bacterial contamination of pathogenic and spoilage in foods such as *S. aureus* and *E. coli* and also increase the utilization of teak leaves.

Starch is a natural polymer that can readily be formed into films. It consists of (1-4) linked α-D-glucopyranosyl units. Natural starch, amylpectin and amylose, consist of this two kinds of chain. The linear polymer, amylose, makes up about 20 wt% of the granule, and the remain 80 wt% consist of branched polymer, amylpectin. Amylose is crystalline which has an average molecular weight of 500,000, while amylpectin is highly branched and have higher molecular-weight [7]. Despite their ease of preparation, starch films have poor physical properties. These can be improved by blending with either synthetic polymers to produce biodegradable materials [8] or other natural polymers in edible packaging. Carrageenan is a water soluble linear biopolymer, increasingly used as natural thickener, formulation stabilizer, or gelling agent in applications ranging from food industry (mainly dairy products) to pharmaceutics [9]. The dairy sector accounts for a large part of the carrageenan applications in food products, such as frozen desserts, chocolate milk, cottage cheese and whipped cream. In addition to this, carrageenans are used in various non-dairy food products, such as instant products, jellies, pet foods, sauces, and non-food products, such as pharmaceutical formulations, cosmetics and oil well drilling fluid [10], [11]. Except starch, carrageenan is, with pectin, the main natural gelling polysaccharide extracted from plants or seaweeds and used as high-value functional ingredient in foods, cosmetics and pharmaceuticals [12].

Carrageenan isomers which already known were κ-, λ-, and τ-carrageenan (κ-kappa, λ-lambda and τ-iota), which has differences in the number and position of the ester sulfate groups belongs to the repeating galactose units. κ-carrageenan has one negative charge per disaccharide with a tendency to form a strong but rigid gel. κ-carrageenan has two and λ-carrageenan has an average of 2.7 charges per disaccharide unit. The gelling power of κ-
carrageenan makes it capable of forming good film [13], [14]. The formation of edible coating with good film forming and mechanical properties can be done by blending starch with κ-carrageenan. Starch must be gelatinized first before blending with carrageenan.

The purpose of this research is to investigate the effect of teak leaf extract as antibacterial to the quality and antibacterial activity of active packaging edible film. So, using this edible film which inhibit the growth and kill pathogens and spoilage bacteria in food will extend the shelf life and maintain the quality of foods.

II. METHODS

A. Materials and Test Isolates

Materials used for the extraction of teak leaf antibacterial are fresh Netherlands teak leaf. Materials used for testing the antibacterial property of the edible film produced in this research were E. coli ATCC 25 922, S. aureus ATCC 29 213, NA and NB which obtained from the Laboratory of Food Microbiology Department of Agricultural Technology UB’s Faculty of Agricultural Technology.

B. Antibacterial Extraction

In this study, the antibacterial compound was extracted from fresh teak leaves using a modified method of Effendi [15], solvent extraction using distilled water and 80 Watt microwave for 1 minute.

Edible film was made by mixing 5 grams tapioca, 1 ml glycerol, carrageenan with various concentration and 100 ml of distilled water. All mixed and heated (temperature 65-70 °C) until the dough gelatinized. After the mixture is cooled to a temperature of 40 °C, teak leaf extract was added. The mixture then spread on a mold, and dried. After the edible film dried, it can be taken from the mold.

C. Parameters Used

The parameters used in this research were the total phenol [16], thickness, tensile strength, film elongation [17], water vapor transmission [18], the antibacterial activity [19], the best treatment [20].

D. Data Analysis

The data were analyzed using Analysis of Varian (ANOVA) followed by Least Significant Difference (LSD) using 5% confidence interval or DMRT (Duncan Multiple Range Test) with a confidence interval of 5%. The best treatment will be taken by the method of multiple attributes [20].

III. RESULT AND DISCUSSION

A. The Thickness of Edible Film

The lowest thickness produced by 3% carrageenan and 10% leaf extract and the highest produced by carrageenan treatment of 7% and 20% leaf extract (Fig. 1). The use of carrageenan increased soluble solids, causing higher thickness [21]. More carrageenan added will increase the edible polymer films, increasing the viscosity of the suspension together with tapioca starch edible film during heating and gelatinization [18]. Edible film thickness is due because of the higher concentration of carrageenan used. The increase of carrageenan will increase in total dissolved solids present in the film-forming solution, so after the drying process will produce thicker films. In general, the thickness effect on tensile strength, elongation, and water vapor transmission rate [4].

B. Elongation

Elongation is the maximum elongation when the edible film start to tear of when it stretched [2]. The use of carrageenan in the production of edible film resulted in the ability to bind water into a well that produced elongation edible film [21]. The results showed 53.67%-59.33% elongation (Fig. 2). Elongation categorized good when elongation more than 50% and less when the elongation less than 10% [21].

The highest elongation obtained from carrageenan concentration of 3% and teak leaf extract by 10%. In carrageenan concentration 5 and 7% decrease in elongation. This is presumably due to the increase in the percentage of solids to water volume [21]. More carrageenan, carrageenan molecules produce a stronger matrix which caused the non-elastic so that elongation decreased [4]. The addition of carvacrol can lower elongation of edible film [22]. The addition of carrageenan affect the reduced elongation [4]. Elongation of edible film is due the higher the concentration of carrageenan used. The carrageenan molecules will form a stronger film matrix so the films lost its elasticity or easily broken (brittle), and consequently the percentage
of extension decreases. Other research showed that the increase of breaking strength of the film will be followed distantly by decrease in the percentage of elongation. Edible films with the smallest elongation usually have a stronger film formed.

C. Water Vapor Transmission

The more the concentration of carrageenan and teak leaf extract the lower water vapor transmission (Fig. 3). From the results obtained showed that water vapor transmission ranges from 10.832 g/m².24 hour - 27.683 g / m².24 hours.

Glycerol is a substance which can improve the properties of edible film as a barrier to water vapor [21]. The lowest water vapor transmission is obtained from the treatment of carrageenan concentration of 7% and 20% leaf extract. The combination of carrageenan molecules, tapioca starch and glycerol which binds both to the edible films caused low water vapor transmission [21]. The water vapor transmission of edible films depends on many factors, such as the integrity of the film, the hydrophilic/hydrophobic ratio, the ratio between crystalline and amorphous zones, and the polymeric chain mobility [22]. One of the major purposes of edible films is to block moisture transfer between the food and the surrounding atmosphere, therefore the WVP should be as low as possible. Permeability is the contribution of diffusivity and solubility of the permeant through the solid matrix, being this parameters changed by the structure of the matrix when using the same permeant

![Image](https://via.placeholder.com/150)

Figure 3. Average water vapor transmission rate of edible film produced from varies carrageenan concentration with addition of varies teakleaf extract concentration.

D. Tensile Strength

Mechanical strength and extensibility are generally required for a film to resist external stress and maintain its integrity when applied to food products. Tensile strength is the maximum tensile stress is acceptable edible film to break up [21]. The results of research show the tensile strength of edible films ranged 0.434 N / cm² - 1,434 N / cm².

Lowest tensile strength obtained from carrageenan concentration of 3% and 10% identity leaf extract and the highest tensile strength obtained from carrageenan concentration of 7% and 20% leaf extract (Fig. 4). In general, the addition of carrageenan will add to the strong bond so the gel formed becomes stronger and caused higher tensile strength. Some research showed that the higher the concentration of carrageenan will increase the value of tensile strength [18]-[23]. The tensile strength of the material arising from the reaction between the polymer bonding between atoms or secondary polymer chains against external forces is given [23]. An increase of tensile strength has been also reported when guar gum (galactomannan) was added to chitosan films, although further increase of concentration of galactomannan in the mixtures led to reduction of TS values [24]. The addition of agar into cassava starch induced an improvement in EB and TS of cassava starch-based films [25]. Different polysaccharide ratios can be used to improve mechanical properties depending on the degree of interaction between the molecules of the components.

![Image](https://via.placeholder.com/150)

Figure 4. Average tensile strenght of edible film formed from varies carrageenan concentration with addition of varies teakleaf extract concentration.

E. Antibacterial Activity of Edible Film

The addition of teak leaf extract showed antibacterial activity on Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC29213 proven by the formation of clear zone diameter 0.12cm-1.26cm and 0.14cm-1.37cm. Other research showed that teak leaf extract added on edible film caused inhibition of *Eschericia coli* and *Staphylococcus aureus*; *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhimium*, *Klebsiella pneumoniae*, *Escherichia coli* and *Serratia marcescens* [27].

Teak leaf extract has antibacterial activity against *Escherichia coli* [26]. Antibacterial contained by edible films containing teak leaf extract more effect on Staphylococcus aureus ATCC 29 213 compared against E.coli ATCC 25922. This is caused by the structure of the cell wall of Gram-positive bacteria such as Staphylococcus aureus is simpler than the cells of Gram-negative bacteria, *Escherichia coli*, so cause antibacterial easier entry into cells of gram-positive bacteria. Gram-positive bacteria have a cell wall of different sensitivity to antibiotics, physical and enzyme treatment compared with gram-negative bacteria which Gram-positive bacteria more sensitive than Gram-negative antibacterial [28]. In order antibacterial effect on Gram-negative bacteria, antibacterial must penetrate the outer membrane of bacterial sheath advance. After that, the antibacterial entry through the cell wall and reaches past the periplasmic enzyme serine protease. The enzyme responsible for the biosynthesis of the cell wall [29].

Carrageenan with the concentration of 7% and 20% leaf extract resulted in the largest inhibition zone (Fig. 5).

The higher the concentration of leaf extract the higher the antibacterial antibacterial activity, meaning that the
bacteria will be killed faster when higher concentrations of antibacterial given [26]. Phenol is a major component in teak leaf extract as antibakteri. Phenol can be damaged by oxidation. The effectiveness of oxidation depending on the amount of O2 in the air and the material properties of carrageenan [6] transmission of O2, as the small molecule of carrageenan fill a void in polymer matrix so as to minimize O2 that affected the oxidized phenols. The hydrophobic nature of O2 and hydrophilic structure carrageenan resulted in O2 difficult to penetrate the polymer carrageenan [30], so phenol in the network are better protected if the edible film [31].

Teak bark effective against *Listeria monocytogenes* and methicillin resistant *Staphylococcus aureus*. The antibacterial compound of teak leaf is 5-hydroxy-1, 4- naphthalenedione (Juglone) [32]. This compound has been found to be inhibitory to oral pathogens, notably *Streptococcus* mutans, *Streptococcus* sanguis, *Porphyromonas gingivalis* and *Prevotella intermedia* [33], [34]. Another study showed the synergistic in-vitro antibacterial activity to formulate new cost effective antimicrobial agent for multi-drug resistant organisms, based on the synergistic activity of Tetracycline with methanol extract of teak. Other research shows maximum synergistic activity against different bacteria both Gram-positive and Gram-negative species. The higher synergistic rate was achieved against *Salmonella* typhimurium (MTCC 98), *Klebsiella pneumonia* (MTCC 432), and lowest synergistic shows against *Pichia pastoris* (MTCC 34), *Escherichia coli* (MTCC 729). No synergistic activity was observed in *Citrobacter freundii* (MTCC 1658) [26]. The antibacterial activity was also tested for leaf, bark and wood extracts of teak against *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), hospital strains of *Salmonella paratyphi* and *Proteus mirabilis* [35].

The use of carrageenan as edible films and coatings already covers various fields of the food industry such as application on fresh and frozen meat, poultry and fish to prevent superficial dehydration [36], ham or sausage-casings [37], granulation-coated powders, dry solid foods, oily foods [34], etc., but also manufacturing soft capsules [39], [40], and especially non-gelatin capsules [41]. Indeed, this protective barrier can also be used in food domain in order to prevent the transfer of moisture, gases, flavours, or lipids and thus to maintain or improve food quality and to increase food product shelf life [42]. Another promising emerging technology that has been applied to various biopolymers, including, carrageenan-based coating, is their use as antimicrobial agent carriers in active packaging systems [14]-[43].

### F. Selection of Best Treatment

The best treatment is obtained by using the method according to the Multiple Attribute [44]. Based on test results, it is obtained with the use of the best treatment karagenan 7% concentration and teak leaf extract 20% (K3E20). It can be shown from the value of each parameter which can be seen Table I.

**TABLE I. THE BEST TREATMENT OF EDIBLE FILM WITH ADDITION OF CARRAGEENAN AND TEAK LEAF EXTRACT**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Perlakuan Terbaik</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketebalan</td>
<td>0,180 (mm)</td>
</tr>
<tr>
<td>Transmisi Uap Air</td>
<td>11,635 (gram/m².24jam)</td>
</tr>
<tr>
<td>Elongasi</td>
<td>54,330 (%)</td>
</tr>
<tr>
<td>Tensile Strenght</td>
<td>1,030 (N/cm²)</td>
</tr>
<tr>
<td>Aktivitas Antibakteri terhadap <em>Eschericia coli</em> ATCC 2922</td>
<td>6,000 (mm)</td>
</tr>
<tr>
<td>Aktivitas Antibakteri terhadap <em>Staphylococcus aureus</em> ATCC 29213</td>
<td>6,500 (mm)</td>
</tr>
</tbody>
</table>

### IV. Conclusion

The addition of carrageenan has a significant influence on the thickness, water vapor transmission and tensile strength edible film. Addition of teak leaf extract has significant impact on the thickness, water vapor transmission of edible film.

The best treatment is obtained on addition of carrageenan concentration as much as 7% and teak leaf extract as much as 20% which results in a thickness of 0.183 mm, 11.635 grams of water vapor transmission per m2.24 hour, 54.334% elongation, 1,034N/cm² tensile strength, 0,600 cm inhibition zone against *E. coli* ATCC25 922 and 0.650 cm inhibition zone *S. aureus* ATCC29213.

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**REFERENCES**


Joni Kusnadi was born in Tanjungkarang, Lampung, Indonesia dated June 12, 1962. He completed the S1 in the Department of Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University in 1986 and graduated S2 in the Department of Biothecnology, Gadjah Mada University in 1999. S3 education was completed in 2008, on the program of agricultural science, Post Graduate UB. In 1988 until now he is a lecturer of Department Completed the S1 in the Department of Agricultural Product Technology, Faculty of Agricultural technology, Brawijaya University, Malang, Indonesia in the field of Food Microbiology study. In the year 2009 until now, he is also the head of the post graduate study program of agricultural product technology at Brawijaya Univegrsity, Malang, Indonesia.