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THE ROLE OF GAMBIER FILTRATE AND RED PALM OIL IN THE FORMATION OF CANNA STARCH BASED-FUNCTIONAL EDIBLE FILM

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ABSTRACT

This study aims to analyze the role of gambier filtrate and red palm oil in the formation of functional edible film and to determine whether the gambier filtrate and red palm oil are synergistic or antagonistic in the formation of functional edible films. The study design used a factorial randomized block design with two treatment factors and each treatment consisted of three levels, namely: gambier filtrate concentration (A): 20, 30, and 40% (v/v) as well as red palm oil concentration (B): 1.2 and 3% (v/v). The observed parameters were thickness, elongation percentage, water vapor transmission rate, antioxidant activity, and antibacterial activity. Gambier filtrate and red palm oil were capable to improve the antioxidant and antibacterial properties of canna starch-based edible film. Gambier filtrate plays a role in increasing the elongation percentage, thickness, and water vapor transmission rate of edible film.

Keywords: antibacterial; antioxidant; edible film; gambier; canna starch

INTRODUCTION

The oxidative reaction and pathogenic microbial contamination are the main factors for the deterioration of food products **(Lee et al., 2004)**. Food packaging is usually used in order to maintain food product quality and to increase the storage life of food products. Most of the food packaging material used currently is plastics packaging material.

In relation to awareness toward environmental deterioration due to plastics packaging waste, then packaging materials had been developed with characteristics of environmentally friendly and safe to be consumed by human-made of biopolymers or edible film **(Zhang et al., 2018)**. The main objectives of edible film application are decreasing weight loss, the danger of physical damage, improvement of appearance, color, flavor, and nutrients of products **(Franssen and Krochta, 2003)**. Edible film function can be improved by incorporating antimicrobial and antioxidant materials to protect the product from deterioration caused by microbial and oxidative deterioration which results in increasing storage life and product safety **(Rojas-Graü et al., 2006; Jung, Chung, and Lee, 2006)**.

Gambier is an extraction product from gambier plant containing catechin compound with a magnitude of 67.55 to 72.02% and gambier extract has other important compounds such as catechin gallate, gallocatechin, gallocatechin gallate, epicatechin, epicatechigallateat, epigallocatechin, epigallocatechin gallate, catechin tannat, and quercetin which have antibacterial and antioxidant characteristics. Catechin compound has weak acid (pKa 1 = 7.72 and pKa 2 = 10.22), it is less soluble in water and very unstable in the open air. It is easily oxidized at pH close to neutral (pH 6.9) and stable at lower pH (pH 2.8 and 4.9). This compound is also easily decomposed by light with a higher reaction rate at low pH (3.45) than at high pH (4.9) **(Pambayun et al., 2007)**.

Red palm oil is palm oil obtained from CPO processing yield without going through blanching process with an objective to maintain its carotenoid content. According to **Van Rooyen et al. (2008)**, red palm oil contains 500 ppm of carotenoid consisting of 37% of α-carotene, 47% of β-carotene, 11.5% of lycopene, and 6.9% of cis- α-carotene. **Bohn (2008)** had described that in addition to having functioned as free radicals catcher, some carotenoids also have vitamin A activity such as α-carotene, β-carotene, γ-carotene and β-cryptoxanthin.

Canna starch is an extraction product from tubers of the canna plant (*Canna edulis* Kerr) as one of the starch sources that have the potential to be developed. Canna starch concentration is 62.92 to 67.32% with amylose content of 21.14 – 24.44% and amylopectin content of 75.56 – 78.86%. Canna starch based-edible film [4% (b/v)] had elongation percentage in the range of 84.4 to 87.78% and water vapor transmission rate in the range of 8.52 to 11.77 $g.m^2.d^{-1}$ **(Santoso et al., 2016)**. **Santoso et al. (2019)** had added that canna starch based-composite edible film incorporated with protein extract of paddy field eel had produced elongation percentage and water vapor transmission rate of 58.84% and 18.85 $g.m^2.d^{-1}$, respectively.

The incorporation of bioactive materials that have antibacterial and antioxidant characteristics on edible film has been widely developed. **Santoso et al. (2018)** had revealed that corn starch based-edible film incorporated with gambier extract had characteristics of antibacterial (diameter of inhibition with a magnitude of 6.67 to 7.67 mm) and antioxidant (IC50 with a magnitude of 258.14 to 469.32 ppm). This finding showed that antioxidant characteristic was classified as very weak with IC50 value much higher than 50 ppm. Therefore, it is important to add biopolymer materials having antioxidant characteristics such as red palm oil.

The research objectives were as follows to analyze the role of gambier powder extract and red palm oil in formation of functional edible film and to determine whether gambier powder extract and red palm oil have synergistic or antagonistic characteristics in the formation of functional edible film.

Scientific hypothesis

The addition of gambier filtrate and red palm oil had a significant effect on the functional properties of the edible film with physical and chemical properties according to Japan International Standards **(JIS, 1975)**.

MATERIAL AND METHODOLOGY

Sample

The edible film made from biopolymer materials such as canna starch, glycerol, and CMC added with gambier filtrate and red palm oil.

Chemical

Canna starch from CV Warung Panganku Jakarta, gambier powder extract was obtained from Indralaya traditional market, red palm oil was obtained from PT Hindoli Banyuasin, South Sumatra, bacterial culture of *Staphylococcus aureus* from Agricultural Product Chemical Laboratory, Sriwijaya University, Indonesia, 1,1 diphenyl 2 picrilhydrazil (DPPH), 6) nutrient agar (NA) media, 7) glycerol, and 8) carboxymethyl cellulose (CMC).

Instruments

Hot plate merk Torrey Pines Scientific, analytical balance (Ohaus Corp. Pine Brook, N.J. USA), vacuum pump (model; DOA-P504-BN), NDH-200 series haze meters, Nipon Denshoku Kogyo Co Ltd, micrometer (Roch) (A281500504, Sisaku SHO Ltd, Japan), testing machine MPY (Type: PA-104-30, Ltd Tokyo, Japan), and water vapor transmission rate tester Bergerlahr.

Laboratory Methods

Laboratory methods were used according to **Santoso et al., 2018**; **ASTM, 1997**; **AOAC, 2012**, **Laohakunjit and Noomhorm, 2004**; **Maesaroh, Kurnia and Anshori, 2018**; **Trisia, Philyria and Toemon, 2018**.

Description of the Experiment

Sample preparation

Processing of gambier powder filtrate was done according to the modified procedure by **Santoso et al. (2018)**. Dry gambier is pounded until smooth by using a mortar and sieved by using 80 mesh siever. Subsequently, 20 g of fine dry gambier is taken and put into a 250 mL Erlenmeyer flask. The next step is the addition of aqua dest up to the border mark of 100 mL and heated at a temperature of 55 to 60 °C for 10 minutes while stirring by using a magnetic stirrer. Finally, the mixture was centrifuged at speed of 1000 rpm and the filtrate is taken with a magnitude of 1/3 part of the total volume.

Processing of functional edible film was done according to the modified procedure by **Santoso et al. (2018)**. Canna starch with a magnitude of 12 g is put into Beaker glass and added with aqua dest up to border mark 300 mL followed by stirring with a magnetic stirrer. Starch suspension is heated by using a hot plate at a temperature of 65 °C while stirring until perfect gelatinization is produced. The next step is the addition of aqua dest with a magnitude of 2% (v/v) from the total volume. Subsequently, gambier filtrate at concentration according to treatments [20, 30, and 40% (v/v)] and addition of CMC 1% (b/v) are put into Beaker glass gradually while constantly stirring. Red palm oil at concentration according to treatments [1, 2, and 3% (v/v)] is added and stirred until a homogenous suspension is developed. Suspension is degassed by using a vacuum pump for about one hour. Suspension with a magnitude of 40 mL is poured and leveled on a petri dish having 20 cm in diameter followed by drying within an oven at a temperature of 65 °C for 24 hours. The edible film is removed from the petri dish and put into a desiccator for one hour and finally, the edible film is ready to be analyzed.

Number of samples analyzed

This research used two treatment factors. Treatment factors were as follows: 1) concentration of gambier filtrate [A_1 = 20%, A_2 = 30% and A_3 = 40% (b/v)] and 2) concentration of red palm oil [B_1 = 1%, B_2 = 2% and B_3 = 3% (v/v)] with the number of samples analyzed as many as 9 samples.

Number of repeated analyzed

Three repetitions for each treatment factor were performed. The total amount of analyzed samples was 27.

Statistical Analysis

This research used a factorial completely randomized design. Treatments that have a significant effect were further tested by using honestly significant different (HSD) test at α = 5%. Data were analyzed by using the software program of SAS version Windows 9 in terms of analysis of variance.

RESULTS AND DISCUSSION

Thickness

The average thickness of the edible film was in the range of
0.133 to 0.210 mm. Treatment of 40% concentration gambier filtrate (v/v) and 3% concentration red palm oil (v/v) had the highest thickness with a magnitude of 0.210 mm, whereas the lowest thickness was found on treatment of 20% concentration gambier filtrate (v/v) and 1% concentration red palm oil (v/v) with magnitude of 0.133 mm. The thickness of the edible film was higher than the edible film based on the starch of durian and jackfruit seeds, which were 0.032 – 0.041 mm and 0.035 – 0.043 mm, respectively **(Wahidin et al., 2021)**. Chitosan edible film incorporated with essential oil from *Chrysanthemum morifolium* and tumeric incorporated alginate edible film has a lower thickness than the resulting edible film, which were 0.05 – 0.15 mm and 0.096 mm, respectively **(Tan et al., 2021; Bojorges et al., 2020)**. The thickness of the

edible film is the same as the edible film thickness of canna starch which was incorporated with the gambier extract, which is 0.143 mm **(Santoso et al., 2019)** and is lower than the edible based on taro tuber starch combined with galangal essential oil, which is 0.3 mm **(Handayani and Nurzanah, 2018)**. The average thickness value of the edible film was shown in Figure 1.

Analysis of variance results showed that treatments of gambier filtrate and red palm oil had a significant effect on the thickness of the functional edible film, whereas interaction of both factors had no significant effect. Results of the HSD test at α = 5% for gambier filtrate treatment were presented in Table 1and Table 2.

HSD test (Table 1) showed that the higher the gambier filtrate concentration, the thicker is the functional edible film. This is due to the fact that gambier filtrate contains catechin compounds which have crystalline form at dry conditions. This crystalline form is a solid that affect edible film thickness, i.e. the higher the total solid content within the edible film matrix, the higher is the edible film thickness. **Santoso et al (2016)** reported that the addition of gambier extract had a significant effect on increasing the thickness of the canna starch-based edible film.

Table 2 (HSD test) showed that the higher the red palm oil concentration, the higher the thickness of the edible film. This is due to the fact that red palm oil contains 44% of palmitic acid which is in solid form at room temperature and has white color. Therefore, the higher the red palm oil concentration, the higher is the thickness of edible film because palmitic acid has an effect on the increase of total solid content within the edible film matrix. **Praseptiangga et al. (2017)** revealed that the incorporation of palmitic acid in the composite semi-refined iota carrageenan-based edible film caused a significant effect increase in thickness.

The role of gambier filtrate and red palm oil significantly increased the edible film thickness, but no synergistic or antagonistic role in interaction treatment of gambier filtrate and red palm oil on the increase of edible film thickness. This can be described by the fact that complex bonds will develop amongst starch-glycerol-gambier filtrate-CMC-red palm oil within an edible film matrix. Catechin compound within gambier filtrate binds to glycerol molecules, starch, and CMC resulting in a low concentration of free catechin compound. In a similar fashion, red palm oil which binds to CMC also decreases the numbers of free palmitic acid within the edible film matrix. The existence of complex bonds which occurred within the edible film matrix causes the effect of catechin compound and red palm oil on edible film thickness to cancel each other out.

Elongation Percentage

The elongation percentage value of the functional edible film was in the range of 58.67 to 136%. The treatment of 20% (v/v) gambier filtrate and 3% (v/v) red palm oil had the lowest elongation percentage, whereas treatment of 40% (v/v) gambier filtrate and 1% (v/v) red palm oil had the highest elongation percentage. The results of this study were higher than the edible films based on alginate, teff-starch, and pumpkin, namely 27.67 – 43.57%, 33.12%, and 13.13-14.47%, respectively **(Lim, Tan and Pui, 2021; Prabhu et al., 2021; Lalnunthari, Devi, and Badwaik, 2020)**. The elongation percentage of this edible film is similar to the results of research by **Kim, Seo and Kim (2018)** and lower than that reported by **Sancakli et al. (2021)**, namely 132% and 258.41%, respectively. AThe average value of elongation percentage for the edible film was shown in Figure 2.

The average value of elongation percentage for the edible film had met the **JIS (1975)** standard with a minimum value of 70%. This was found on treatments of A_2B_1, A_2B_2, A_2B_3, A_3B_1, A_3B_2 and A_3B_3. The elongation percentage of canna starch-based-edible film was better than that of corn starch-based-edible film in which both were incorporated with gambier filtrate. **Santoso et al. (2018)** had described that corn starch containing high amylose as a raw material of edible film incorporated with gambier filtrate had produced low elongation percentage values in the range of 13.33 to 16.67%. This is primarily related to the composition of amylose and amylopectin within corn starch. It is known that amylopectin molecules had an effect on elasticity increment of edible film and the opposite happened to amylose molecules.

Analysis of variance results showed that treatments interaction of red palm oil and gambier filtrate had no significant effect on elongation percentage of the edible film, whereas gambier filtrate treatments had a significant effect on elongation percentage of edible film. Results of HSD test at α = 5% for gambier filtrate treatment related to elongation percentage of edible film was presented in Table 1.

HSD test (Table 1) showed that the higher the gambier filtrate concentration, the higher is the elongation percentage of functional edible film. It is known that gambier filtrate contains a catechin compound with a hydroxyl group (OH) as its active group. The higher the gambier filtrate concentration, the higher is the numbers of OH group that is capable to bind free water within matrix of edible film. According to **Santoso et al. (2017)**, the use of extract gambier in edible film formulation has a function to increase the elasticity of edible film because the catechin compound has a hydroxyl group that is capable to bind free water within the matrix of edible film.

Water Vapor Transmission Rate

The water vapor transmission rate of the edible film was in the range of 26.03 to 26.8 $g.m^2.d^{-1}$ and it did not fulfill the **JIS (1975)** standard (maximum of 10 $g.m^2.d^{-1}$). The edible film with the highest water vapor transmission rate was found on the A_3B_1 treatment (26.03 $g.m^2.d^{-1}$) and the lowest one was found on the A_1B_3 treatment (26.80 $g.m^2.d^{-1}$). The value of the vapor transmission rate of edible film produced was higher than the carrageenan-based edible film with added sorbitol of 6.83 $g.m^2.d^{-1}$ **(Rahmawati, Arief and Satyantini, 2019)**, gelatin edible films incorporated with casein phosphopeptides were 4.5 – 7.9 $g.m^2.d^{-1}$ **(Khedri et al., 2021)**, and carboxymethyl chitosan-pullulan edible films enriched with galangal essential oil of 0.185 – 0.290 $g.m^2.d^{-1}$ **(Zhou et al., 2021)**. The results of this study were lower than those reported by **Li et al. (2021)**, namely in the amount of 31.10 – 38.02 $g.m^2.d^{-1}$. Average value of water vapor transmission rate for the edible film was presented in Figure 3.

Analysis of variance results showed that gambier filtrate treatment had a significant effect on water vapor transmission rate, whereas red palm oil treatment and treatment interaction of gambier filtrate and red palm oil had no significant effect. Results of the HSD test related to gambier filtrate treatment toward water vapor transmission rate were shown in Table 1.

Table 1 HSD test (α = 5%) related to concentration of gambier filtrate treatment on thickness, elongation percentage, water vapor transmission rate, antioxidant activity and antibacterial activity of functional edible film.

Treatment	Thickness (mm)	Elongation percentage (%)	Water vapor transmission rate ($g.m^2.d^{-1}$)	Antioxidant activity (IC_{50} ppm)	Antibacterial activity (inhibition diameter, mm)
A_1 (20%)	0.154 ±0.025a	65.11 ±6.05a	26.19 ±0.21a	196.60 ±3.33a	2.19 ±0.20a
A_2 (30%)	0.172 ±0.023b	91.56 ±7.67b	26.52 ±0.20b	190.06 ±2.40b	2.69 ±0.49b
A_3 (40%)	0.199 ±0.015c	127.33 ±8.35c	26.66 ±0.17c	182.04 ±6.69c	3.18 ±0.47c

Note: Numbers followed by the letters in the same columns are not significantly different.

Table 2 HSD test (α = 5%) related to concentration of red palm oil treatment on antioxidant activity and antibacterial activity of functional edible film.

Concentration of red palm oil (%)	Antioxidant activity (IC_{50} ppm)	Antibacterial activity (inhibition diameter, mm)
B_1 (1%)	194.13 ±5.64a	3.08 ±0.62a
B_2 (2%)	188.49 ±7.45b	2.67 ±0.52b
B_3 (3%)	186.09 ±9.09c	2.31 ±0.35c

Note: Numbers followed by the letters in the same columns are not significantly different.

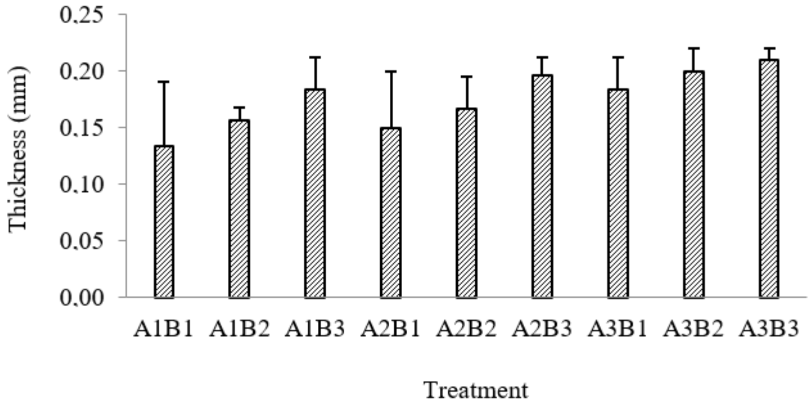

Figure 1 Average thickness value of functional edible film.

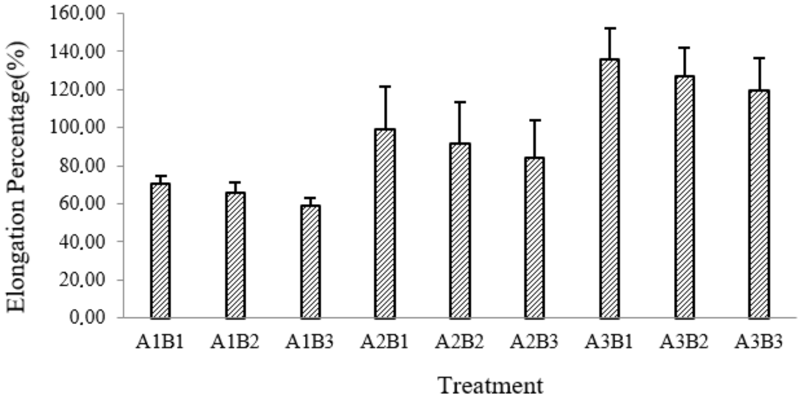

Figure 2 Average value of elongation percentage of functional edible film.

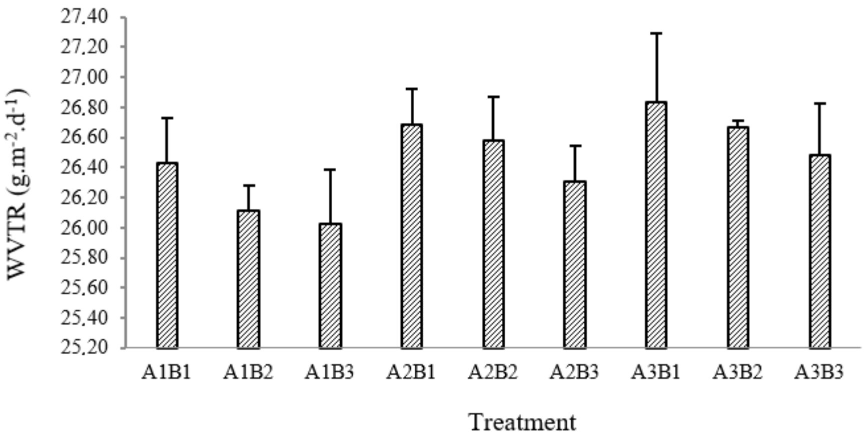

Figure 3 Average value of water vapor transmission rate of functional edible film.

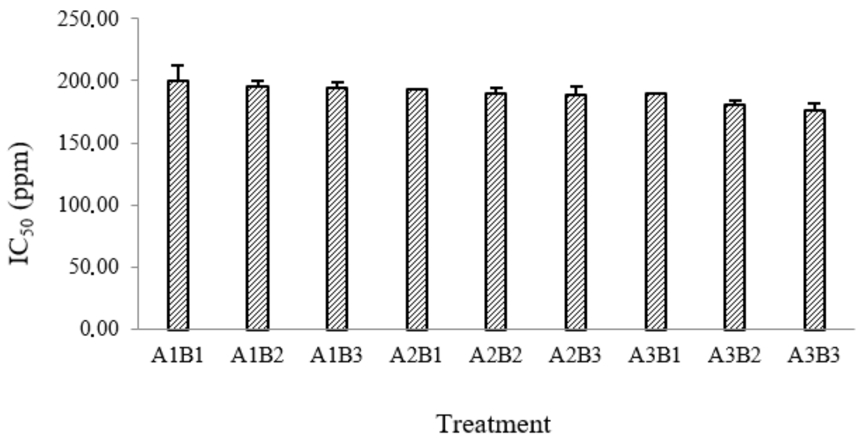

Figure 4 Average value of IC_{50} of functional edible film.

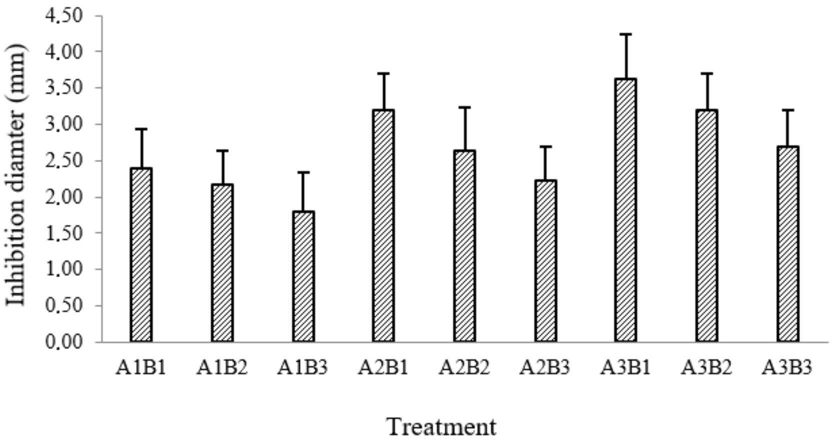

Figure 5 Average value of inhibition diameter of functional edible film functional.

Results of the HSD test at α = 5% (Table 1) showed that the higher the concentration of gambier filtrate, the higher is the water vapor transmission rate. This is due to the fact that gambier filtrate decreases inter-molecular and trans-molecular interactions of starch, increases free space amongst starch chains, and increases polymer mobility. In addition, the high value of water vapor transmission rate is due to a hydrophilic group within gambier filtrate that will decrease matrix density of edible film resulting in free space within film matrix which facilitates water vapor diffusion. **Santoso et al. (2018)** had described that gambier filtrate addition to the formulation of corn starch-based-edible film had increased water vapor transmission rate. This is related to catechin compound content within gambier filtrate. It is known that the catechin compound contains a hydroxyl group (OH) having the hydrophilic characteristic.

Antioxidant Activity

The average value of IC_{50} for the edible film was in the range of 176.11 to 200.34 ppm. A_1B_1 treatment had produced the highest IC_{50} and the lowest one was found on A_3B_3 treatment. These antioxidant activity values were classified as weak because the IC_{50} value was higher than 50 ppm. The IC_{50} values in this research were lower than the study results by **Santoso et al. (2018)** using canna starch-based-edible film with IC_{50} of 258.14 – 469.32 ppm. These research results were higher than the study results by **Yerramathi et al. (2021)** with IC_{50} of 50.42 – 77.41 ppm and **Jiang et al. (2021)** with IC_{50} of 87.41 ppm. The average value of IC_{50} for the edible film was presented in Figure 4.

Analysis of variance results showed that treatments of gambier filtrate and red palm oil had a significant effect on antioxidant activity of functional edible film, whereas interaction of both factors had no significant effect on antioxidant activity of functional edible film. Results of HSD test at α = 5% for gambier filtrate and red palm oil treatments were presented in Table 1 and Table 2, respectively.

Results of the HSD test (Table 1) showed that the higher the gambier filtrate concentration, the lower is the IC_{50} value.

It means that the antioxidant property of functional edible film will increase with the increase of gambier filtrate concentration. It is known that gambier filtrate contains epigallocatechin gallate compound and the addition of this compound in the sodium alginate and CMC-based edible films increases antioxidant activity **(Ruan et al., 2019)**. Table 2 (HSD test) showed that the higher the red palm oil concentration, the lower is the IC_{50} value, or the higher the red palm oil concentration, the higher is the antioxidant property of the edible film. According to **Goon et al. (2019)**, red palm oil contains such as tocotrienol, tocopherol, and carotenes.

Antibacterial Activity

Results of antibacterial activity on functional edible film produced a diameter of inhibition in the range of 2 to 3.6 mm. A_3B_1 treatment had the highest value of inhibition diameter with a magnitude of 3.6 mm and A_1B_3 treatment had the lowest value of inhibition diameter with a magnitude of 2 mm. The inhibition diameter value of functional edible film produced in this research was classified as a low antibacterial activity because it was less than 5 mm. The results of this study are lower than that reported by **Çakmak et al. (2020)** and **Sharma, Dhanjal, and Mittal (2017)** in a research on whey protein-based edible film which was incorporated with essential oil and cinnamon oil, namely 5.5 mm and 13.37 mm, respectively. Compared to the research of **Dinika et al (2020)** on starch-based edible film added with papaya leaf extract, it was also lower. The antibacterial activity of this edible film was similar to that produced by **Zerihun et al. (2016)** in a study of banana peel edible film incorporated with ginger. The average value of inhibition diameter for the functional edible film was presented in Figure 5.

Analysis of variance results showed that treatments of gambier filtrate and red palm oil had a significant effect on antibacterial of functional edible film, whereas interaction of both factors had no significant effect on antibacterial activity of functional edible film. Results of HSD test at α = 5% for gambier filtrate and red palm oil treatments toward inhibitory power diameter of the edible film were presented in Table 1 and Table 2, respectively.

Table 1 (HSD test) showed that the higher the gambier filtrate concentration, the higher is the inhibition diameter. It means that the antibacterial property of functional edible film will increase with the increase of gambier filtrate concentration. It is described previously that gambier filtrate contains catechin compound. According to **Ma et al. (2019)**, catechin compound not only has antioxidant property but also has aan ntibacterial property. Results of the HSD test (Table 2) showed that the higher the red palm oil concentration, the higher is the antibacterial property of the edible film. It is known that red palm oil has hydrophobic or non-polar properties so that bacteria are incapable to grow and develop because food-destroying bacteria usually have hydrophilic characteristics.

CONCLUSION

The addition of the gambier filtrate and red palm oil can form an edible film with antibacterial and antioxidant properties, the inhibition diameter value was 2,0 – 3.6 mm and the IC_{50} value was 176.11 – 200.34 ppm, respectively. They have a role in increasing the elongation percentage, thickness, and water vapor transmission rate of edible film, but the water vapor transmission rate of the edible film has not yet been capable to fulfill the JIS (1975) standard.

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