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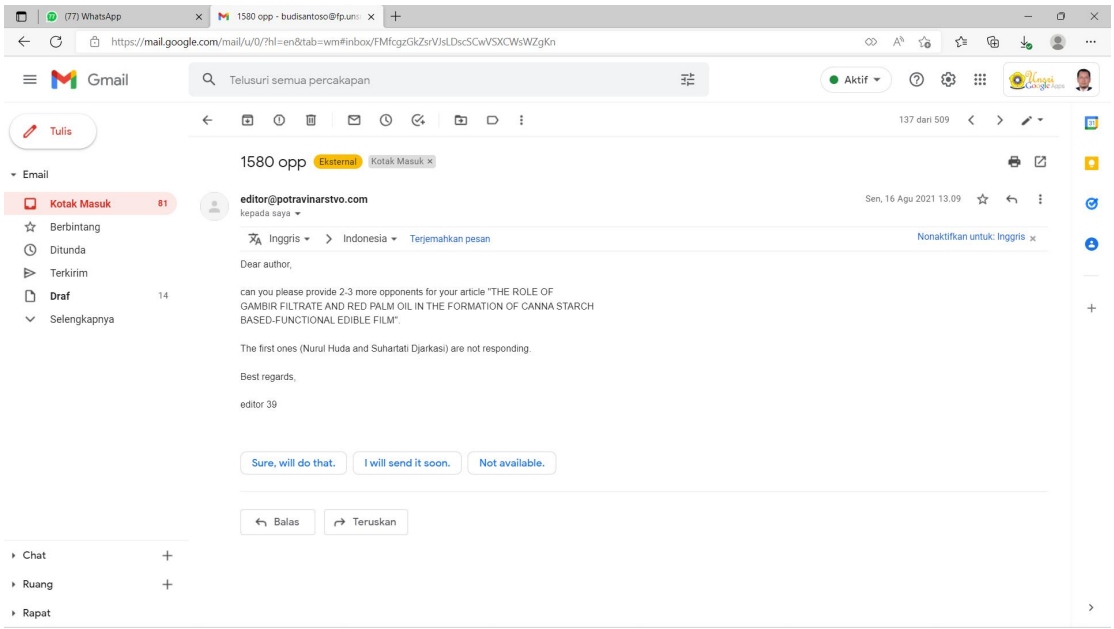

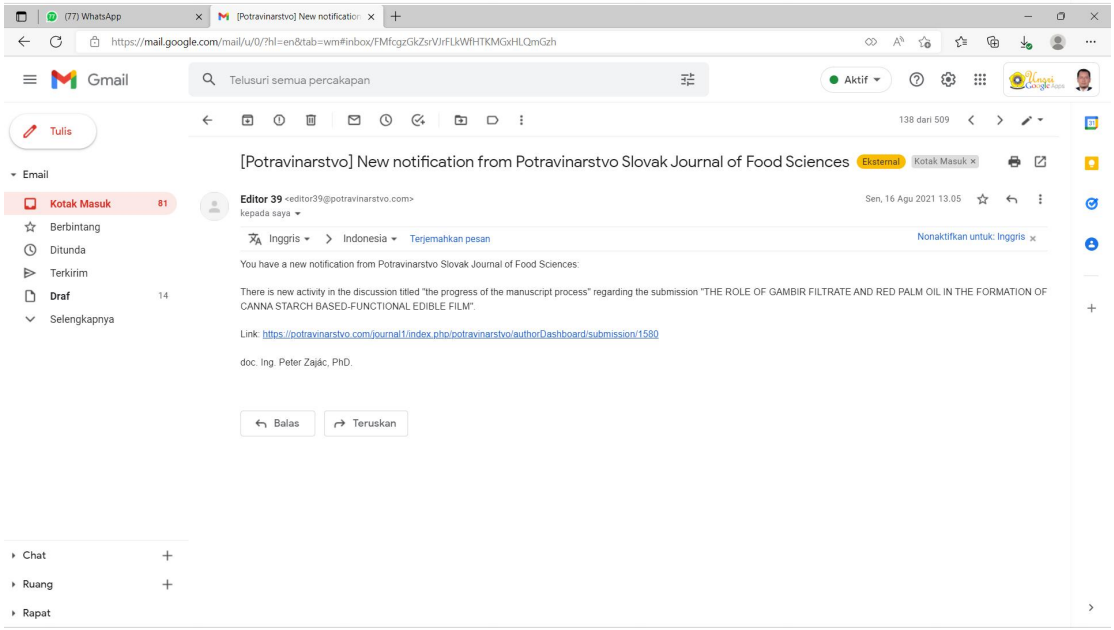

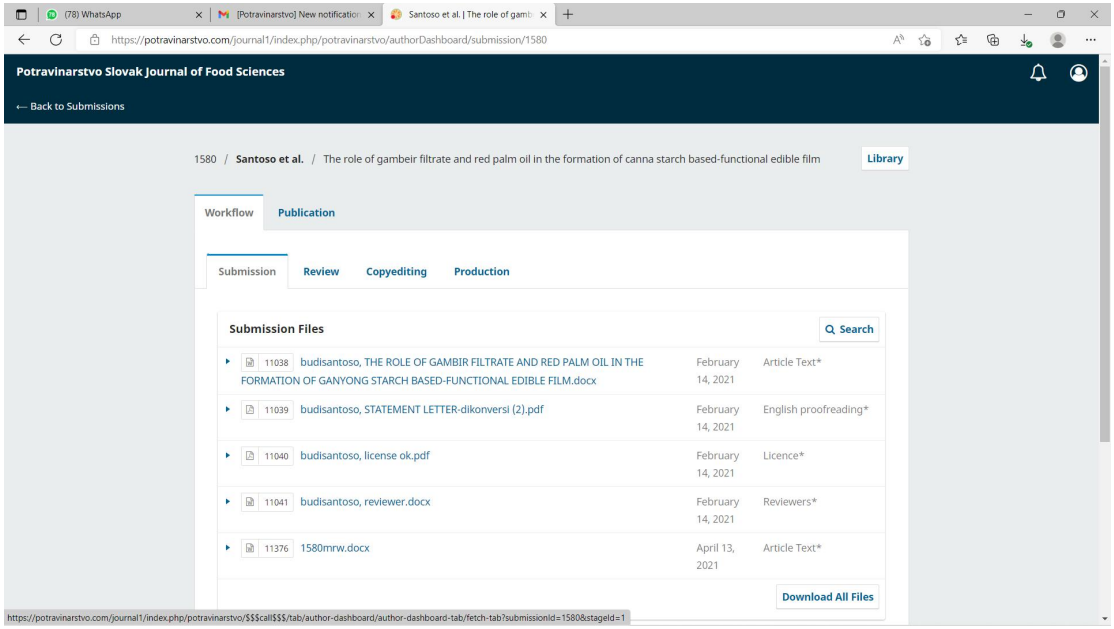

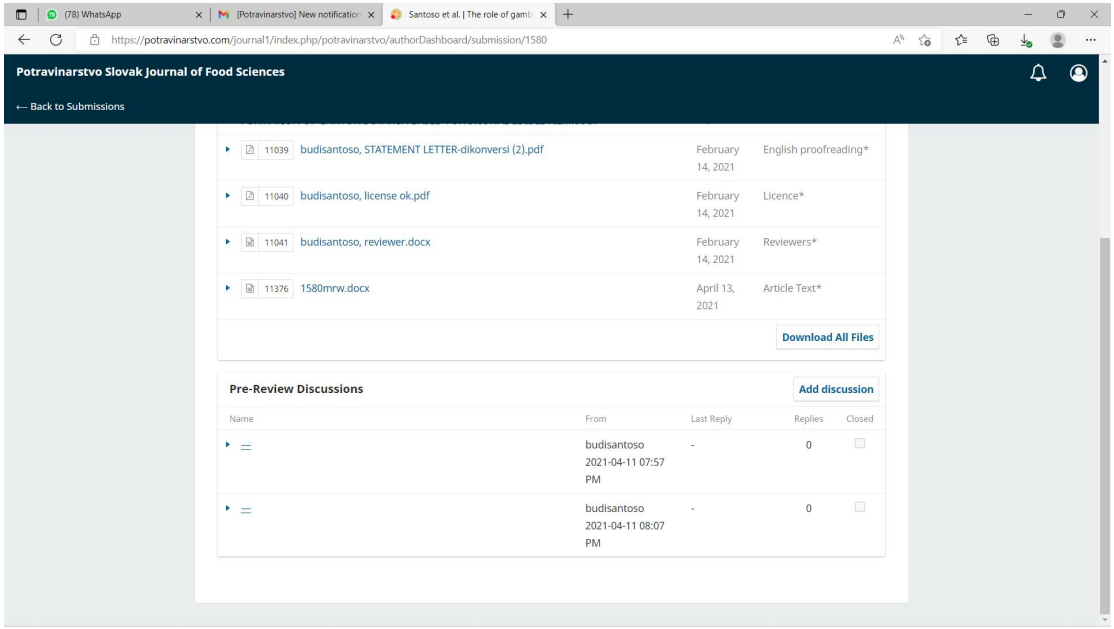

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THE ROLE OF GAMBIR FILTRATE AND RED PALM OIL IN THE FORMATION OF CANNA STARCH BASED-FUNCTIONAL EDIBLE FILM

Budi Santoso[1*], Doni Andrian Saragih[1], Gatot Priyanto[1] and Hermanto Hermanto[1]

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Major revision is required

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ABSTRACT

This study aims to analyze the role of gambier powder and red palm oil filtrate in the formation of functional edible film and to determine whether the gambier powder and red palm oil filtrate are synergistic or antagonistic in formation of functional edible films. The study design used 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 not capable to improve the antioxidant and antibacterial properties of edible films with a strong category. Gambier filtrate plays a role in increasing the elongation percentage, thickness, and water vapor transmission rate of edible film, but the transmission rate of edible film water vapor did not meet JIS 1975 standard.

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

INTRODUCTION

The oxidative reaction and pathogenic microbial contamination are the main factors for deterioration of food products (**Lee et al., 2004**). Food packaging is usually used in order to maintain food product quality and to increase storage life of food product. Most of 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 environmental 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, danger of physical damage, improvement of appearance, color, flavor and nutrients of products **(Fransen and Kroctha, 2003)**. Edible film function can be improved by incorporating antimicrobial and antioxidant materials to protect product from deterioration caused by microbial and oxidative deterioration which results in increasing storage life and product safety **(Rojas, et al., 2006 and Jung, Chung, and Lee, 2006).**

Gambier is an extraction product from gambier plant containing catechin compound with magnitude of 67.55 to 72.02 percent **(Pambayun, 2001). Lucida, Bakhtiar, and Putri (2007)** had described that the main component of catechin compound is polyphenol which have antibacterial and antioxidant characteristics. **Pambayun et al. (2007)** and **Hayani (2003)** showed that in addition to catechin compound, gambier extract has other important compounds such as catechin galat, galo catechin, galo catechin galat, epicatechin, epicatechin galat, epigalo catechin, epigalo catechin galat, catechin tanat and quercetine. According to **Lucida (2006)**, catechin compound has weak acid (pKa 1 = 7.72 and pKa 2 = 10.22), less soluble in water and very unstable on 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 higher reaction rate at low pH (3.45) than at high pH (4.9).

Red palm oil is palm oil obtained from CPO processing yield without going through blanching process with an objective to maintain its carotenoid content **(Ayustaningworo, 2012)**. **Bohn (2008)** had described that in addition to have function as free radicals catcher, some carotenoids also have vitamin A activity such as α-caroten, β-caroten, γ-caroten and β-cryptoxanthin. According to **Van Royan et al. (2008)**, red palm oil contains 500 ppm of carotenoid consisting of 37% of α-caroten, 47% of β-caroten, 11.5% of lycopen and 6.9% of cis- α-caroten.

Canna starch is an extraction product from tubers of canna plant (*Canna edulis* Kerr) as one of starch sources that has 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%. **(Santoso et al., 2007)**. **Santoso et al. (2016)** had showed that 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 11.77g.m^2.d^{-1}. **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 18.85g.m^2.d^{-1}, respectively.

Incorporation of bioactive materials that have antibacterial and antioxidan 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 magnitude of 6.67 to 7.67mm) and antioxidant (IC_{50} with magnitude of 258.14 to 469.32ppm). This finding showed that antioxidant characteristic was classified as very weak with IC_{50} 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 folows: 1) To analyze the role of gambier powder extract and red palm oil in formation of functional edible film and 2) To determine whether gambier powder extract and red palm oil have synergistic or antagonistic characteristic in 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 edible film with physical and chemical properties according to Japan International Standards in 1975.

MATERIAL AND METHODOLOGY

Sample

Edible film made from biopolymer materials such as cane 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) Gliserol, 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), haze meter seri NDH – 200, 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

Santoso et al., 2018; ASTM, 1997; AOAC, 2012, Orak, 2006, Laohakunjit and Noomhorm, 2004; Maesaroh et al., 2018; and Trisia et al., 2018

Description of the Experiment

Sample preperation

Processing of gambier powder filtrate

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 mortar and sieved by using 80 mesh siever. Subsequently, 20 grams of fine dry gambier is taken and put into 250 mL Erlenmeyer flask. The next step is addition of aquadest up to border mark of 100 mL and heated at temperature of 55 to 60°C for 10 minutes while stirring by using magnetic stirrer. Finally, the mixture was centrifuged at speed of 1000 rpm and filtrate is taken with magnitude of 1/3 part of total volume.

Processing of functional edible film

Processing of functional edible film was done according to the modified procedure by Santoso et al. (2018). Canna starch with magnitude of 12g is put into Beaker glass and added with aquadest up to border mark 300 mL followed by stirring with magnetic stirrer. Starch suspension is heated by using hot plate at temperature of 65°C while stirring until perfect gelatinization is produced. The next step is addition of aquadest with magnitude of 2% (v/v) from total volume. Subsequently, gambier powder 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 homogenous suspension is developed. Suspension is degassed by using vacuum pump for about one hour. Suspension with magnitude of 40 mL is poured and leveled on petri dish having 20 cm in diameter followed by drying within oven at temperature of 65°C for 24 hours. Edible film removed from petri dish and put into desiccator for one hour and finally 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 powder 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 repeated for each treatment factors. The total sample analyzed was 27 samples

Statistical Analysis

This research used factorial completely randomized design. Treatments which have significant effect were further tested by using Honestly Significant Different (HSD) test at α = 5%. Data was analyzed by using software program of SAS version Windows 9 in term of analysis of variance.

RESULTS AND DISCUSSION

Thickness

Average thickness of 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 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 0.133mm. The thickness of the edible film was higher than the edible film based on starch of durian and jackfruit seeds, which were 0.032-0.041mm and 0.035-0.043mm, respectively (Wahidin et al. 2021). Chitosan edible film incorporated with essential oil from *Chrysanthemum morifolium* and tumeric incorporated alginate edible film have a lower thickness than the resulting edible film, which were 0.05-0.15mm and 0.096mm, respectively (Tan et al. 2021 and 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 0.3mm (Handayani and Nurzanah, 2018).

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

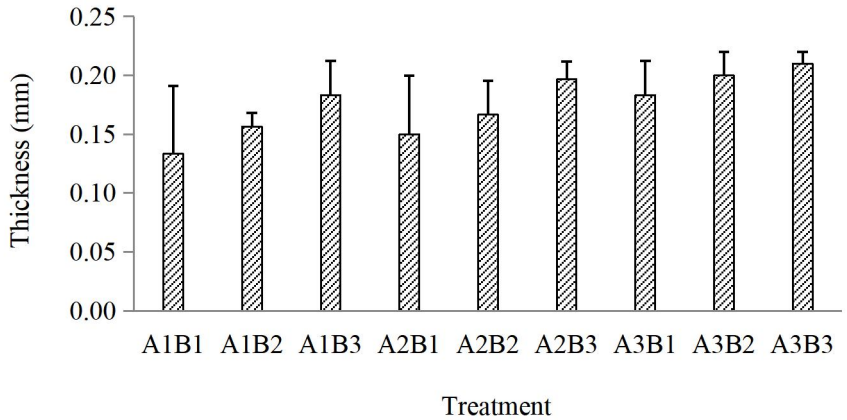

Figure 1. Average thickness value of functional edible film

HSD te: in edible film matrix, gambier filtrate concentration, the thicker is the functional edible film. This is due to the fact that gambier filtrate contains catechin compound which have crystalline form at dry condition. This crystalline form is a solid that affect edible film thickness, i.e. the 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 1. HSD test (α = 5%) related to concentration of gambier powder 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 ($g.m^2.d^{-2}$)	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

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

Table 2 (HSD test) showed that the higher the red palm oil concentration, the higher is the thickness of edible film. This is due to the fact that red palm oil contains 44 % 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 effect on the increase of total solid content within edible film matrix. Praseptingga 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.

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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

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

The role of gambier filtrate and red palm oil is significantly increase 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 edible film matrix. Catechin compound within gambier filtrate binds to glycerol molecules, starch and CMC resulting in low concentration of free catechin compound. In similar fashion, red palm oil which bind to CMC also decrease the numbers of free palmitic acid within edible film matrix. The existence of complex bonds which occured within edible film matrix cause the effect of catechin compound and red palm oil on edible film thickness cancel each other out.

Elongation Percentage

Elongation percentage value of 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. Hasil ini lebih tinggi dibanding edible film berbasis alginate yang diinkorporasi dengan hawthorn berry extrat, teff-starch based edible film, dan edible film yang terbuat dari pumpkin industry by product dengan persen pemanjangan berturut-turut sebesar 27.67-43.57%, 33,12%, dan 13.13-14.47% (Lim, Tan, and Pui, 2021; Prabhu et al., 2021; Lalnunthari, Devi, and Badwaik, 2020). Persen pemanjangan edible film ini serupa dengan Kim, Seo, and Kim (2018) dalam penelitian yuba films incorporated with various types of additives memilki nilai persen pemanjangan sebesar 132%. serta lebih rendah dibanding edible film berbasis alginate dengan persen pemanjangan sebesar 258.41% (Sancakli et al. 2021). Average value of elongation percentage for edible film was shown in Figure 2.

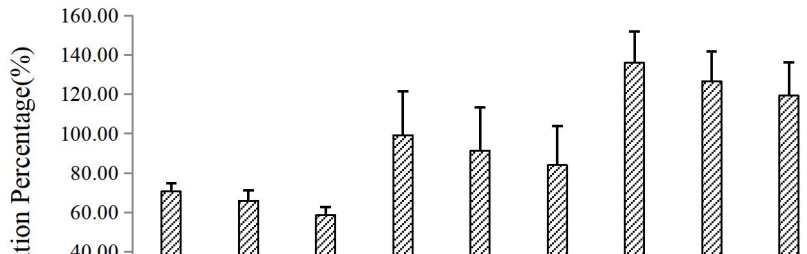

Figure 2. Average value of elongation percentage of functional edible film

Analysis of variance results showed that treatments interaction of red palm oil and gambier filtrate had no significant effect on elongation percentage of edible film, whereas gambier filtrate treatments had 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 catechin compound with hydroxyl group (OH) as its active group. The higher the gambier filtrate concentration, the higher is the numbers of OH group that capable to bind free water within matrix of edible film. According to **Santoso et al. (2017)**, the use of esktrat gambir in edible film formulation has function to increase elasticity of edible film because catechin compound has hydroxyl group that capable to bind free water within matrix of edible film.

Average value of elongation percentage for edible film had met the JIS-1975 standard with 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. Elongation percentage of ganyong 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 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 composition of amylose and amylopectin within corn starch. It is known that amylopectin molecules had effect on elasticity increment of edible film and the opposite happened to amylose molecules.

Water Vapor Transmission Rate

Water vapor transmission rate of edible film was in the range of 26.03 to 26.8 $g.m^2.d^{-1}$ and it was not fulfill the JIS-1975 standard (minimum of 10 $g.m^2.d^{-1}$). Edible film with the highest water vapor transmission rate was found on A_3B_1 treatment (26.03$g.m^2.d^{-1}$) and the lowest one was found on A_1B_3 treatment (26.80$g.m^2.d^{-1}$). Nilai laju transmisi uap edible film yang dihasilkan lebih tinggi dibanding edible film berbasis karagenin yang ditambahkan sorbitol sebesar 6.83 $g.m^2.d^{-1}$ (Rahmawati, Arief, and Satyantini, 2019), gelatin edible films incorporated with casein phosphopetides sebesar 4.5-7.9 $g.m^2.d^{-1}$ (Khedri et al., 2021), dan carboxymethyl chitosan-pullulan edible films enriched with galangal essential oil sebesar 0.185-0.290 $g.m^2.d^{-1}$ (Zhou et al., 2021). Hasil penelitian ini lebih rendah dibanding yang dilaporkan oleh Li et al. (2021) tentang laju transmisi uap air edible film berbasis carboxymethyl cellulose, dioscorea opposite, glycerol, and ZnO nanoparticle sebesar 31.10-38.02$g.m^2.d^{-1}$. Average value of water vapor transmission rate for edible film was presented in Figure 3.

Analysis of variance results showed that gambier filtrate treatment had 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 HSD test related to gambier filtrate treatment toward water vapor transmission rate were shown in Table 1.

Results of 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, high value of water vapor transmission rate is due to 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 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 catechin compound contains hydroxyl group (OH) having hydrophilic characteristic.

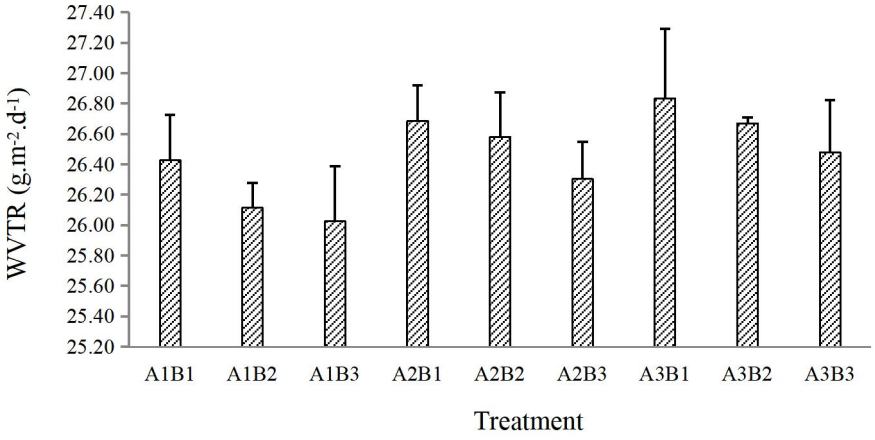

Figure 3. Average value of water vapor transmission rate of functional edible film

Antioxidant Activity

Average value of IC_{50} for *edible film* was in the range of 176.11 to 200.34ppm. 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 higher than the study results by Santoso *et al.* (2018) using canna starch based-edible film with IC_{50} of 258.14-469.32 ppm and lower than the study results by **Yerramathi et al. (2021)** with IC_{50} of 50.42- 77.41ppm. Average value of IC_{50} for *edible film* was presented in Figure 4.

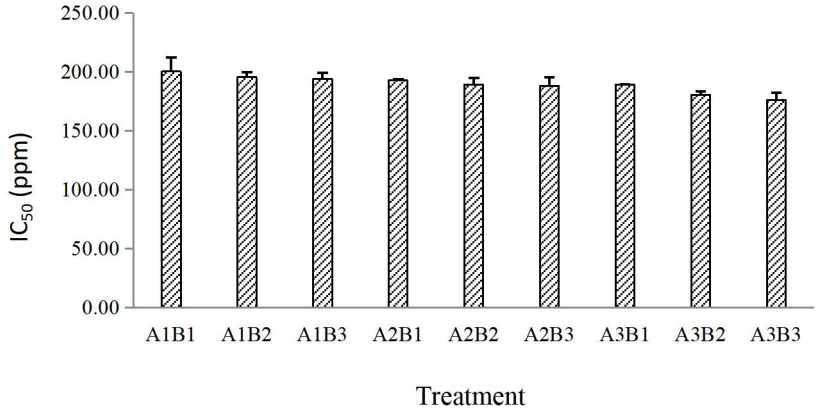

Figure 4. Average value of IC_{50} of functional edible film

Analysis of variance results showed that treatments of gambier filtrate and red palm oil had 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 HSD test (Table 1) showed that the higher the gambir filtrate concentration, the lower is the IC_{50} value. It means that antioxidant property of functional edible film will increase with the increase of gambir filtrate concentration. It is known that gambier filtrate contains epigallocatechin gallate compound and according to Ruan et al (2019) penambahan senyawa ini dalam edible film berbasis sodium alginate dan CMC meningkatkan aktivitas antioksidan. 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 edible film. According to **Manorama et al. (1996)**, red palm oil contains total carotene of 500 ppm and Edem (2002) had stated that red palm oil contains E vitamin of 559-1000 ppm consisting of 18-22 % tocopherol and 78-82 % tocotrienol, respectively.

Antibacterial Activity

Results of antibacterial activity on functional edible film produced diameter of inhibition in the range of 2 to 3.6 mm. A_3B_1 treatment had the highest value of inhibition diameter with magnitude of 3.6 mm and A_1B_3 treatment had the lowest value of inhibition diameter with magnitude of 2 mm. Inhibition diameter value of functional *edible film* produced in this research was classified as low antibacterial activity because it less than 5 mm. Cakmat et al. (2020) menjelaskan bahwa edible film berbasis whey protein yang diinkorporasikan minyak essensial oil memiliki aktivitas antibakteri lebih tinggi dibanding hasil penelitian sebesar 0.1-5.5mm.

Average value of inhibition diameter for functional edible film was presented in Figure 5.

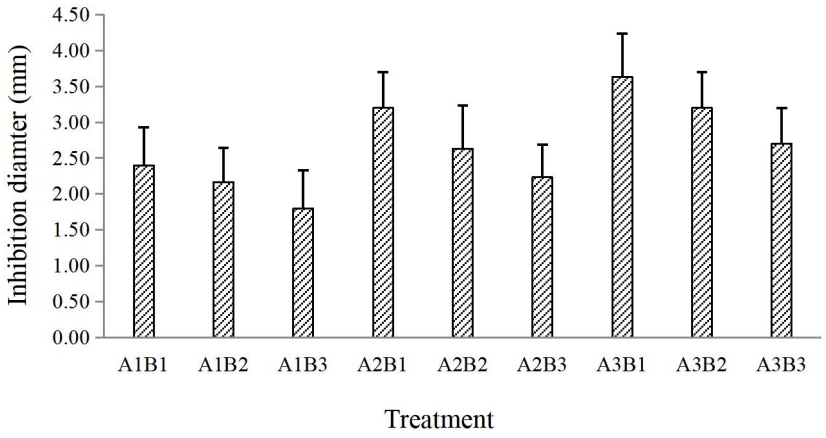

Figure 5. Average value of inhibition diameter of functional edible

Analysis of variance results showed that treatments of gambier filtrate and red palm oil had 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 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 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 **Pambayun et al. (2007)**, catechin compound not only has antioxidant property, but also has antibacterial property. Results of HSD test (Table 2) showed that the higher the red palm oil concentration, the higher is the antibacterial property of edible film. It is known that red palm oil has hydrophobic or non-polar property so that bacteria are incapable to grow and develop because food-destroying bacteria usually has hydrophilic characteristic.

CONCLUSION

The role of gambier filtrate and red palm oil was incapable to increase antioxidant and antibacterial properties of edible film with strong category. Gambier filtrate has a role in increasing elongation percentage, thickness and water vapor transmission rate of edible film, but water vapor transmission rate of edible film has not yet capable to fulfill the JIS 1975 standard.

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The authors declare no conflict of interest.

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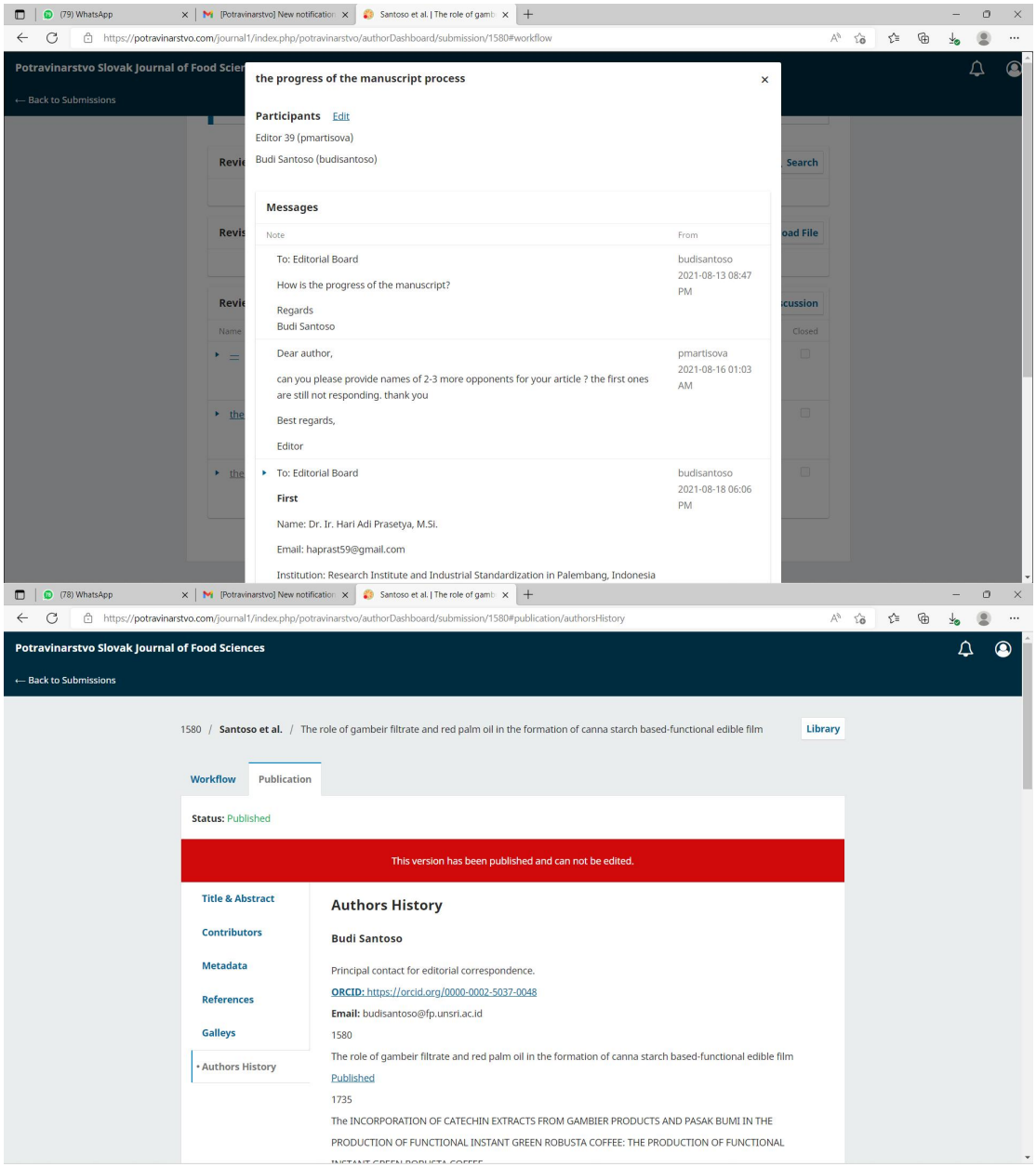

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