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The effect of incorporation of gambier filtrate and rosella flower petals extract on mechanical properties and antioxidant activity of canna starch based active edible film

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ABSTRACT

The research objective was to analyse the incorporation effect of gambier filtrate and rosella flower petals extract on mechanical properties and antioxidant activity of canna starch-based active edible film. This research used an experimental method consisting of two treatments, namely gambier filtrate (A): A1 = 3, A2 = 4, and A3 = 5 (% v/v), as well as rosella flower petals extract (B): B1 = 2, B2 = 4 and B3 = 6 (% v/v) and each treatment was replicated three times. The results showed that the two treatment interactions significantly influenced elongation percentage, water vapour transmission rate, and antioxidant activity. The edible film's thickness, tensile strength, and water vapour transmission rate were 0.096-0.124 mm, 1.89-3.38 MPa, and 12.99-17.04 $g.m^{-2}.d^{-1}$, respectively. The edible film contains an antioxidant compound of the strong category with IC50 values of 34.53 to 48.02 ppm. Treatment of A3B2 [gambier filtrate 5% (v/v) and rosella flower petals extract 4% (v)] was the best treatment. This edible film is generally suitable for application as a packaging material for food having high lipid content to inhibit the oxidation process of that food.

Keywords: antioxidant, edible film, gambier, thickness, rosella.

INTRODUCTION

Due to chemical, biochemical and microbiological reactions, food materials will be accelerated with the existence of oxygen gas, water vapour, sunlight, and temperature. Oxygen gas and sunlight are external factors that cause rancidity reactions in food having high lipid content. To avoid this reaction, packaging materials are required that inhibit oxygen gas and sunlight and inhibit rancidity reaction with the availability of antioxidant compounds in that packaging materials. The edible film is one of the food packaging materials that can be formulated by adding antioxidant compounds from synthesis and natural materials and has barrier properties against oxygen gas and sunlight.

The study results by **[12]** showed that polysaccharides edible film incorporated with essential oil and the herbal extract could improve the mechanical properties of edible film and increase the shelf life of meat and sensory quality to increase nutritional value through inhibition of oxidation reaction. **[8]** showed that potato starch edible film added with potato skins had an antioxidant property with antioxidant activity and phenolic compound content of 24-55% and 10-22 mg $GAE.g^{-1}$, respectively. Some authors **[3]** reported that adding turmeric extract on alginate edible film could produce an edible film with an antioxidant property with a DPPH value of 38.28ppm.

Gambier extract is produced from the gambier plant (*Uncaria gambir* Roxb) by processing the leaves and young twigs using hot water, pressing liquid precipitation and drying the sediment **[22]**. Gambier extract contains a catechins compound with 98% **[23]**. Moreover, **[22]** showed that the catechins compound in gambier extract had semi-polar properties and contained compounds with antioxidant and antibacterial properties. The extract was applied to inhibit the oxidation reaction in cassava chips **[7]**. Gambier extract was also used by **[26]** in canna starch-based edible film, but its antioxidant activity was still low. Besides gambier extract, rosella flower petals extract is produced from the flower petals of the rosella plant (*Hibiscus sabdariffa*), which are dried at 40 °C, and crushed in a blender, and extracted. Rosella flower petals extract also contains anthocyanin with a strong antioxidant property with IC50 values of 50 to 100 ppm **[5]**. Seaweed syrup added with rosella flower petals extract contains an anthocyanin compound of 0.625 g.100 mL^{-1} **[13]**.

Edible film development conducted by researchers currently continues to increase from year to year through the use of natural materials containing antioxidant and antibacterial properties such as curcumin **[24]**, black chokeberry extract **[15],** and some plants extract containing phenolic compounds **[31]**. But until now, no edible film is incorporated with two natural materials with antioxidant properties such as gambier filtrate and rosella flower petals extract. This research objective was to analyse the incorporation effect of gambier filtrate, and rosella flower petals extract on mechanical properties and antioxidant activity of canna starch-based active edible film.

Scientific Hypothesis

The addition of gambir catechin extract has a significant effect on increasing the functional properties of edible film.

MATERIAL AND METHODOLOGY

Samples

The edible film is made from biopolymer materials such as canna starch, glycerol, and CMC with incorporated gambier filtrate and rosella flower petals extract.

Chemicals

Olive oil from PT HNI Indonesia, carboxymethyl cellulose (CMC), 2,2–diphenyl-1-picrylhydrazyl (DPPH), and nutrient agar (NA) obtained from the Laboratory of Chemical Agricultural Products, Faculty of Agriculture, Sriwijaya University, Indonesia.

Biological Material

Gambier (*Uncaria gambir* Roxb) extract from Babat Toman Village, Banyuasin District, South Sumatra, Indonesia. Rosella (*Hibiscus sabdariffa*) flower petals from PT HNI Indonesia. Canna (*Canna edulis* Ker) starch from Industri Lingkar Organik Sleman, Yogyakarta, Indonesia.

Instruments

Drying oven, magnetic stirrer, incubator, vacuum pump (model; DOA-P504-BN), spectrophotometer, haze meter (serie NDH – 200, Nipon Denshoku Kogyo Co., Ltd.), micrometre (Roch, A281500504, Sisaku SHO Ltd, Japan), testing machine MPY(type: PA-104-30. Ltd. Tokyo, Japan), water vapour transmission rate tester of Bergerlahr cup method, hot plate (Torrey Pines Scientific brand) and analytical balance (Ohaus Corp. Pine Brook, N. J. USA).

Laboratory Methods

The edible film-making process was done according to the modified procedure by **[26]**. Parameters of thickness, percent elongation, tensile strength, and water vapour transmission rate of the edible film were measured referring to **[1]** by using the tool haze meter (serie NDH – 200, Nipon Denshoku Kogyo Co., Ltd.), micrometre (Roch, A281500504, Sisaku SHO Ltd, Japan), testing machine MPY(type: PA-104-30. Ltd. Tokyo, Japan), water vapour transmission rate tester of Bergerlahr cup method, hot plate (Torrey Pines Scientific brand), respectively, while for the antioxidant activity parameters measured using the 2,2–diphenyl-1-picrylhydrazyl (DPPH) method **[20]**.

Description of the Experiment

Sample preparation:

Gambier filtrate production

Gambier extract is crushed until fine using mortar and subsequently is sieved using an 80 mesh siever. Weighing of fine gambier extract 40 (% w/v) and then put it into a volumetric glass and added with aquadest until 100 mL boundary mark. The suspension was stirred using a magnetic stirrer for 10 minutes, filtered and using Whatman No. 1 filter paper, and centrifuged at 1000 rpm, followed by the filtrate.

Edible film production

Canna starch as much as 4 g is put into Beaker glass of 250 mL in size, and aquadest water is added up to the mark of 100 mL. Starch suspension is stirred by using a magnetic stirrer while being heated by using a hotplate at a temperature of 65 °C until perfect gelatinisation is obtained. Gelatinised starch suspension is added with 1% glycerol (v/v), in which the stirring process and heating are maintained. Suspension is added with gambier filtrate according to treatments 3, 4, and 5% (v/v) until homogenous mixture and then added with rosella flower petals extract according to treatments 2, 4, and 6% (v/v). After homogenizing edible film suspension, CMC as much as 1% (w/v) is added gradually while maintaining temperature and stirring. Subsequently, olive oil as much as 1% (v/v) is added while stirring. Edible film suspension is vacuum treated using a vacuum pump for 1 hour. Edible film suspension of as much as 40 mL is poured into a petri dish with a diameter of 15 cm and then dried within a drying oven at 60 °C for 24 hours. The edible film is released from the petri dish and put into a desiccator for 1 hour. Finally, the edible film is ready to be analysed.

Number of samples analyzed: The number of analysed was 9.

Number of repeated analyses: Three repeated analysed were performed for each treatment factor. The total sample analysed was 27 samples.

Number of experiment replication: The number of experiment replication as many as 9 samples.

Design of the experiment: Treatment factors consisted of gambier filtrate (A): A1 = 3; A2 = 4 and A3 = 5 (% v/v) and rosella flower petals extract (B): B1 = 2, B2 = 4 and B3 = 6 (% v/v).

Statistical Analysis

This research used a factorial, completely randomised design. Treatments with significant effects were further tested using the HSD test at α = 5%. Research results were analysed using the analysis of variance (ANOVA) method with the aid of the SAS program-Windows version 9.

RESULTS AND DISCUSSION

The edible film had thickness in the range of 0.096 to 0.124 mm, and these values fulfilled the Japan Industrial Standard (JIS, 1975) of 0.25 mm maximum. The highest thickness of this edible film is similar to the thickness of tapioca starch-based edible film, which is incorporated with kelakai leaves extract with a magnitude of 0.124 mm **[21]**and the lowest thickness is similar to the thickness of edible alginate film, which is incorporated with curcumin extract with a magnitude of 0.096 mm **[3]**. This result is lower than the thickness of the edible film obtained from the study, with an average value of 0.26 mm **[27]**. This is also higher than the thickness of edible film made from catfish surimi with a size of 0.049 mm **[30]**.

Treatment of gambier filtrate at 5% (v/v) concentration combined with rosella flower petals extract at 2% (v/v) concentration (A_3B_1) produced the highest thickness. In contrast, the lowest thickness was found in treatment of gambier powder filtrate at 3% (v/v) concentration combined with rosella flower petals extract at 6% (v/v) concentration (A_1B_3). The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film was presented in Figure 1.

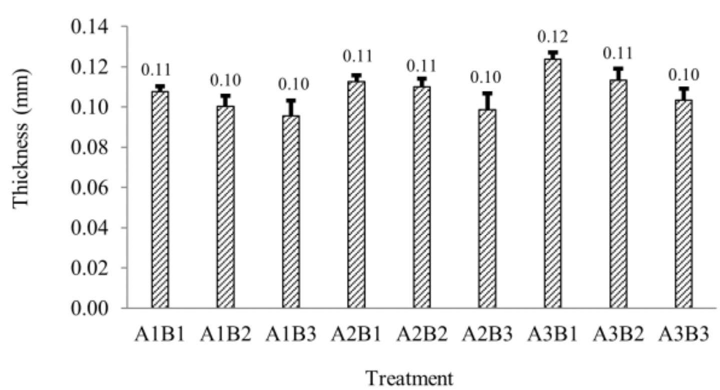

Figure 1 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film.

Treatment of gambier filtrate and rosella flower petals extract significantly affected edible film thickness, but their interactions had no significant effect. Results of honestly significant different (HSD) test for the impact of gambier filtrate concentration on active edible film thickness were shown in Table 1.

Active edible film thickness increases according to the increase of gambier filtrate concentration. It is known that gambier filtrate contains a catechin compound with semi-polar characteristics, which includes solids that are insoluble in water—the amount of these solids affected the increase of active edible film thickness. The results of this study are the same as those produced by **[28],** which explains that the edible film thickness of sugar palm fruit had increased according to the rise of plasticiser concentration in which plasticiser is polymers that make up the edible film matrix that affects on the increase of total soluble solids within edible film suspension.

Table 1 Results of HSD test for the effect of gambier powder filtrate concentration on active edible film thickness, elongation percentage, tensile strength, water vapor transmission rate, and antioxidant activity.

Treatment	Thickness (mm)	Elongation percentage (%)	Tensile strength (MPa)	Water vapor transmission rate (g.m^{-2}.day^{-1})	Antioxidant activity (IC_{50}) ppm
A_1 (3% v/v)	0.101 ±0.006a	17.94 ±2.94a	3.27 ±0.19a	16.50 ±0.22a	45.99±2.06a
A_2 (4% v/v)	0.107 ±0.007b	22.85 ±2.58b	2.44 ±0.13b	15.32 ±2.19ab	39.51±1.33b
A_3 (5% v/v)	0.113 ±0.010c	32.00 ±6.86c	1.96 ±0.06c	14.27 ±2.08b	35.61 ±1.29c

Note: Numbers followed by the same letter at the same column are not significantly different (p >0.05).

The HSD test in Table 2 showed that concentration increase of rosella flower petals extracts had decreased active edible film thickness. This is because rosella is hydrophilic or polar which affects the decrease in the thickness of the edible film. This statement is supported by **[2]** which states that rosella flower petals extract contains anthocyanin compounds, which are polar molecules.

Table 2 Results of HSD test for the effect of rosella flower petals extract concentration on active edible film thickness, elongation percentage, water vapor transmission rate, and antioxidant activity.

Treatment	Thickness (mm)	Elongation percentage (%)	Water vapor transmission rate (g.m^{-2}.day^{-1})	Antioxidant activity (IC_{50}) ppm
B_1 (2% (v/v)	0.115 ±0.008a	19.90 ±5.01a	14.25 ±2.17a	41.97 ±5.58a
B_2 (4% (v/v)	0.108 ±0.006b	25.88 ±5.99b	15.49 ±2.19ab	40.27 ±5.43b
B_3 (6% (v/v)	0.099 ±0.004c	27.01 ±10.51c	16.35 ±0.31b	38.88 ±4.73c

Note: Numbers followed by the same letter at the same column are not significantly different (p >0.05).

Elongation percentage

The produced elongation percentage of active edible film was in the range of 14.90 to 38.62%. This elongation percentage was lower than the JIS standard (1975) which sets out of minimum 70%. Still, it was higher compared to millet starch edible film added with clove essential oil with a magnitude of 5.67% **[11]** and edible films based on the pumpkin with a magnitude of 13.13 – 14.47% **[16]** as well lower than a composite edible film of palm starch and chitosan which is incorporated with olive oil with a magnitude of 224.6% **[10]** and edible films based on alginate namely 27.67 – 43.57% **[18]**. The highest and the lowest elongation percentages of the active edible film were found on A_3B_3 and A_1B_1 treatments, respectively. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film was shown in Figure 2.

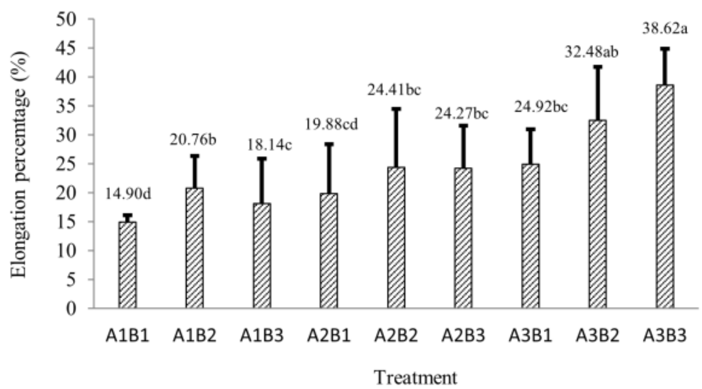

Figure 2 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film.

Edible film elongation percentage was significantly affected by treatments of gambier filtrate, rosella flower petal extract and their interaction. HSD test at a 5% level (Table 1) showed that the higher the gambier filtrate concentration, the higher the edible film elongation percentage. It is previously mentioned that the catechin compound has semi-polar characteristics and part of the catechin with polar characteristics affects the addition of hydrophilic compound in the edible film suspension. which causes the increase in edible film elongation percentage increased. Active edible film elongation percentage increased according to the rise of rosella flower petal extract concentration (Table 2). This was also influenced by the addition of hydrophilic compound as in gambier filtrate because rosella flower petal extract contains water-soluble anthocyanin compound. **[11]** showed that the elongation percentage of millet edible film had decreased with the increase of clove essential oil concentration. It is known that clove essential oil has hydrophobic characteristics and this can be interpreted that the hydrophobic component decreases edible film elongation percentage. In contrast, the hydrophilic component increases the edible film elongation percentage.

This edible film is formed by several materials consisting of canna starch, glycerol, gambier filtrate, rosella flower petals extract, CMC and olive oil. Edible film matrix is formed by complex bonds amongst these constituent materials. This complex bond consist of canna starch-glycerol-gambier filtrate-rosella flower petals extract-CMC-olive oil. Constituent materials of this edible film are divided into three hydrophilic components: canna starch, glycerol, gambier filtrate and rosella flower petals extract; CMC as an emulsifier as olive oil as a hydrophobic component. The hydrophilic component was more dominant in forming of edible film matrix than other components. This cause interaction treatment of A_3B_3 had produced the highest elongation percentage.

Tensile strength

The produced tensile strength of the active edible film was in the range of 1.89 to 3.38 MPa. A1B1 treatment (gambier filtrate of 3% v/v and rosella flower petals extract of 2% v/v) produced the active edible film with the highest tensile strength. In contrast, the lowest was found in A_3B_3 treatment (gambier filtrate of 5% v/v and rosella flower petals extract of 6% v/v). The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film was shown in Figure 3.

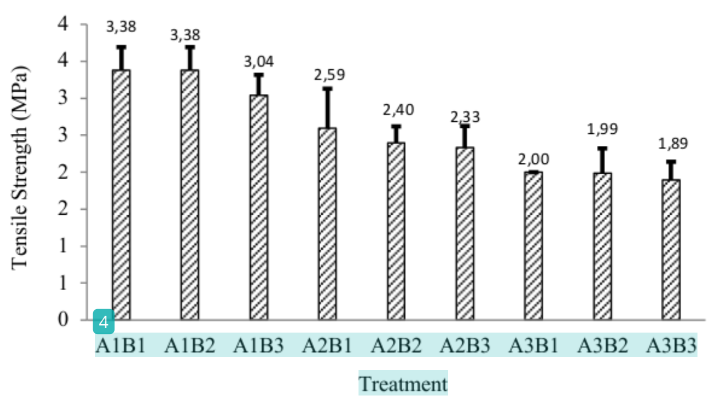

Figure 3 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film.

Analysis of variance results showed that gambier filtrate treatment had a significant effect on the tensile strength of the active edible film. In contrast, the treatment of rosella flower petals extract and both treatment's interactions had no significant effect on the tensile strength of the active edible film. HSD test at the 5% level in Table 1 showed that the higher the gambier filtrate concentration, the lower the tensile strength of the active edible film. This is related to the catechin compound that has semi-polar characteristics as mentioned previously. The tensile strength of the edible film is influenced by its constituent components in which components having hydrophilic characteristics such as sorbitol will decrease the tensile strength of the edible film. In contrast, a hydrophobic component or non-polar component will increase the tensile strength of the edible film. In addition, the tensile strength of the edible film is inversely proportional to the elongation percentage, namely, the higher the tensile strength, the lower the elongation percentage (Table 1). This is the general theory that applies to edible film as stated by **[29]** that increasing the elongation percentage of the edible film will cause lower tensile strength of the edible film.

The tensile strength of edible film according to the standard of JIS 1975 (Japanese Industrial Standart) is a minimum of 0.39226 MPa. The tensile strength of the produced edible film from several treatments combination was in the range of 1.89 to 3.38 MPa and all the produced edible films fulfilled the JIS standard. These tensile strength values are higher compared to the tensile strength of edible film from sweet potato starch as reported by **[6]** with a magnitude of 0.75 MPa. They are lower compared to the tensile strength of edible film from breadfruit starch, as reported by **[34]**, with a magnitude of 93.43 MPa.

Water vapour transmission rate

The water vapour transmission rate of the produced active edible film was in the range of 12.85 to 17.04 $g.m^{-2}.d^{-1}$ and higher than the JIS 1975 standard with a maximum value of 10 $g.m^{-2}.d^{-1}$. The water vapour transmission rate of this active edible film was higher (12.99 to 17.04 $g.m^{-2}.d^{-1}$) than alginate edible film added with turmeric extract (1.37 $g.m^{-2}.d^{-1}$) as reported by **[3]**. It was lower than canna-based edible film added with gambier extract (20.23 $g.m^{-2}.d^{-1}$) as written by **[26]**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapour transmission rate of the active edible film was shown in Figure 4.

Analysis of variance results showed that treatments of gambier filtrate and rosella flower petals extract and their interaction had a significant effect on the water vapour transmission rate of active edible film. Further test in Table 1 showed that the water vapour transmission rate of the active edible film had decreased with gambier filtrate concentration. This is influenced by semipolar characteristics of catechin compounds within gambier filtrate. The addition of essential oil from lemon and bergamot to protein isolate edible film could decrease water vapour transmission rate **[4]**. In addition, the water vapour transmission rate of edible film decrease with the increase of edible film thickness (Table 1). This is because the thicker the edible film, the more difficult for water vapour to penetrate the edible film.

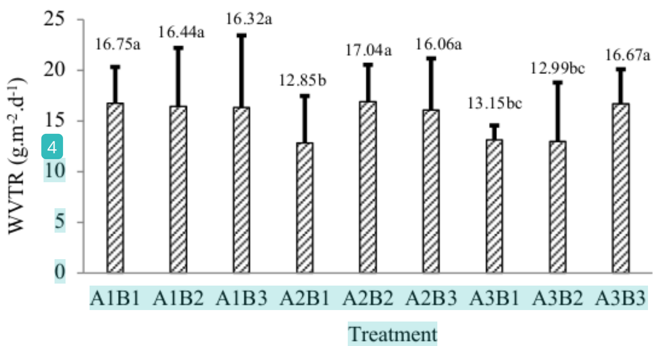

Figure 4 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapor transmission rate of the active edible film.

The HSD test at the 5% level in Table 2 showed the opposite results with rosella flower petals extract. The higher the concentration of rosella flower petals extracts, the higher the water vapour transmission rate of active edible film. It is previously mentioned that rosella flower petals extract has hydrophilic characteristics, which make it easier for water vapour to penetrate the edible film. **[17]** reported that adding a hydrophobic component in form of sunflower oil to green bean starch edible film could decrease water vapour transmission rate. The opposite is true for the addition of a hydrophilic component.

Figure 4 shows that treatments A2B1, A3B1, and A3B2 had lower water vapour transmission rates than other treatments. This is due to the gambier filtrate's influence containing semipolar catechin compounds which the rosella flower petal extract is polar. Thus, the combination of higher gambier filtrate than rosella flower petal extract, the lower the water vapour transmission rate of the edible film produced.

Antioxidant activity

The produced active edible film had an antioxidant activity with IC_{50} values in 34.53 to 48.02 ppm. The higher the IC_{50} value, the lower the antioxidant properties, and *vice versa*. The most increased antioxidant activity was found in the A_3B_3 treatment, whereas the lowest was found on the A1B1 treatment. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity (IC_{50}) of the active edible film was shown in Figure 5.

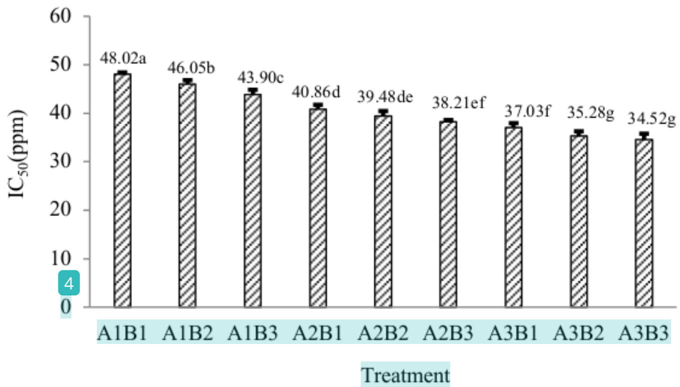

Figure 5 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity (IC_{50}) of the active edible film.

The IC_{50} value of this edible film was similar to edible film incorporated with turmeric extract with an IC_{50} value of 38.28 ppm as reported by **[3]**. **[19]** had said that polyvinyl alcohol edible film added with curcumin had an antioxidant activity of 35.16 ppm and **[15]** had reported that edible alginate film incorporated with black chokeberry extract had the antioxidant activity of 32.96 ppm. However, the IC_{50} value of this edible film was higher than potato starch edible film included with *Salvia officinalis* essential oil with a magnitude of 68.35 ppm as reported by **[28]** **[33]** with IC_{50} of 50.42-77.41 ppm and **[14]** with IC_{50} of 87.41 ppm.

The IC_{50} value of the active edible film is significantly influenced by treatments of gambier filtrate and rosella flower petals extract and their interaction. The increase of gambier filtrate concentration results in the increase of the antioxidant activity of active edible film, as presented in Table 1. The IC_{50} value had decreased with the rise of gambier filtrate concentration. The increase in antioxidant activity is due to catechin compound content within gambier filtrate. **[25]** had described that gambier extract has potential as a drug that contains antioxidant, anthelmintic, antibacterial and antidiabetic. Results of the HSD test at a 5% level (Table 2) showed that the increase of rosella flower petals extracts results in the growth of antioxidant activity of active edible film as indicated by the decrease of IC_{50} value. This is due to the anthocyanin compound available in rosella flower petals extract. **[5]** reported that rosella flower petals contain an anthocyanin compound with antioxidant characteristics with IC_{50} values in the range of 50 to 100 ppm.

CONCLUSION

The mechanical properties of the active edible film fulfilled JIS 1975 standard, especially in terms of thickness, tensile strength, and water vapour transmission rate. However, the elongation percentage has not met the standard. The active edible film has antioxidant characteristics of the strong category with IC50 values in the range of 34.53 to 48.02 ppm. In general, this edible film is feasible to be applied as packaging material for high lipid foods to inhibit the oxidation process in those foods.

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