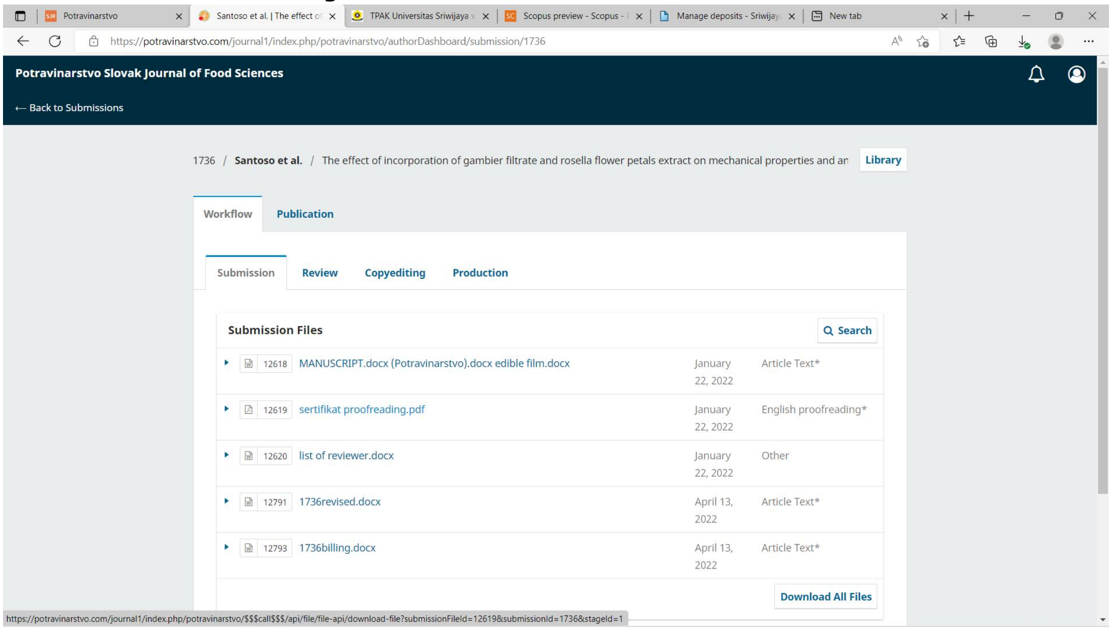
KORESPONDENSI : **The effect of incorporation of gambier filtrate and rosella flower petals extract on mechanical properties and antioxidant activity of canna starch based active edible film**



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# 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 were replicated three times. The results showed that the two treatment interaction significantly influenced elongation percentage, water vapor transmission rate, and antioxidant activity. The value of edible film's thickness, tensile strength, and water vapour transmission rate of the edible film were 0.096-0.124mm, 1.89-3.38MPa, and 12.99-17.04g.m-2.d-1, respectively. The edible film contains an antioxidant compound of the strong category with IC50 values in 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 of food having high lipid content to inhibit the oxidation process of that food.

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

## INTRODUCTION
Food material deteriotation due to chemical, biochemical and microbiological reactions, food materials will be accelerated with the existence of oxygen gas, water vapor, sunlight, and temperature. Oxygen gas and sunlight are external factors that cause rancidity reaction on food having high lipid content. To avoid this reaction, packaging materials are required that are capable of inhibiting 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 property against oxygen gas and sunlight.

The study results by **[12]** showed that polysaccharides edible film incorporated with essential oil and the herbal extract was capable of improving the mechanical properties of edible film and increasing 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 10-22mg GAE/g, respectively. **[3]** **had** reported that the addition of turmeric extract on alginate edible film could produce an edible film having 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 precipitation of liquid, and drying the sediment **[22]**. Gambier extract contains a catechins compound with a magnitude of 98% **[23]**. Moreover, **[22]** showed that the catechins compound in gambier extract had semi-polar property and contained compounds with antioxidant and antibacterial properties. The extract was applied to inhibit the oxidation reaction that occurred 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, crushed in a blender, and extracted. Rosella flower petals extract is also contains anthocyanin having a strong antioxidant property with $IC_{50}$ values in the range of 50 to 100ppm **[5]**. Seaweed syrup added with rosella flower petals extract contains an anthocyanin compound of 0.625 g/100mL **[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 compound **[31]**. But until now, there is no edible film 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

Edible film making process was done according to the modified procedure by **[26]**. Parameters of thickness, percent elongation, tensile strength, and water vapor 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:**

**Instant green coffee**

Green coffee beans were dried to a moisture content of 12% and ground using a grinder. The powder was filtered using an 80-mesh sieve, after which water was added at a temperature of 100 °C and a ratio of 1:2, stirred, left for 10 minutes, and later filtered using a filter cloth to obtain the filtrate. Moreover, maltodextrin (10% w/w) and egg white (20% w/w) were added to the filtrate, mixed using a mixer for 10 minutes at high speed to form foam, and spread out on an aluminium pan lined with Polypropylene plastic. The mixture was dried in a carbine dryer at 60 ℃ for 4 hours, blended, and filtered using an 80-mesh filter to obtain a green coffee powder.

**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 100mL boundary mark. The suspension was stirred using a magnetic stirrer for 10 minutes and filtered using Whatman No. 1 filter paper, and centrifuged at 1000rpm, followed by taking the filtrate.

**Edible film production**

Canna starch weighing 4 g is put into Beaker glass of 250mL in size, and aquadest water is added up to the mark of 100mL. 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 mixtured 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 by by using a vacuum pump for 1 hour. Edible film suspension as much as 40 mL is poured into a petri dish with having diameter of 15 cm and then dried within a drying oven at 60 ºC for 24 hours. The edible film is released from the petri dish and then put into a desiccator for 1 hour. Finally, the edible film is ready to be analysed.

**Number of samples analyzed:** The number of analysed asa many as samples was 9.

**Number of repeated analyses:** Three repeated analysis 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 that have significant effects were further tested by using an honestly significant different (HSD) test at α = 5%. Data of research results was analysed using analysis of variance (ANOVA) method with the aid of SAS program-Windows version 9.

## RESULTS AND DISCUSSION

The edible film had thickness in the range of 0.096 to 0.124mm and these values had fulfilled the Japan Industrial Standard (JIS, 1975) of 0.25mm in maximum. The highest thickness of this edible film is similar to thickness of tapioca starch-based edible film, which is incorporated with kelakai leaves extract with a magnitude of 0.124mm **[21]**and the lowest thickness is similar to thickness of alginate edible film which is incorporated with curcumin extract with a magnitude of 0.096mm **[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 0.049mm **[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 on 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.

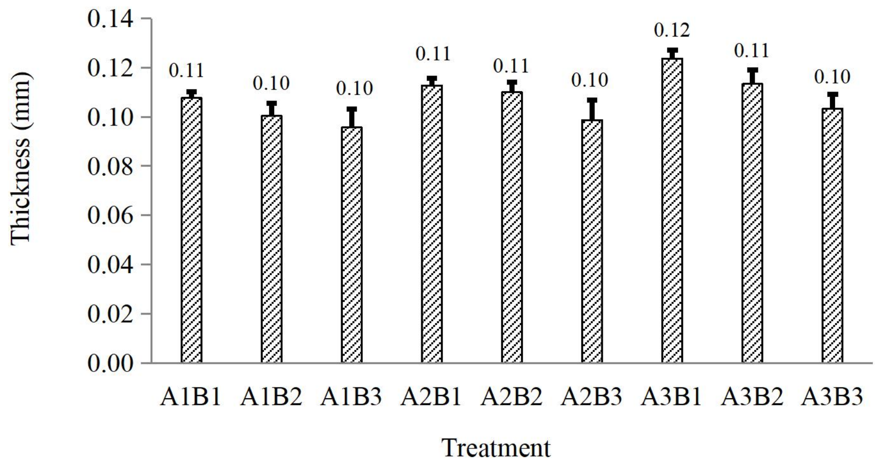

**Figure 1** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film.

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

**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).

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

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.

**Tabel 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 |

**Remarks:** 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) that 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 pumpkin with 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.

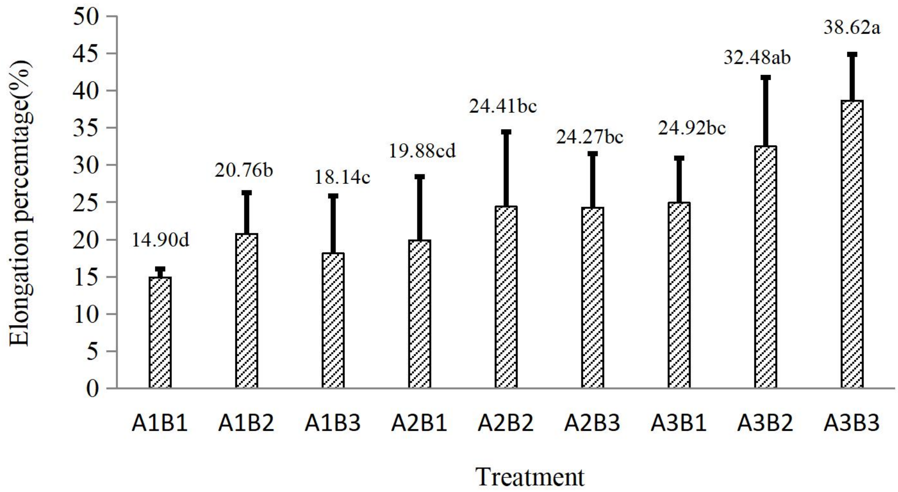


**Figure 2**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film.

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 characteristic affects the addition of hydrophilic compound in the edible film suspension. which causes the increase of 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, hydrophilic component increases 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 emulsifier as olive oil as 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 3.38MPa. $A_1B_1$ treatment (gambier filtrate of 3%v/v and rosella flower petals extract of 2% v/v) had produced the active edible film with the highest tensile strength. In contrast, the lowest was found on $A_3B_3$ 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.

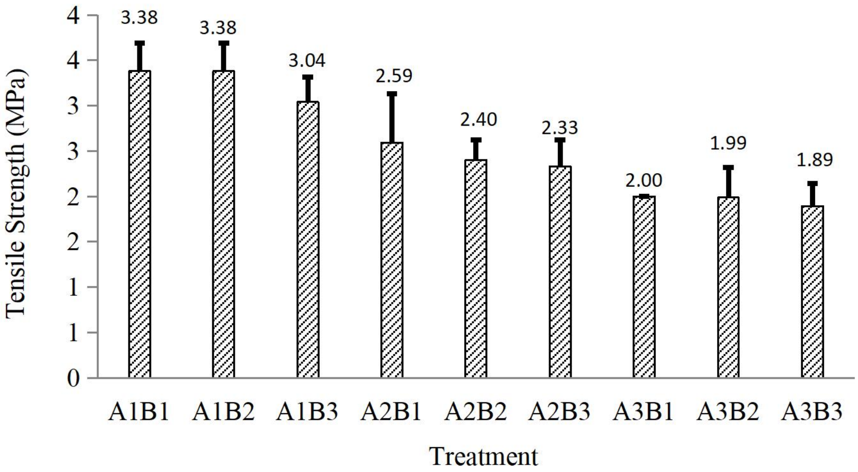


**Figure 3** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film.

Analysis of variance results showed that gambier filtrate treatment had a significant effect on tensile strength of the active edible film. In contrast, treatment of rosella flower petals extract and both treatments interaction had no significant effect on 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, 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 elongation percentage, namely, the higher the tensile strength, the lower the elongation percentage (Table 1). This is by the general theory that applies to edible film as stated by **[29]** that increasing 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 minimum of 0.39226MPa. Tensile strength of the produced edible film from several treatments combination was in the range of 1.89 to 3.38MPa and all the produced edible films had fulfilled the JIS standard. These tensile strength values are higher compared to tensile strength of edible film from sweet potato starch as reported by **[6]** with magnitude of 0.75MPa. They are lower compared to the tensile strength of edible film from breadfruit starch, as reported by **[34]**, with magnitude of 93.43MPa.

**Water vapor transmission rate**

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

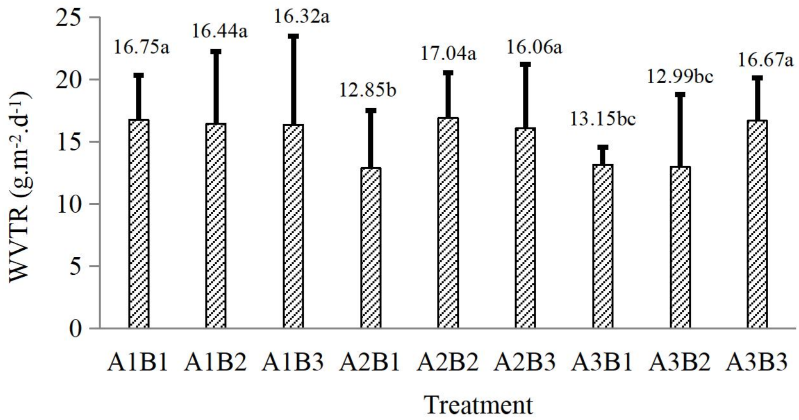


**Figure 4** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapor transmission rate of the active edible film.

The HSD test at 5% level in Table 2 showed the opposite results with rosella flower petals extract. The higher the concentration of rosella flower petals extract, the higher the water vapor 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 edible film. **[17]** reported that adding a hydrophobic component in form of sun flower oil to green bean starch edible film could decrease water vapor transmission rate. The opposite is true for the addition of a hydrophilic component.

Figure 4 shows that treatments $A_2B_1$, $A_3B_1$, and $A_3B_2$ 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 vapor 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 48.02ppm. The higher the $IC_{50}$ value, the lower the antioxidant properties, and vice versa. The most increased antioxidant activity was found on the $A_3B_3$ treatment, whereas the lowest was found on the $A_1B_1$ 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.

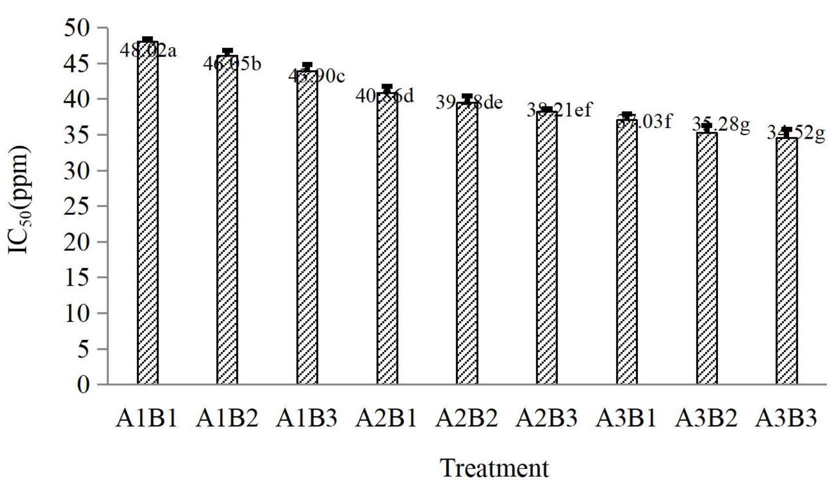

**Figure 5**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity ($IC_{50}$) of the active edible film.

The $IC_{50}$ value of this edible film was similar to edible film incorporated with turmeric extract with an $IC_{50}$ value of 38.28ppm as reported by **[3]**. **[19]** had said that polyvinyl alcohol edible film added with curcumin had an antioxidant activity of 35.16ppm and **[15]** had reported that edible alginate film incorporated with black chokeberry extract had antioxidant activity of 32.96ppm. However, the $IC_{50}$ value of this edible film was higher than potato starch edible film included with *Salvia officinalis* essential oil with magnitude of 68.35ppm as reported by **[23], [33]** with $IC_{50}$ of 50.42-77.41ppm and **[14]** with $IC_{50}$ of 87.41ppm.

$IC_{50}$ 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 of antioxidant activity is due to catechin compound content within gambier filtrate. **[25]** had described that gambier extract has potential as a drug that contain 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 100ppm.

## CONCLUSION

Mechanical properties of the active edible film had fulfilled JIS 1975 standard, especially in terms of thickness, tensile strength and water vapor transmission rate. However, the elongation percentage has not met the standard. Edible film has antioxidant characteristics of the strong category with $IC_{50}$ values in the range of 34.53 to 48.02ppm. In general, This edible film is feasible to be applied as packaging material for high lipid foods to inhibit oxidation process in those foods.

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The authors declare no conflict of interest.

**Ethical Statement:**
This article does not contain any studies that would require an ethical statement.Contact

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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

***Budi Santoso, Dwi Ambar Waty, Umi Rosidah, Hermanto Hermanto***

## 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 vapor transmission rate, and antioxidant activity. The edible film's thickness, tensile strength, and water vapour transmission rate were 0.096-0.124mm, 1.89-3.38MPa, and 12.99-17.04g.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 vapor, 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 was capable of improving the mechanical properties of edible film and increasing 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 10-22mg GAE/g, respectively. **[3]** reported that the addition of 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

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flower petals extract is produced from the flower petals of the rosella plant (*Hibiscus sabdariffa*), which are dried at 40°C, crushed in a blender, and extracted. Rosella flower petals extract also contains anthocyanin having a strong antioxidant property with $IC_{50}$ values in the range of 50 to 100ppm **[5]**. Seaweed syrup added with rosella flower petals extract contains an anthocyanin compound of 0.625 g/100mL **[13]**.

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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 compound **[31]**. But until now, there is no edible film 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.

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**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.), micrometer (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).

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**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 vapor 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]**.

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**Description of the Experiment**

**Sample preparation:**

**Instant green coffee**

Green coffee beans were dried to a moisture content of 12% and ground using a grinder. The powder was filtered using an 80-mesh sieve, after which water was added at a temperature of 100 °C and a ratio of 1:2, stirred, left for 10 minutes, and later filtered using a filter cloth to obtain the filtrate. Moreover, maltodextrin (10% w/w) and egg white (20% w/w) were added to the filtrate, mixed using a mixer for 10 minutes at high speed to form foam, and spread out on an aluminium pan lined with Polypropylene plastic. The mixture was dried in a carbine dryer at 60 °C for 4 hours, blended, and filtered using an 80-mesh filter to obtain a green coffee powder.

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**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 100mL boundary mark. The suspension was stirred using a magnetic stirrer for 10 minutes, filtered using Whatman No. 1 filter paper, and centrifuged at 1000rpm, followed by the filtrate.

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**Edible film production**

Canna starch as much as 4g is put into Beaker glass of 250mL in size, and aquadest water is added up to the mark of 100mL. 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 as much as 40 mL is poured into a petri dish with having diameter of 15 cm and then dried within a drying oven at 60 ºC for 24 hours. The edible film is released from the petri dish and then put into a desiccator for 1 hour. Finally, the edible film is ready to be analysed.

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

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

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**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).

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**Statistical Analysis**

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

**RESULTS AND DISCUSSION**

The edible film had thickness in the range of 0.096 to 0.124mm and these values had fulfilled the Japan Industrial Standard (JIS, 1975) of 0.25mm in maximum. The highest thickness of this edible film is similar to thickness of tapioca starch-based edible film, which is incorporated with kelakai leaves extract with a magnitude of 0.124mm **[21]**and the lowest thickness is similar to thickness of alginate edible film which is incorporated with curcumin extract with a magnitude of 0.096mm **[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 0.049mm **[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 on 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.

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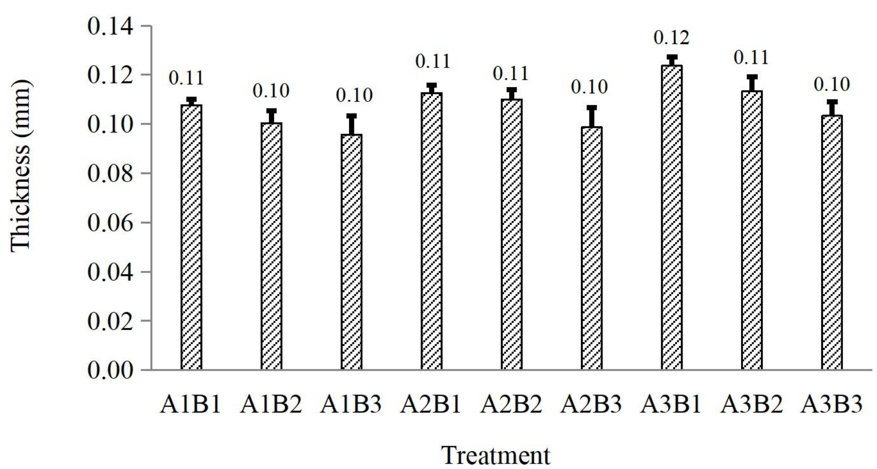


**Figure 1** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film.

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

**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 ($p>0.05$).

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

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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.

**Tabel 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 |

**Remarks:** 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) that 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 pumpkin with magnitude of 13.13-1_4.47% **[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.

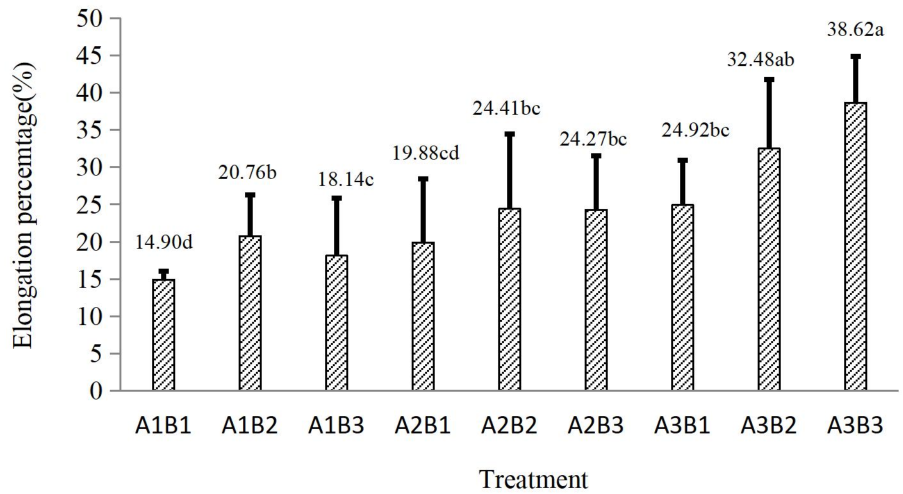


**Figure 2**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film.

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 characteristic affects the addition of hydrophilic compound in the edible film suspension. which causes the increase of 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, hydrophilic component increases 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 emulsifier as olive oil as 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 3.38MPa. $A_1B_1$ treatment (gambier filtrate of 3%v/v and rosella flower petals extract of 2% v/v) had produced the active edible film with the highest tensile strength. In contrast, the lowest was found on $A_3B_3$ 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.

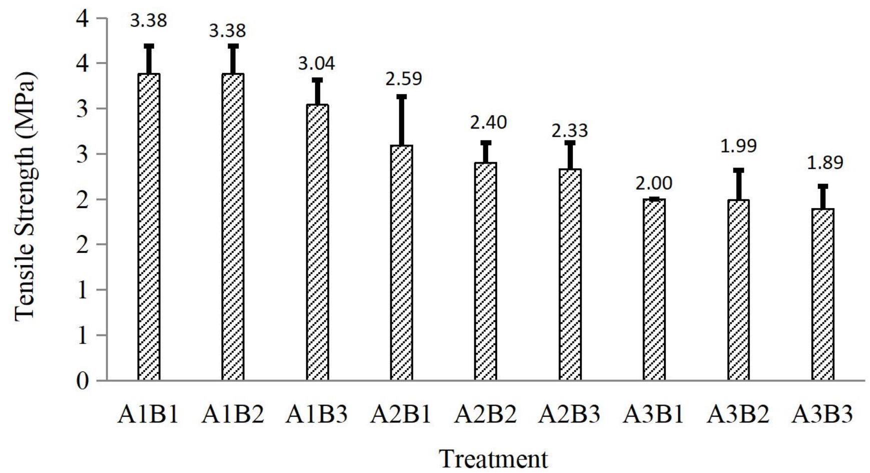


**Figure 3** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film.

Analysis of variance results showed that gambier filtrate treatment had a significant effect on tensile strength of the active edible film. In contrast, treatment of rosella flower petals extract and both treatments interaction had no significant effect on 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, 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 elongation percentage, namely, the higher the tensile strength, the lower the elongation percentage (Table 1). This is by the general theory that applies to edible film as stated by **[29]** that increasing 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 minimum of 0.39226MPa. Tensile strength of the produced edible film from several treatments combination was in the range of 1.89 to 3.38MPa and all the produced edible films had fulfilled the JIS standard. These tensile strength values are higher compared to tensile strength of edible film from sweet potato starch as reported by **[6]** with magnitude of 0.75MPa. They are lower compared to the tensile strength of edible film from breadfruit starch, as reported by **[34]**, with magnitude of 93.43MPa.

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**Water vapor transmission rate**

The water vapor 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$g.m^{-2}.d^{-1}$. 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 vapor 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 vapor transmission rate of active edible film. Further test in Table 1 showed that the water vapour transmission rate of 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 vapor transmission rate **[4]**. In addition, the water vapor 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.

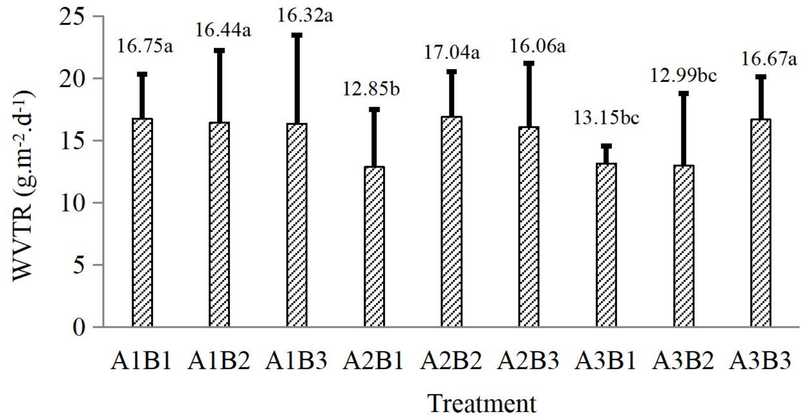


**Figure 4** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapor transmission rate of the active edible film.

The HSD test at 5% level in Table 2 showed the opposite results with rosella flower petals extract. The higher the concentration of rosella flower petals extract, the higher the water vapor 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 edible film. **[17]** reported that adding a hydrophobic component in form of sun flower oil to green bean starch edible film could decrease water vapor transmission rate. The opposite is true for the addition of a hydrophilic component.

Figure 4 shows that treatments $A_2B_1$, $A_3B_1$, and $A_3B_2$ 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 vapor 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 48.02ppm. The higher the $IC_{50}$ value, the lower the antioxidant properties, and vice versa. The most increased antioxidant activity was found on the $A_3B_3$ treatment, whereas the lowest was found on the $A_1B_1$ 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.

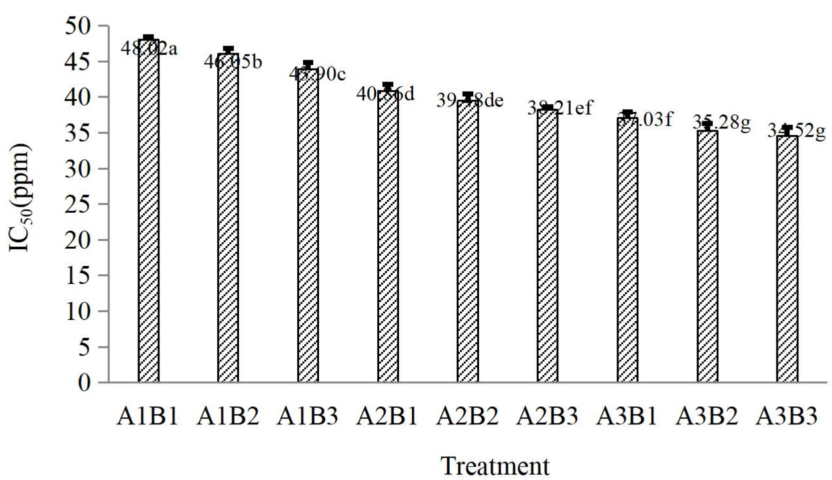

**Figure 5**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity ($IC_{50}$) of the active edible film.

The $IC_{50}$ value of this edible film was similar to edible film incorporated with turmeric extract with an $IC_{50}$ value of 38.28ppm as reported by **[3]**. **[19]** had said that polyvinyl alcohol edible film added with curcumin had an antioxidant activity of 35.16ppm and **[15]** had reported that edible alginate film incorporated with black chokeberry extract had antioxidant activity of 32.96ppm. However, the $IC_{50}$ value of this edible film was higher than potato starch edible film included with *Salvia officinalis* essential oil with magnitude of 68.35ppm as reported by **[23], [33]** with $IC_{50}$ of 50.42-77.41ppm and **[14]** with $IC_{50}$ of 87.41ppm.

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$IC_{50}$ 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 of antioxidant activity is due to catechin compound content within gambier filtrate. **[25]** had described that gambier extract has potential as a drug that contain 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 100ppm.

## CONCLUSION

Mechanical properties of the active edible film had fulfilled JIS 1975 standard, especially in terms of thickness, tensile strength and water vapor transmission rate. However, the elongation percentage has not met the standard. Active edible film has antioxidant characteristics of the strong category with $IC_{50}$ values in the range of 34.53 to 48.02ppm. In general, This edible film is feasible to be applied as packaging material for high lipid foods to inhibit oxidation process in those foods.

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**Conflict of Interest:**
The authors declare no conflict of interest.

**Ethical Statement:**
This article does not contain any studies that would require an ethical statement.Contact

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| Authors | **Budi Santoso[1)]*, Dwi Ambar Wati[1)], Umi Rosidah[1)], and Hermanto[1]** |
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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 were replicated three times. The results showed that the two treatment interaction significantly influenced elongation percentage, water vapor transmission rate and antioxidant activity. The values of thickness, tensile strength, and water vapour transmission rate of the edible film were 0.096-0.124mm, 1.89-3.38MPa and 12.99-17.04g.m-2.d-1, respectively. The edible film contains an antioxidant compound of the strong category with IC50 values in 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 of food having high lipid content to inhibit the oxidation process of that food.

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

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## INTRODUCTION

Food materials deterioration due to chemical, biochemical and microbiological reactions will be accelerated with the existence of oxygen gas, water vapor, sunlight and temperature. Oxygen gas and sunlight are external factors that cause rancidity reaction on food having high lipid content. To avoid this reaction, packaging materials are required that are capable of inhibiting 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 property against oxygen gas and sunlight.

The study results by **[12]** showed that polysaccharides edible film incorporated with essential oil and the herbal extract was capable of improving the mechanical properties of edible film and increasing 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 10-22mg GAE/g, respectively. **[3]** had reported that addition of turmeric extract on alginate edible film could produce an edible film having 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 precipitation of liquid, and drying the sediment **[22]**. Gambier extract contains a catechins compound with a magnitude of 98% **[23]**. Moreover, **[22]** showed that the catechins compound in gambier extract had semi-polar property and contained compounds with antioxidant and antibacterial properties. The extract was applied to inhibit oxidation reaction that occurred 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, crushed in a blender and extracted. Rosella flower petals extract is also contains anthocyanin having a strong antioxidant property with $IC_{50}$ values in the range of 50 to 100ppm **[5]**. Seaweed syrup added with rosella flower petals extract contains an anthocyanin compound of 0.625 g/100mL **[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 compound [31]. But until now, there is no edible film 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

**Sample**

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

**Chemical**

Gambier extract from Babat Toman Village, Banyuasin District, South Sumatra, Indonesia, rosella flower petals from PT HNI Indonesia, canna starch from Industri Lingkar Organik Sleman, Yogyakarta, Indonesia, 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

**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**

Edible film making process was done according to the modified procedure by **[26]**. Parameters of thickness, percent elongation, tensile strength, and water vapor transmission rate of edible film were measured referring to **[1]** while for the antioxidant activity parameters and according to the method **[20]**.

**Description of the Experiment**

**Sample preparation**

**Gambier filtrate production**

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

**Edible film production**

Canna starch as much as 4g is put into Beaker glass of 250mL in size, and aquadest water is added up to the mark of 100mL. Starch suspension is stirred by using a magnetic stirrer while being heated by using 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 mixed 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 by using a vacuum pump for 1 hour. Edible film suspension as much as 40 mL is poured into a petri dish with having diameter of 15 cm and then dried within a drying oven at 60 ºC for 24 hours. The edible film is released from the petri dish and then put into desiccator for 1 hour. Finally, the edible film is ready to be analysed.

**Number of samples analysed**

Treatment factors consisted of gambier filtrate (A): $A_1$=3; $A_2$=4 and $A_3$=5 (%v/v) and rosella flower petals extract (B): $B_1$=2, $B_2$=4 and $B_3$=6 (%v/v) with the number of samples analysed as many as 9 samples.

**Number of repeated analysed**

Three repeated for each treatment factor. The total sample analysed was 27 samples.

**Statistical Analysis**

This research used a factorial completely randomised design. Treatments that have significant effects were further tested by using an honestly significant different (HSD) test at $\alpha$ = 5%. Data of research results was analysed using analysis of variance (ANOVA) method with the aid of SAS program-Windows version 9.

## RESULTS AND DISCUSSION

**Thickness**

The edible film had thickness in the range of 0.096 to 0.124mm and these values had fulfilled the Japan Industrial Standard (JIS, 1975) of 0.25mm in maximum. The highest thickness of this edible film is similar to thickness of tapioca starch-based edible film, which is incorporated with kelakai leaves extract with a magnitude of 0.124mm **[21]**and the lowest thickness is similar to thickness of alginate edible film which is incorporated with curcumin extract with a magnitude of 0.096mm **[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 0.049mm **[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 on 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.

Treatment of gambier filtrate and rosella flower petals extract had a significant effect on edible film thickness, but their interactions had no significant effect. Results of honestly significant different (HSD) test for the effect of gambier filtrate concentration on active edible film thickness was 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 having semi-polar characteristics so that it includes solids that are insoluble in water. The amount of these solids effected on the increase of active edible film thickness. **[28]** showed that edible film thickness of sugar palm fruit had increased according to the rise of plasticizer concentration in which plasticizer is polymers that make up the edible film matrix that affect on the increase of total soluble solids within edible film suspension.

HSD test in Table 2 showed that concentration increase of rosella flower petals extracts had decreased active edible film thickness. **[2]**reported that rosella flower petals extract contains anthocyanin compounds, which are polar molecules. It decreasing active edible film thickness as previsouly discussed that the increase of edible film thickness is due to the addition of total solids within edible film solution.

**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) that 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 pumpkin with 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.

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 characteristic affects the addition of hydrophilic compound in the edible film suspension. which causes the increase of 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, hydrophilic component increases 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 emulsifier as olive oil as 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 3.38MPa. $A_1B_1$ treatment (gambier filtrate of 3%v/v and rosella flower petals extract of 2% v/v) had produced the active edible film with the highest tensile strength. In contrast, the lowest was found on $A_3B_3$ 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.

Analysis of variance results showed that gambier filtrate treatment had a significant effect on tensile strength of the active edible film. In contrast, treatment of rosella flower petals extract and both treatments interaction had no significant effect on 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, 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 elongation percentage, namely, the higher the tensile strength, the lower the elongation percentage (Table 1). This is by the general theory that applies to edible film as stated by **[29]** that increasing 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 minimum of 0.39226MPa. Tensile strength of the produced edible film from several treatments combination was in the range of 1.89 to 3.38MPa and all the produced edible films had fulfilled the JIS standard. These tensile strength values are higher compared to tensile strength of edible film from sweet potato starch as reported by **[6]** with magnitude of 0.75MPa. They are lower compared to the tensile strength of edible film from breadfruit starch, as reported by **[34],** with magnitude of 93.43MPa.

**Water vapor transmission rate**

The water vapor 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 $10 g.m^{-2}.d^{-1}$. 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 vapor 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 vapor transmission rate of active edible film. Further test in Table 1 showed that the water vapour transmission rate of 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 vapor transmission rate **[4]**. In addition, the water vapor 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.

The HSD test at 5% level in Table 2 showed the opposite results with rosella flower petals extract. The higher the concentration of rosella flower petals extract, the higher the water vapor 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 edible film. **[17]** reported that adding a hydrophobic component in form of sun flower oil to green bean starch edible film could decrease water vapor transmission rate. The opposite is true for the addition of a hydrophilic component.

Figure 4 shows that treatments $A_2B_1$, $A_3B_1$, and $A_3B_2$ 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 vapor 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 48.02ppm. The higher the $IC_{50}$ value, the lower the antioxidant properties, and vice versa. The most increased antioxidant activity was found on the $A_3B_3$ treatment, whereas the lowest was found on the $A_1B_1$ 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.

The $IC_{50}$ value of this edible film was similar to edible film incorporated with turmeric extract with an $IC_{50}$ value of 38.28ppm as reported by **[3]. [19]** had said that polyvinyl alcohol edible film added with curcumin had an antioxidant activity of 35.16ppm and **[15]** had reported that edible alginate film incorporated with black chokeberry extract had antioxidant activity of 32.96ppm. However, the $IC_{50}$ value of this edible film was higher than potato starch edible film included with *Salvia officinalis* essential oil with magnitude of 68.35ppm as reported by **[23], [33]** with $IC_{50}$ of 50.42-77.41ppm and **[14]** with $IC_{50}$ of 87.41ppm

$IC_{50}$ 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 of antioxidant activity is due to catechin compound content within gambier filtrate. **[25]** had described that gambier extract has potential as a drug that contain 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 100ppm.

**CONCLUSION**

Mechanical properties of the active edible film had fulfilled JIS 1975 standard, especially in terms of thickness, tensile strength and water vapor transmission rate. However, the elongation percentage has not met the standard. Active edible film has antioxidant characteristics of the strong category with $IC_{50}$ values in the range of 34.53 to 48.02ppm. In general, This edible film is feasible to be applied as packaging material for high lipid foods to inhibit oxidation process in those foods.

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The authors declare no conflict of interest.

**Ethical Statement:**
This article does not contain any studies that would require an ethical statement.

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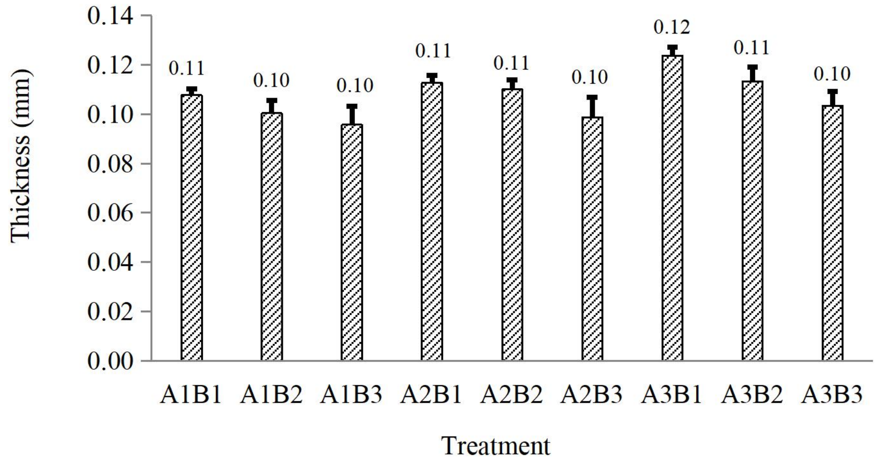


**Figure 1**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film.

**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 |

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

**Tabel 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 |

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

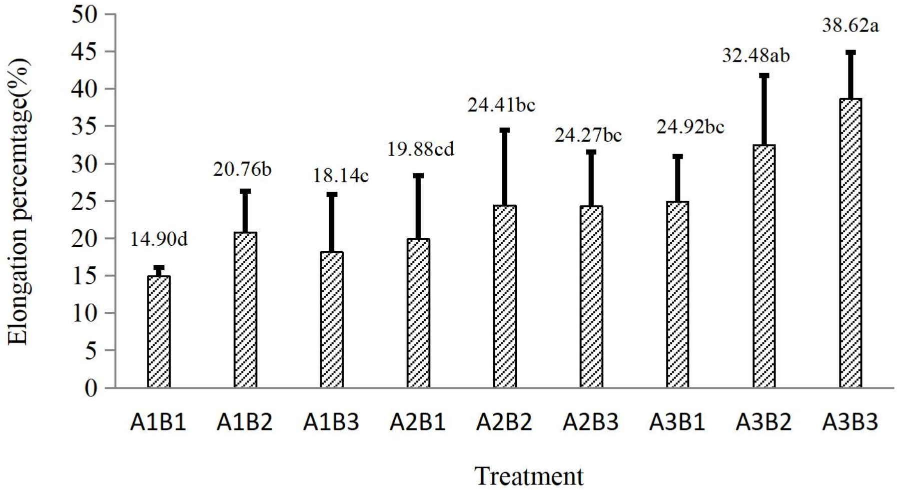


**Figure 2**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film.

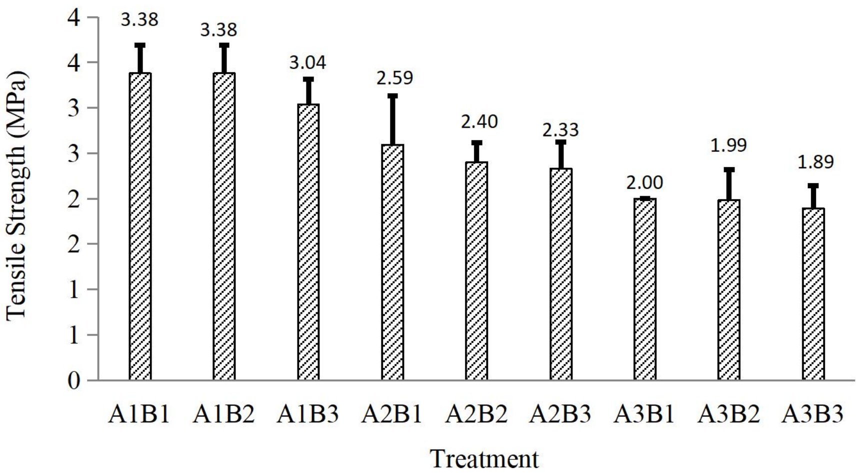


**Figure 3**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film.

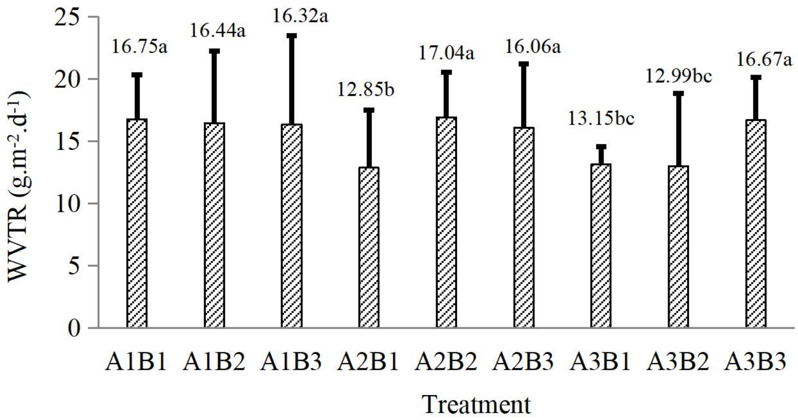


**Figure 4.** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapor transmission rate of the active edible film.

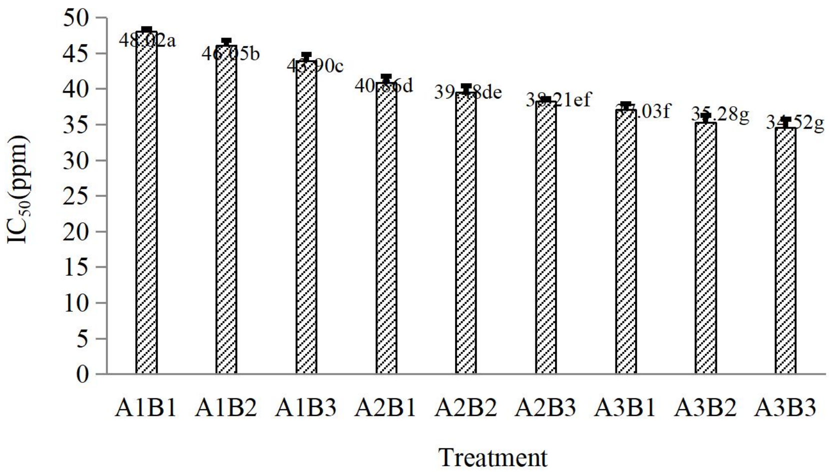


**Figure 5**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity ($IC_{50}$) of the active edible film.

**Effect of incorporation of gambier fitrate 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 were replicated three times. The results showed that the two treatments interaction significantly influenced elongation percentage, water vapour transmission rate and antioxidant activity. The values of thickness, tensile strength, and water vapour transmission rate of the edible film were 0.096-0.124mm, 1.89-3.38MPa and 12.99-17.04g.m-2.d-1, respectively. The edible film contains an antioxidant compound of the strong category with IC50 values in 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 of food having high lipid content to inhibit the oxidation process of that food.

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

## INTRODUCTION

Food materials deterioration due to chemical, biochemical and microbiological reactions will be accelerated with the existence of oxygen gas, water vapor, sunlight and temperature. Oxygen gas and sunlight are external factors that cause rancidity reaction on food having high lipid content. To avoid this reaction, packaging materials are required that are capable of inhibiting 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 property against oxygen gas and sunlight.

The study results by **Hasmeni et al. (2020)** showed that polysaccharides edible film incorporated with essential oil and the herbal extract was capable of improving the mechanical properties of edible film and increasing the shelf life of meat and sensory quality to increase nutritional value through inhibition of oxidation reaction. **Gebrechristos et al. (2020)** 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 10-22mg GAE/g, respectively. **Bojorges et al. (2020)** had reported that the addition of turmeric extract on alginate. Edible film could produce an edible film having 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, precipitation of liquid, and drying the sediment (**Pambayun et al., 2007**). Gambier extract contains a catechins compound with a magnitude of 98% (**Yeni et al., 2017**). Moreover, **Pambayun et al. (2007)** showed that the catechins compound in gambier extract had semi-polar property and contained compounds with antioxidant and antibacterial properties. The gambier extract was applied to inhibit oxidation reaction that occurred in cassava chips **(Firdausni et al., 2020)**. Gambier extract was also used by **Santoso et al. (2019)** 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, crushed in a blender and extracted. Rosella flower petals extract is also contains anthocyanin having a strong antioxidant property with $IC_{50}$ values in the range of 50 to 100ppm **(Djaeni et al., 2017)**. Seaweed syrup added with rosella flower petals extract contains an anthocyanin compound of 0.625 g/100mL **(Irsyad et al., 2017)**.

Edible film development conducted by researchers currently continues to increase from year to year

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**Scientific Hypothesis**

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

## MATERIAL AND METHODOLOGY

**Sample**

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

**Chemical**

Gambier extract from Babat Toman Village, Banyuasin District, South Sumatra, Indonesia, rosella flower petals from PT HNI Indonesia, canna starch from Industri Lingkar Organik Sleman, Yogyakarta, olive oil from PT HNI, Indonesia, carboxymethyl cellulose (CMC), 2,2–diphenyl-1-picrylhydrazyl (DPPH) and nutrient agar (NA).

**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**

Santoso et al., 2019; ASTM, 1997; AOAC, 2012; Maesaroh, Kurnia and Anshori, 2018.

**Description of the Experiment**

**Sample preparation**

**Gambier filtrate production**

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

**Edible film production**

The edible film making process was done according to the modified procedure by **Santoso et al. (2019).** Canna starch as much as 4g is put into Beaker glass of 250mL in size, and aquadest water is added up to the mark of 100mL. 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 mixed and then added with rosella flower petals extract according to treatments 2, 4 and 6%(v/v). After homogenising 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 by using a vacuum pump for 1 hour. Edible film suspension as much as 40 mL is poured into a petri dish with having diameter of 15 cm and then dried within a drying oven at 60ºC for 24 hours. The edible film is released from the petri dish and then put into desiccator for 1 hour. Finally, the edible film is ready to be analysed.

**Number of samples analysed**

Treatment factors consisted of gambier filtrate (A): $A_1$=3; $A_2$=4 and $A_3$=5 (%v/v) and rosella flower petals extract (B): $B_1$=2, $B_2$=4 and $B_3$=6 (%v/v) with the number of samples analysed as many as 9 samples.

**Number of repeated analysed**

Three repeated for each treatment factor. The total sample analysed was 27 samples.

**Statistical Analysis**

This research used a factorial completely randomised design. Treatments that have significant effects were further tested by using an honestly significant different (HSD) test at α = 5%. Data of research results was analyzed using analysis of variance (ANOVA) method with the aid of SAS program-Windows version 9.

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## RESULTS AND DISCUSSION

**Thickness**

The edible film had thickness in the range of 0.096 to 0.124mm, and these values had fulfilled the Japan Industrial Standard (JIS, 1975) of 0.25mm in 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 0.124mm **(Nata et al., 2020)**, and the lowest thickness is similar to the thickness of alginate edible film which is incorporated with curcumin extract with a magnitude of 0.096mm **(Bojorges et al., 2020).**

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 on treatment of gambier powder filtrate at

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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.

Treatment of gambier filtrate and rosella flower petals extract had a significant effect on edible film thickness, but their interactions had no significant effect. Results of honestly significant different (HSD) test for the effect of gambier filtrate concentration on active edible film thickness was 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 having semi-polar characteristics so that it includes solids that are insoluble in water. The amount of these solids affected the increase of active edible film thickness. **Sitompul and Zubaidah (2017)** showed that edible film thickness of sugar palm fruit had increased according to the rise of plasticizer concentration in which plasticizer is polymers that make up the edible film matrix that affect the increase of total soluble solids within edible film suspension.

HSD test in Table 2 showed that concentration increase of rosella flower petals extracts had decreased active edible film thickness. **Amperawati et al. (2019)** reported that rosella flower petals extract contains anthocyanin compounds, which are polar molecules. It affects decreasing active edible film thickness as previsouly discussed that the increase of edible film thickness is due to the addition of total solids within edible film solution.

**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) that 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% **(Hashimi et al., 2020)** 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% **(Hasan et al., 2020)**. 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.

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 it also has polar characteristics. Part of the catechin compound with polar characteristics affects the addition of hydrophilic compound in edible film suspension, which causes the increase of edible film elongation percentage. 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. **Hashimi *et al.* (2020)** 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, hydrophilic component increases 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 consists 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 emulsifier as olive oil as hydrophobic component. The hydrophilic component was more dominant in forming 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 3.38MPa. $A_1B_1$ treatment (gambier filtrate of 3%v/v and rosella flower petals extract of 2% v/v) had produced the active edible film with the highest tensile strength. In contrast, the lowest was found on $A_3B_3$ 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.

Analysis of variance results showed that gambier filtrate treatment had a significant effect on tensile strength of the active edible film. In contrast, treatment of rosella flower petals extract and both treatments interaction had no significant effect on 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, 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 elongation percentage, namely, the higher the tensile strength, the lower the elongation percentage (Table 1). This is by the general theory that



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applies to edible film as stated by **Supeni *et al.* (2015)** that increasing 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 0.39226MPa. Tensile strength of the produced edible film from several treatments combination was in the range of 1.89 to 3.38MPa, and all the produced edible films had 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 **Fatnasari *et al.* (2018)** with a magnitude of 0.75MPa. They are lower compared to the tensile strength of edible film from breadfruit starch, as reported by **Yulistiani *et al.* (2019)**, with magnitude of 93.43MPa.

## Water vapor transmission rate

The water vapor transmission rate of the produced active edible film was in the range of 12.85 to 17.04g.m$^{-2}$.d$^{-1}$ and higher than the JIS 1975 standard with a maximum value of 10g.m$^{-2}$.d$^{-1}$. Water vapour transmission rate of this active edible film was higher (12.99 to 17.04g.m$^{-2}$.d$^{-1}$) than alginate edible film added with turmeric extract (1.37g.m$^{-2}$.d$^{-1}$), as reported by **Bojoges et al. (2020)**. It was lower than canna based edible film added with gambier extract (20.23g.m$^{-2}$.d$^{-1}$) as written by **Santoso et al. (2019).** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapor 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 vapor transmission rate of active edible film. Further test in Table 1 showed that the water vapor transmission rate of 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 the water vapor transmission rate **(Cakmat et al., 2020)**. In addition, the water vapor transmission rate of edible film decreased 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.

The HSD test at 5% level in Table 2 showed the opposite results with rosella flower petals extract. The higher the concentration of rosella flower petals extract, the higher the water vapor 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 edible film. **Lee et al. (2020)** reported that adding a hydrophobic component in the form of sun flower oil to green bean starch edible film could decrease water vapor transmission rate. The opposite is true for the addition of a hydrophilic component.

Figure 4 shows that treatments $A_2B_1$, $A_3B_1$, and $A_3B_2$ had lower water vapour transmission rates than other treatments. This is due to the gambier filtrate's influence containing semipolar catechin compounds while the rosella flower petal extract is polar. Thus, the combination of higher gambier filtrate than rosella flower petal extract, the lower the water vapor 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 48.02ppm. The higher the $IC_{50}$ value, the lower the antioxidant properties, and vice versa. The most increased antioxidant activity was found on the $A_3B_3$ treatment, whereas the lowest was found on the $A_1B_1$ 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.

The $IC_{50}$ value of this edible film was similar to edible film incorporated with turmeric extract with an $IC_{50}$ value of 38.28ppm as reported by **Bojorges et al. (2020). Ma et al. (2017)** had said that polyvinyl alcohol edible film added with curcumin had an antioxidant activity of 35.16ppm, and **Kim et al. (2018)** had reported that edible alginate film incorporated with black chokeberry extract had an antioxidant activity of 32.96ppm. However, the $IC_{50}$ value of this edible film was lower than potato starch edible film included with *Salvia officinalis* essential oil with magnitude of 68.35ppm as reported by **Pirouzifard et al. (2019).**

$IC_{50}$ 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 IC50 value had decreased with the rise of gambier filtrate concentration. The increase of antioxidant activity is due to catechin compound content within gambier filtrate. **Saad et al. (2020)** had described that gambier extract has potential as a drug that contain 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. **Djaeni et al. (2017)** reported that rosella flower petals contain an anthocyanin compound with antioxidant characteristics with $IC_{50}$ values in the range of 50 to 100ppm.

## CONCLUSION

Mechanical properties of the active edible film had fulfilled JIS 1975 standard, especially in terms of thickness, tensile strength and water vapor transmission rate. However, the elongation percentage has not met the standard. Active edible film has antioxidant characteristics of the strong category with $IC_{50}$ values



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in the range of 34.53 to 48.02ppm. In general, this edible film is feasible to be applied as packaging material for high lipid foods to inhibit oxidation process in those foods.

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The authors declare no conflict of interest.

**Ethical Statement:**
This article does not contain any studies that would require an ethical statement.

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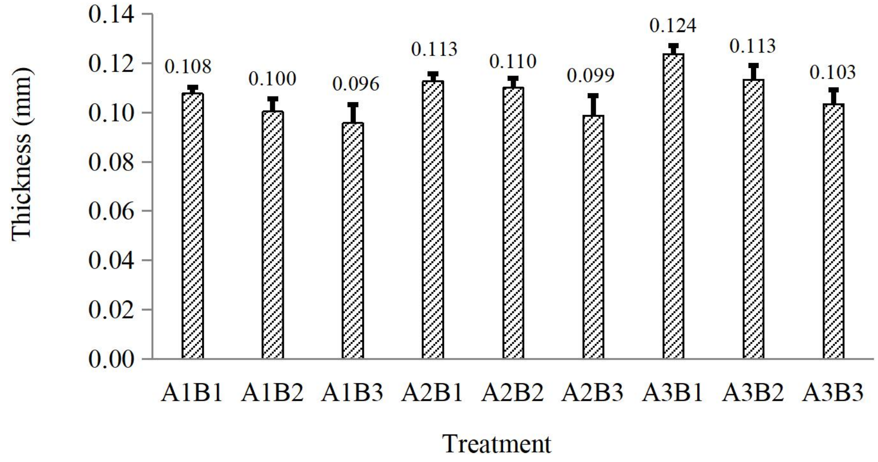


**Figure 1**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film.

**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 |

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

**Tabel 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 |

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

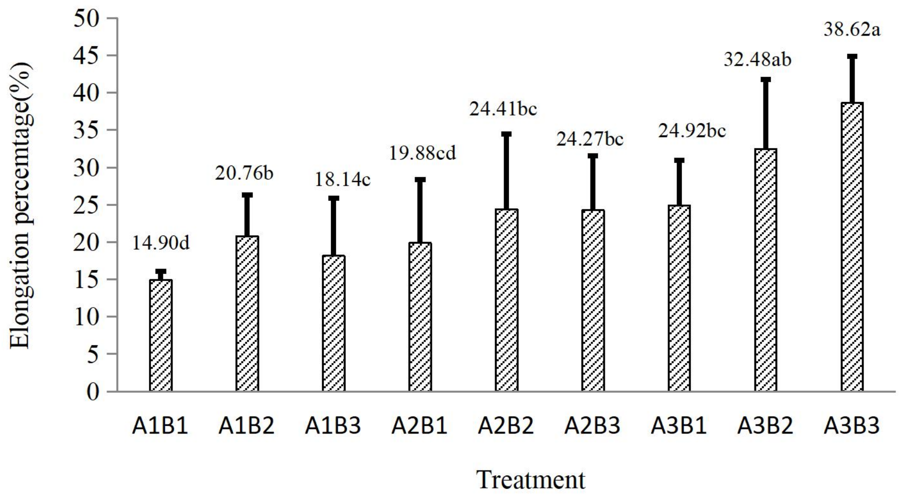


**Figure 2**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film.

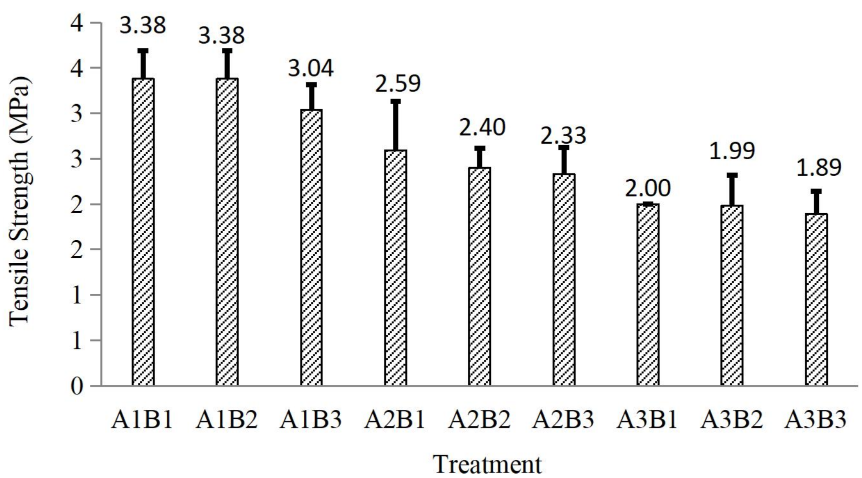


**Figure 3**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film.

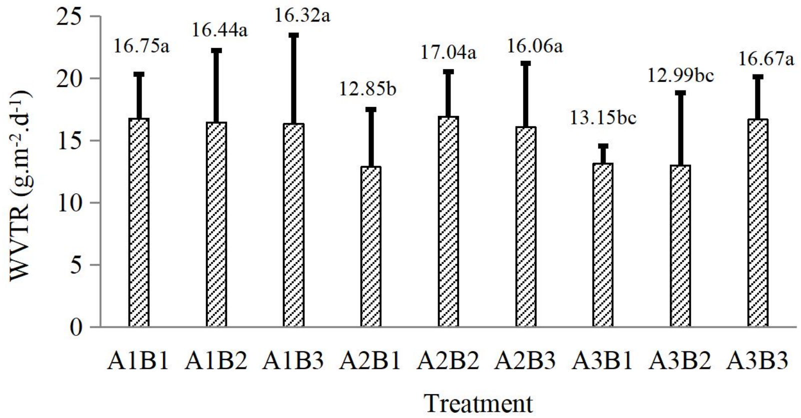


**Figure 4.** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapor transmission rate of the active edible film.

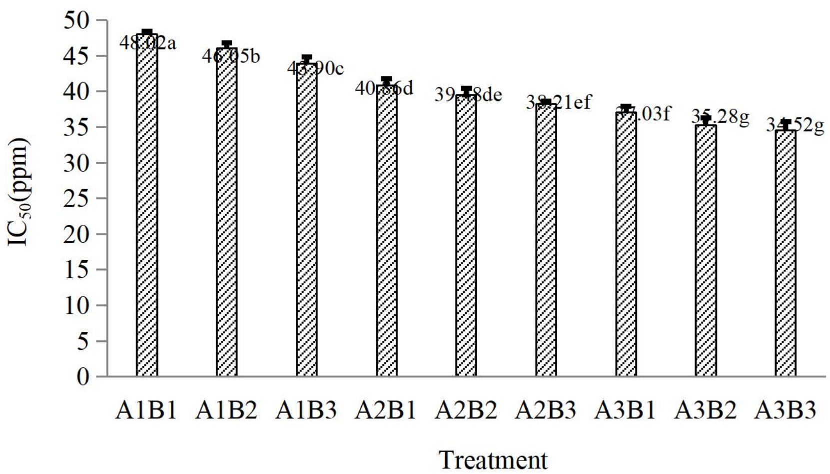


**Figure 5**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity ($IC_{50}$) of the active edible film.

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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~~starch based~~ active edible film

*Budi Santoso, Dwi Ambar Waty, Umi Rosidah, Hermanto Hermanto*

## 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~~were~~ replicated three times. The results showed that the two treatment interactions~~interaction~~ significantly influenced elongation percentage, water vapor transmission rate, and antioxidant activity. The ~~values of~~ edible film's thickness, tensile strength, and water vapour transmission rate ~~of the edible film~~ were 0.096-0.124mm, 1.89-3.38MPa, and 12.99-17.04g.m-2.d-1, respectively. The edible film contains an antioxidant compound of the strong category with IC50 values of~~in~~ 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~~of~~ food having high lipid content to inhibit the oxidation process of that food.

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

## INTRODUCTION

Due~~Food materials deterioration due~~ to chemical, biochemical and microbiological reactions, food materials will be accelerated with the existence of oxygen gas, water vapor, sunlight, and temperature. Oxygen gas and sunlight are external factors that cause rancidity reactions~~reaction~~ in~~on~~ food having high lipid content. To avoid this reaction, packaging materials are required that inhibit~~are capable of inhibiting~~ 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~~property~~ against oxygen gas and sunlight.

The study results by **[12]** showed that polysaccharides edible film incorporated with essential oil and the herbal extract was capable of improving the mechanical properties of edible film and increasing 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 10-22mg GAE/g, respectively. **[3]** ~~had~~ reported that the addition of turmeric extract on alginate edible film could produce an edible film with~~having~~ 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~~precipitation of liquid~~, and drying the sediment **[22]**. Gambier extract contains a catechins compound with ~~a magnitude of~~ 98% **[23].** Moreover, **[22]** showed that the catechins compound in gambier extract had semi-polar properties~~property~~ and contained compounds with antioxidant and antibacterial properties. The extract was applied to inhibit the oxidation reaction ~~that occurred~~ 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, crushed in a blender, and extracted. Rosella flower petals extract ~~is~~ also contains anthocyanin having a strong antioxidant property with $IC_{50}$ values in the range of 50 to 100ppm **[5]**. Seaweed syrup added with rosella flower petals extract contains an anthocyanin compound of 0.625 g/100mL **[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 compound **[31]**. But until now, there is no edible film 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~~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.), micrometer~~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~~Edible~~ film making process was done according to the modified procedure by **[26]**. Parameters of thickness, percent elongation, tensile strength, and water vapor 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:**

**Instant green coffee**

Green coffee beans were dried to a moisture content of 12% and ground using a grinder. The powder was filtered using an 80-mesh sieve, after which water was added at a temperature of 100 °C~~oC~~ and a ratio of 1:2, stirred, left for 10 minutes, and later filtered using a filter cloth to obtain the filtrate. Moreover, maltodextrin (10% w/w) and egg white (20% w/w) were added to the filtrate, mixed using a mixer for 10 minutes at high speed to form foam, and spread out on an aluminium pan lined with Polypropylene plastic. The mixture was dried in a carbine dryer at a temperature of 60 °CoC for 4 hours, blended, and filtered using an 80-mesh filter to obtain a green coffee powder.

**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 100mL boundary mark. The suspension was stirred using a magnetic stirrer for 10 minutes, filtered and filtered using Whatman No. 1 filter paper, and centrifuged at 1000rpm, followed by taking the filtrate.

**Edible film production**

Canna starch as much as 4g is put into Beaker glass of 250mL in size, and aquadest water is added up to the mark of 100mL. 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 mixturemixed 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 by using a vacuum pump for 1 hour. Edible film suspension as much as 40 mL is poured into a petri dish with having diameter of 15 cm and then dried within a drying oven at 60 ºC for 24 hours. The edible film is released from the petri dish and then put into a desiccator for 1 hour. Finally, the edible film is ready to be analysed.

**Number of samples analyzed:** The number of analysed samples analysed as many aswas 9 samples.

**Number of repeated analyses:** Three repeated analysis 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 that have significant effects were further tested by using an honestly significant different (HSD) test at $\alpha = 5\%$. Data of research results was analysed using analysis of variance (ANOVA) method with the aid of SAS program-Windows version 9.

## RESULTS AND DISCUSSION

The edible film had thickness in the range of 0.096 to 0.124mm and these values had fulfilled the Japan Industrial Standard (JIS, 1975) of 0.25mm in maximum. The highest thickness of this edible film is similar to thickness of tapioca starch-based edible film, which is incorporated with kelakai leaves extract with a magnitude of 0.124mm **[21]**and the lowest thickness is similar to thickness of alginate edible film which is incorporated with curcumin extract with a magnitude of 0.096mm **[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 0.049mm **[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 on 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.

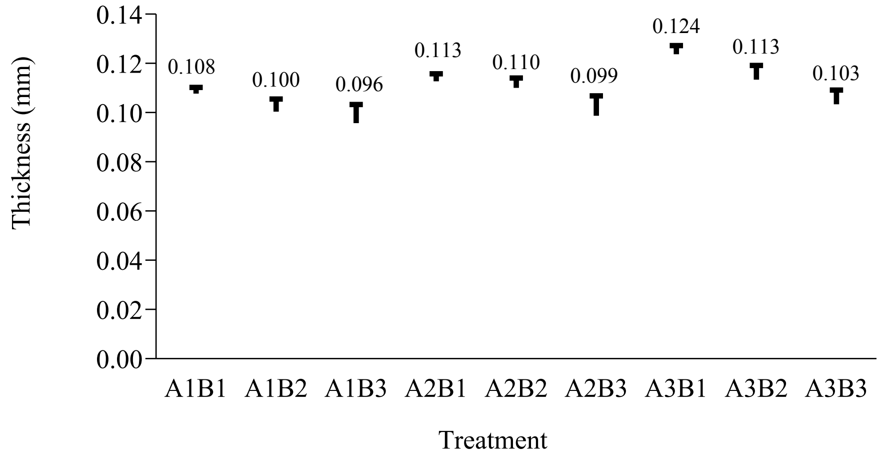

**Figure 1** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film.

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

**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).

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

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.

**Tabel 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 |

**Remarks:** 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) that 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 pumpkin with 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.

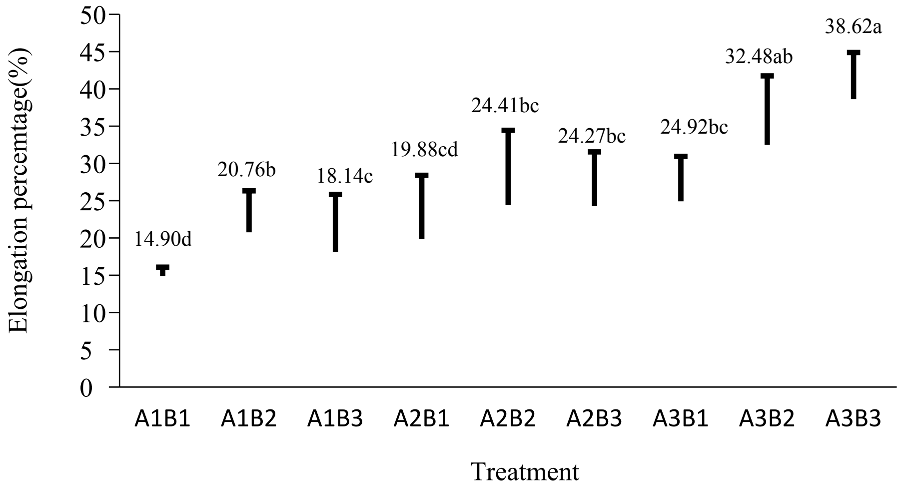


**Figure 2**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film.

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 characteristic affects the addition of hydrophilic compound in the edible film suspension. which causes the increase of 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, hydrophilic component increases 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 emulsifier as olive oil as 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 3.38MPa. $A_1B_1$ treatment (gambier filtrate of 3%v/v and rosella flower petals extract of 2% v/v) had produced the active edible film with the highest tensile strength. In contrast, the lowest was found on $A_3B_3$ 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.

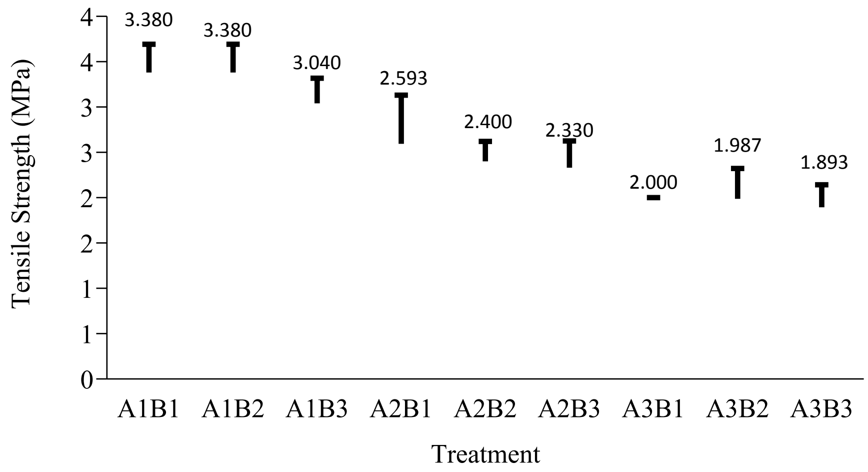


**Figure 3** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film.

Analysis of variance results showed that gambier filtrate treatment had a significant effect on tensile strength of the active edible film. In contrast, treatment of rosella flower petals extract and both treatments interaction had no significant effect on 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, 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 elongation percentage, namely, the higher the tensile strength, the lower the elongation percentage (Table 1). This is by the general theory that applies to edible film as stated by **[29]** that increasing 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 minimum of 0.39226MPa. Tensile strength of the produced edible film from several treatments combination was in the range of 1.89 to 3.38MPa and all the produced edible films had fulfilled the JIS standard. These tensile strength values are higher compared to tensile strength of edible film from sweet potato starch as reported by **[6]** with magnitude of 0.75MPa. They are lower compared to the tensile strength of edible film from breadfruit starch, as reported by **[34]**, with magnitude of 93.43MPa.

**Water vapor transmission rate**

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

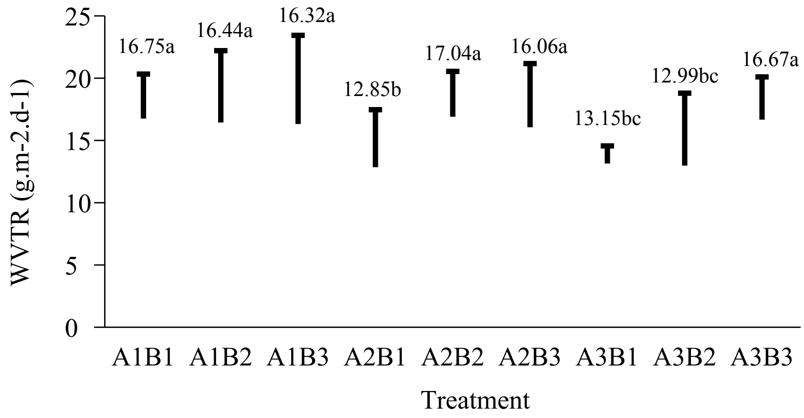


**Figure 4** The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapor transmission rate of the active edible film.

The HSD test at 5% level in Table 2 showed the opposite results with rosella flower petals extract. The higher the concentration of rosella flower petals extract, the higher the water vapor 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 edible film. **[17]** reported that adding a hydrophobic component in form of sun flower oil to green bean starch edible film could decrease water vapor transmission rate. The opposite is true for the addition of a hydrophilic component.

Figure 4 shows that treatments $A_2B_1$, $A_3B_1$, and $A_3B_2$ 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 vapor 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 48.02ppm. The higher the $IC_{50}$ value, the lower the antioxidant properties, and vice versa. The most increased antioxidant activity was found on the $A_3B_3$ treatment, whereas the lowest was found on the $A_1B_1$ 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.

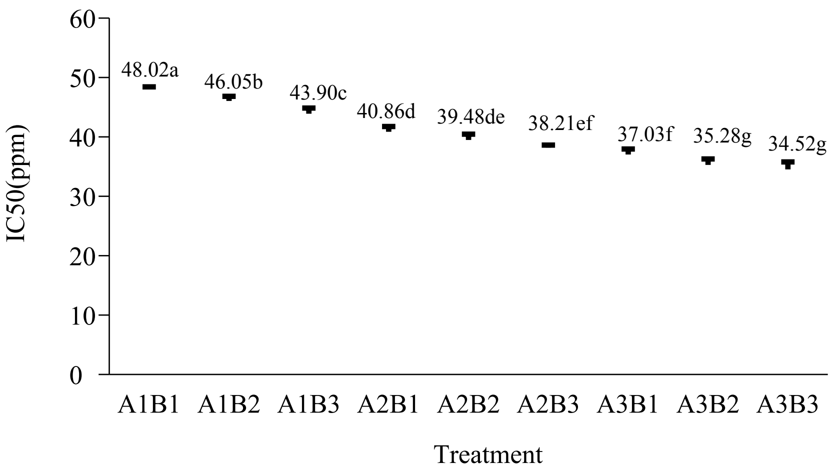

**Figure 5**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity ($IC_{50}$) of the active edible film.

The $IC_{50}$ value of this edible film was similar to edible film incorporated with turmeric extract with an $IC_{50}$ value of 38.28ppm as reported by **[3]**. **[19]** had said that polyvinyl alcohol edible film added with curcumin had an antioxidant activity of 35.16ppm and **[15]** had reported that edible alginate film incorporated with black chokeberry extract had antioxidant activity of 32.96ppm. However, the $IC_{50}$ value of this edible film was higher than potato starch edible film included with *Salvia officinalis* essential oil with magnitude of 68.35ppm as reported by **[23], [33]** with $IC_{50}$ of 50.42-77.41ppm and **[14]** with $IC_{50}$ of 87.41ppm.

$IC_{50}$ 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 of antioxidant activity is due to catechin compound content within gambier filtrate. **[25]** had described that gambier extract has potential as a drug that contain 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 100ppm.

## CONCLUSION

Mechanical properties of the active edible film had fulfilled JIS 1975 standard, especially in terms of thickness, tensile strength and water vapor transmission rate. However, the elongation percentage has not met the standard. Active edible film has antioxidant characteristics of the strong category with $IC_{50}$ values in the range of 34.53 to 48.02ppm. In general, This edible film is feasible to be applied as packaging material for high lipid foods to inhibit oxidation process in those foods.

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**Conflict of Interest:**

The authors declare no conflict of interest.

**Ethical Statement:**

This article does not contain any studies that would require an ethical statement.Contact

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## Manuscript Title

Effects of the Incorporation of Gambier Filtrate and Rosella Flower Petals Extract on the Mechanical Properties and Antioxidant Activity of Canna Starch-Based Active Edible Film

## Author(s)

Budi Santoso

## Date Issued

**July 12, 2021**

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**INVOICE: no.: 202229**

**Seller**
Ing.Peter Zajác - HACCP Consulting
Slivková 12
951 01 Nitrianske Hrnčiarovce,
Slovak Republic
ID no.: 41062191
TAX ID: 1071118180
VAT ID: Not VAT

Listed in register: 430-24632

Bank name: Vseobecna uverova banka, Mlynske nivy 1, 829 90 Bratislava 25, Slovakia,
Bank code: 0200,
SWIFT code: SUBASKBX,
Account no.: IBAN: SK3502000000001794485058,
We are not a VAT payer.

**Buyer**
Budi Santoso/Budi
Jl. Dahri Sembayu Komp. Taman Sembayu No 124 Rt 23 Rw 003, Kelurahan Sungai Buah, Kecamatan Ilir II, Palembang City, South Sumatera, Indonesia

Payment form: BANK TRANSFER
Inovice date: 9.6.2022
Payment due date: 30.6.2022
Supply date: 9.6.2022

| Product | Unit price | Quantity | Total price |
|---|---|---|---|
| **Article 1735, 1736 in the Potravinarstvo Slovak Journal of Food Sciences:** | 250 | 2 pc | € 500,00 |

**Total amount to pay: € 500,00**

Issued by: Peter Zajác

HACCP CONSULTING
Ing. Peter Zajác IČO: 41 062 191
Slivková 12
Nitrianske Hrnčiarovce 951 01

Signature

REFERENCE : S10PBG00048722

NO. TRX. : 85921 980766 96962 TRAN 22/06/2022 11:19:54
NO. REK. : 000001000000105 SIMSEM GIRO INTERNAL -
JUMLAH : IDR 509,630- 1568
010 - PALEMBANG

NO. TRX. : 85921 980766 96962 TRAN 22/06/2022 11:19:54
NO. REK. : 010360420801001 PENDAPATAN PROPISI KU
JUMLAH : IDR 35,000 1568
010 - PALEMBANG

NO. TRX. : 85921 980766 96962 TRAN 22/06/2022 11:19:54
NO. REK. : 010360462010001 Pendapatan Restitusi B
JUMLAH : IDR 474,630 1568
010 - PALEMBANG

NO. TRX. : 85921 980766 96962 TRAN 22/06/2022 11:19:54
NO. REK. : 000001000000105 SIMSEM GIRO INTERNAL -
JUMLAH : IDR 7,910,500- 1568
010 - PALEMBANG

NO. TRX. : 85921 980766 96962 TRAN 22/06/2022 11:19:54
NO. REK. : 010333200101001 KU YAKIR EUR
JUMLAH : EUR 500 1568
010 - PALEMBANG

PT. BANK NEGARA INDONESIA (Persero), Tbk
CABANG : PALEMBANG

IBOC - Maintenance (S10)

Teller ID : 85921
Date : 22/06/2022
Time : 11:36:00

Sender's Reference:
:20:S10PBG00048722
Bank Operation Code:
:23B:CRED
Value Date/Currency/Interbank Settled Amount:
:32A:220622EUR500,
Ordering Customer:
:50K:BUDI SANTOSO
JL PERUMAHAN RAKYAT NO 1
KOMP TAMAN SEMBAYU INDONESIA
Ordering Institution:
:52A:BNINIDJAXXX
Details Of Charges:
:71A:OUR

BANK NEGARA INDONESIA (PERSERO) Tbk
BNI
KANTOR CAPEM
UNSRI

**BNI**

Validasi

Tanggal/ Date 22/06/2022

# Formulir Kiriman Uang
*Remittance Application*

Penerima/ *Beneficiary* — Penduduk/ *Resident* — Bukan Penduduk/ *Non Resident*

Nama/ *Name* : Ing. peter Zajac – HACCP consulting

Alamat/ *Address* : SLIVKOVA 12, 951 01 NITRIANSKE

Telepon/ *Phone* : HRNCIAROVCE SLOVAK REPUBLIC

Kota/ *City* : Negara/ *Country* :

Bank Penerima/ *Beneficiary Bank* : VSEOBECNA UVEROVA BANKA MLYNSKENIVY 1, 829 90 BRATISLAVA 25

Kota/ *City* : Negara/ *Country* : SWIFT CODE : SUBASKBX SLOVAKIA

No. Rek./ *Acc. No.* : IBAN : SK3502000000001794485058

Jenis Pengiriman/ *Type of Transfer* : LLG/ *Clearing* RTGS ✓ Draft SWIFT SUBASKBX

Sumber Dana/ *Source of fund* : Tunai/ *Cash* Cek/ BG No. ✓ Debit Rek./ *Debit Acc. No* 1000000105

Mata Uang/ *Currency* : IDR USD

Jumlah Dana yang dikirim/ *Amount Transfer* : € 500

| Jumlah/ *Amount* | Kurs/ *Rate* | Nilai/ *Total Amount* |
|---|---|---|
| € 500 | Rp. 15.821 | Rp. 7.910.500 |

Pengirim/ *Remitter* ✓ Penduduk/ *Resident* — Bukan Penduduk/ *Non Resident*

Nama/ *Name* : BUDI SANTOSO

Nama Alias/ *Alias Name* :

No. ID KTP/SIM/ Passport/ KITAS : 1671061006750015

Alamat/ *Address* : LEMABANG

Telepon/ *Phone* : +6281278536 31

Kota/ *City* : PLG Negara/ *Country* : INDONESIA

Tujuan Transaksi (*Transaction Purpose*) : BIAYA PENERBITAN JURNAL INTERNASIONAL

Berita (*Message*) :

| Biaya/ *Charge* | Valas/ *Foreign Currency* | Kurs/ *Amount* | Nilai/ *Total Amount* |
|---|---|---|---|
| Komisi/ *Commission* Pengiriman/ *Handling* | € 30 | Rp. 15.821 | Rp. 474.630 |
| Bank Koresponden / *Correspondent Bank* | | | |

Jumlah Biaya / *Amount Charge* : Rp. 35.000

Total yang dibayarkan/ *Total Amount* : Rp. 8.420.130

Terbilang/ *Amount in Words* : Delapan juta empat ratus dua puluh ribu seratus tiga puluh rupiah

Biaya dari bank koresponden dibebankan ke rekening/ *Correspondent bank charges are for account of* : Penerima/ *Beneficiary* Pengirim/ *Remitter* Sharing

Saya menyetujui sepenuhnya syarat-syarat yang tercantum pada halaman belakang formulir ini / *I unconditionally accept all the terms and conditions on the reverse form.*

Pejabat Bank/ *Bank Officer* — Teller — Pemohon/ *Applicant*

KMU 1 05M/07/11

Lembar 1 Bank

**BNI**

Tanggal/ Date : 27/06/2022

Validasi:

# Formulir Kiriman Uang

*Remittance Application*

Penerima/ *Beneficiary* ☐ Penduduk/ *Resident* ☐ Bukan Penduduk/ *Non Resident*

Nama/ *Name* : Ing. peter Zajac - HACCP consulting

Alamat/ *Address* : Šlivkova 12, 95101 Nitrianske

Telepon/ *Phone* : Hanciarovce Slovak Republic

Kota/ *City* : Negara/ *Country* :

Bank Penerima/ *Beneficiary Bank* : VSEOBECNA UVEROVA BANKA A.S. MLYNSKE NIVY 1, 82990 BRATISLAVA 25

Kota/ *City* : Negara/ *Country* : SLOVAKIA

SWIFT CODE : SUBASKBX

No. Rek./ *Acc. No.* : IBAN : SK3502000000001794405058

Pengirim/ *Remitter* ☑ Penduduk/ *Resident* ☐ Bukan Penduduk/ *Non Resident*

Nama/ *Name* : BUDI SANTOSO

Nama Alias/ *Alias Name* :

No. ID : 1671061006750015

KTP/SIM/ *Passport/ KITAS*

Alamat/ *Address* : LEMABANG

Telepon/ *Phone* : +6281278536[illegible]

Kota/ *City* : PLG Negara/ *Country* : INDONESIA

Tujuan Transaksi (*Transaction Purpose*) : BIAYA PENERBITAN JURNAL INTERNASIONAL

Berita (*Message*) :

Biaya dari bank koresponden dibebankan ke rekening/ *Correspondent bank charges are for account of* :

☐ Penerima/ *Beneficiary* ☐ Pengirim/ *Remitter* ☐ Sharing

Jenis Pengiriman/ *Type of Transfer* : ☐ LLG/ *Clearing* ☐ RTGS ☐ Draft ☑ SWIFT ☐ ........ SUBASKBX

Sumber Dana/ *Source of fund* :

☐ Tunai/ *Cash* ☐ Cek/ *BG No.* ..........

☑ Debit Rek./ *Debit Acc. No.* 1000000105

Mata Uang/ *Currency* : ☐ IDR ☐ USD ☐ ..........

Jumlah Dana yang dikirim/ *Amount Transfer* : € 500

| Jumlah / *Amount* | Kurs/ *Rate* | Nilai/ *Total Amount* |
|---|---|---|
| € 500 | Rp 15.721,41 | Rp 7.860.705 |

| Biaya/ *Charge* | Valas/ *Amount in Foreign Exchange* | Kurs/ *Amount* | Nilai/ *Total Amount* |
|---|---|---|---|
| Komisi/ *Commission* | € 30 | Rp 15.721,41 | Rp 471.642,30 |
| Pengiriman/ *Handling* | | | |
| Bank Koresponden / *Correspondent Bank* | | | |
| Jumlah Biaya / *Amount Charge* : | | | Rp 35.000 |
| Total yang dibayarkan/ *Total Amount* | | | Rp 8.4[illegible] |

Terbilang/ *Amount in Words* [illegible]

Saya menyetujui sepenuhnya syarat-syarat yang tercantum pada halaman belakang formulir ini / *I unconditionally accept all the terms and conditions on the reverse form.*

PT. BANK NEGARA INDONESIA (PERSERO) Tbk KANTOR CAPEM UNSRI

Pejabat Bank/ *Bank Officer* Teller Pemohon/ *Applicant*

Sah jika ada cetakan data komputer atau tanda tangan yang berwenang/ *The application form will be valid if there is a computerized validation or the authorized signature.*

- Transaksi oleh Walk In Customer (WIC) di atas Rp. 100 juta atau nilai yang setara dengan itu wajib mengisi form PMN (KYC)/ *Transaction by Walk In Customer amounting exceeds Rp. 100.000.000,- (one hundred million rupiahs) or equivalent value must fill in the PMN (KYC) Form.*
- Transaksi oleh bukan penduduk di atas USD 10.000 atau ekuivalen wajib mengisi form LLD1 / *Transactional by non-resident amounting over US $ 10,000 or its equivalent must fill in the LLD 1 Form.*

Lembar 3 : Nasabah

### Notifications

- [Potravinarstvo] Editor Decision 2022-06-08 04:13 PM

**Reviewer's Attachments**

- Search

Potravinarstvo Slovak Journal of Food Sciences

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| Name | Date | Component |
| --- | --- | --- |
| No Files | | |

**Review Discussions**

- Add discussion

| Name | From | Last Reply | Replies | Closed |
| --- | --- | --- | --- | --- |
| No Items | | | | |

# [Potravinarstvo] Editor Decision

2022-06-08 04:13 PM

Budi Santoso, Dwi Ambar Wati, Umi Rosidah, Hermanto:

We have reached a decision regarding your submission to Potravinarstvo Slovak Journal of Food Sciences, "the EFFECT OF INCORPORATION OF GAMBIER FITRATE AND ROSELLA FLOWER PETALS EXTRACT ON MECHANICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF CANNA STARCH BASED ACTIVE EDIBLE FILM".

Our decision is to: Article accepted for publication.