Reaktor

p-ISSN 0852 – 0798
e-ISSN 2407 – 5973

Website: http://ejournal.undip.ac.id/index.php/reaktor/

Reaktor, Vol. 22 No. 3, December Year 2022, pp. 113-121

Preparation and Characterization of Velvet Beans-Based Edible Film Fortified with Green Tea Extract as Antioxidant Agent

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(Received: 15 September 2022; Published: 09 January 2023)

Abstract

Health and environmental issues have long been linked to the use of plastic food packaging. For that reason, the edible film as food packaging derived from organic and edible materials could be one of the best solutions. The combination of protein velvet bean (Mucuna pruriens) and gelatin – alginate was carried to modify the structure of the film through crosslinking mechanism. In this study, green tea extract (GTE) was also added as antioxidant agent. The objectives of this research are to investigate the effect of gelatin, alginate, and GTE on the mechanical properties of edible film. In addition, the antioxidant property was examined using DPPH method; while the antimicrobial property was determined following the nutrient agar method. Furthermore, the functional group and morphology of film were also observed using the FTIR and SEM, respectively. The main procedure of this study comprised (i) isolation of protein from velvet beans, (ii) modification of edible film, and (iii) addition of GTE. The SEM result shows a compact structure of the edible film. The presence of GTE indicated by FTIR spectra provides the antioxidant and antimicrobial activity to the film. The optimum composition of gelatin, alginate, and GTE which meets, Japanese Industrial Standard (JIS) standard are 5 %w/w, 3%w/w, and 2%v/w, respectively.

Keywords: *edible films, velvet bean, plasticizer, antioxidant, antimicrobial*

How to Cite This Article: Soewito, L.B., Fatikah, A.F., Tanuwidjaja, I.N., Sabrilla, T.S., Prasetyaningrum, A., and Handayani N.A., (2022), Preparation and Characterization of Velvet Beans-Based Edible Film Incorporated with Green Tea Extract as Antioxidant Agent, Reaktor, 22(3), pp. 113-121, https://doi.org/10.14710/reaktor. 22. 3. 113 - 121

INTRODUCTION

The demand for the use of plastic as food packaging continues to increase every year. However, many health concerns have been related to the use of food packaging plastic, mainly due to bisphenol-A (BPA) and phthalates exposure contained in plastic materials (Ilmiawati et al., 2017). In addition, according to the Indonesian Aromatic and Plastic Olefin Industry Association (INAPLAS), the use of plastic packaging accounts (for 65% of national plastic consumption) was concerning (Purwoko & Wibowo, 2018). One alternative solution to solve these problems is substitution of plastic with edible film. The edible film is a thin layer that can be directly eaten and used to protect food against moisture, oxygen, light, lipids, and solutes. It also improves handling in storage, decomposes naturally, and has a high aesthetic value. Some researcher uses staple food resources as the main ingredients to produce an edible film. However, this can disrupt national food security. Therefore, the edible film production should be made using non-essential functional components.

One of the potential commodities is velvet bean (*Mucuna pruriens*), which has a high protein content. However, the utilization of velvet beans has not yet been optimized due to cyanide acid (HCN), which is toxic to humans. Thus, it is not categorized as the main staple food. Further, it is suitable as main ingredients for making edible films. Previous research reported that velvet bean-based edible films have weak mechanical properties (tensile strength, elongation, and water vapor transmission rate-WVTr) and do not comply with the standards of the Japanese Industrial Standard (JIS) (Kunarto, 2006). According to JIS, the maximum thickness of the edible film and WVTr are 0.25 mm and 10 g/m^2.24 hours, respectively. At the same time, the minimum value of tensile strength and elongation at break are 0.39 MPa and 70%, respectively. A similar study resulted edible films reported that tensile strength and elongation at break value met standard of JIS, but no other analysis was carried out (Hardipramesti, 2017). Based on previous research, velvet beans-based edible film requires modifications to strengthen the mechanical properties of the edible film by adding some functional ingredients.

The combination of velvet beans and gelatin form a more complex matrix (Dou et al., 2018). Glycerol and alginate were also applied to improve tensile strength and elongation properties (Gao et al., 2017; Park et al., 2021). Calcium chloride was used as a crosslinking agent to strengthen the chemical structure of edible films. Furthermore, green tea leaf extract (GTE) was added to provide antioxidant property. This study aimed to investigate the effect of gelatin, alginate, and GTE concentration on mechanical properties (elongation at break, tensile strength, WVTr, and film thickness) and physical properties (morphology-SEM) of velvet-based-edible films. The functional groups and antimicrobial properties of the edible film were also reported.

MATERIALS AND METHOD

Materials

The main ingredients, velvet beans, were purchased from Wonogiri, Central Java. Green tea leaves were provided by PT. Gunung Subur Sejahtera (Karanganyar, Indonesia), as antioxidant source. Gelatin, glycerol, alginate, and calcium chloride were supplied from Merck. Bacterial culture (*Escherichia coli* and *Staphylococcus aureus*) is provided by LabDeal (Bandung, Indonesia).

Preparation of velvet bean flour

Velvet beans were peeled, washed, and soaked in water (the mass ratio of water and beans is 3:1) for 48 hours. The water was changed every 8 hours to remove the HCN content. Further, the beans were dried in an oven at 50ºC for 24 hours. Later, the velvet ground and velvet beans were sieved using an 80-mesh sieve and then stored as a powder. The sieve results in powder mixed with 1:10v/w distilled water. The solution was stirred without heating with a pH adjustment of 9. The mixture was centrifuged for 15 minutes at a speed of 3000 rpm. The filtrate obtained from the centrifugation results was re-adjusted at a pH of 4 and then centrifuged again. The residue in the centrifuge tube was taken as protein isolate.

Green Tea Leaf Solution Preparation

The solution was heated with a stirrer in a temperature range of 50-60ºC for 15 minutes. Dry green tea leaves were soaked into distilled water (in 1:20w/v) and stirred vigorously. Then, the resulting solution (filtrate) was separated from the leaves using Whatman paper no.42 over a vacuum filtration device (Pasrija & Anandharamakrishnan, 2015).

Edible Film Production

The composition of protein isolate, gelatin, glycerol, alginate, and GTE used in edible film production are shown in Table 1. Firstly, protein isolate, gelatin, GTE (4%v/w), and distilled water (1:1v/v) were mixed and stirred at 70-80ºC for 30 minutes. Afterward, 60%w/v glycerol was added and stirred until the obtainment of a homogeneous phase. Alginate was then added under continuous mixing (30 min). Next, 2%w/v of calcium chloride was added and mixed until homogeneous. The solution was then molded on a Teflon with a film dimension of about 5×2 cm. The printed film was immersed in 0.4 M calcium chloride solution for 5 minutes. The film printed was dried and then keep in a desiccator for 24 hours to avoid re-moisturization. Further, this film was analyzed for mechanical properties. The experiment was repeated with variations of alginate and GTE concentration based on the best mechanical properties of the previous experiment.

Antioxidant Activities

The antioxidant activity of edible film with variations of green tea extract was analyzed using the DPPH (2,2-diphediphenyl-1-picrylhydrazyl) method based on the research conducted by Chen et al. (2019). This analysis was carried out by mixing the edible film in solution form as much as 0.2 mL with 2 mL of 0.2 m methanol of the DPPH solution. The mixture was then stirred and incubated in a dark room at room temperature for 30 minutes. Then, the mixture was centrifuged at 8000 rpm for 5 minutes. Finally, the absorbance of the prepared sample was read using spectrophotometry (Helios Gamma) at 517 nm wavelength.

Antibacterial Activity

Nutrient Agar (NA) is used as a medium for testing microbial activity. In this experiment, a predetermined amount of NA was dissolved and sterilized by heating. Then, the resulting solution was let to cool to about 50ºC. Cultures from *E. coli* (gram-negative) and *S. aureus* (gram-positive) were suspended in a medium of 60 µL NA for every 20 ml of NA.

Table 1. Composition of protein isolate, gelatin, glycerol, alginate, and GTE used in edible film production

Film Samples	Composition				
	Protein Isolate (gr)	Gelatin (%w/w)	Glycerol (%v/w)	Alginate (%w/w)	GTE (%v/w)
1	5	5	60	3	4
2	5	10	60	3	4
3	5	15	60	3	4
4	5	20	60	3	4
5	5	OG	60	2	4
6	5	OG	60	3	4
7	5	OG	60	4	4
8	5	OG	60	5	4
9	5	OG	60	OA	0
10	5	OG	60	OA	2
11	5	OG	60	OA	4
12	5	OG	60	OA	6
13	5	OG	60	OA	8

OG: Optimum gelatin variation based on mechanical strength value; OA: Optimum alginate variation based on mechanical strength value

After the media is formed, the bioplastic film was cut with a 5 mm diameter and placed on the sterilized culturing media. The prepared substrate was then incubated at a temperature of 37°C for 24 hours with the position of the plate upside down. After incubation, measuring the distance of the inhibition from the edge of the film and calculation are carried out three times to get the average value as the final result (Amalia et al., 2020).

Texture Analysis

The film thickness was measured using a micrometer with an accuracy of 0.0001 mm. Measurements were made at five different points on the film. Then, the average thickness was calculated, which approximately describes the thickness of the film. Tensile strength and elongation were analyzed using a universal testing machine. The analysis was carried out by cutting the film sample to an elongated shape (20×50 mm^2), then placing the film by clamping it on a tensile test machine. Then the machine was operated, and the retraction was carried out at 100 mm/min speed until the film broke. The initial thickness, width, and length of the film are recorded first. Then the tensile strength value is obtained by determining the stress distribution in the cross-sectional area. The area is obtained by multiplying the initial width by the initial thickness of the film sample. Meanwhile, elongation is calculated by dividing the difference between the final and initial lengths by the initial length of the film (Warkoyo et al., 2021)

$$Average\ thickness\ (t) = \underline{t} = \frac{t_1 + t_2 + t_3 + t_4 + t_5}{5}$$

$$\tau = Fmax/A$$

where

τ = Tensile strength (MPa)

F_{max} = maximum tension (N)

A = cross-sectional area (mm^2)

Water Vapor Transmission (WVT) Rate Analysis

The WVT rate aims to analyze the barrier film refers to the ASTM E96/E96M-16 (2016) procedure. A 30 mL cup or 5 cm diameter glass is filled with 10 grams of silica gel, then the film is glued to the glass, and the initial mass of the cup is calculated. Then the cup/glass is placed in a controlled desiccator at 50-55% RH at a temperature of 27-28°C (in the adjustment of conditions, the RH in the glass is always lower than outside the glass). Water vapor transmission was determined by weighing the cup/glass every 24 hours for seven days. Slope (change of mass in each time interval) is calculated to determine the value of WVTr and converted to units of time in hours (Roy & Rhim, 2021).

$$WVTr = \frac{slope}{sample\ area} \cdot \frac{1\ day}{24\ hour} \left(\frac{gram}{m^2 .\ hour}\right)$$

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis of the edible film that has been posted with a mass of 2 mg is entered into a disk containing 20 mg of KBr salt. Calculations are based on the Perkin-Elmer Spectrum One FTIR Spectrophotometer method in the wave range of 4000 – 400cm^{-1} at a resolution of 2cm^{-1} (Chen et al., 2019).

RESULT AND DISCUSSION

Effect of antioxidant (GTE) concentration on Antioxidant Activity, and antimicrobial activity

Green Tea Extract (GTE) has numerous antioxidant compounds, namely epigallocatechin-3-gallate and epicatechin-3-gallate, which both include flavonoid monomers belonging to the type of catechins (Castro et al. 2019; Pluta-Kubica et al. 2021). The previous analysis of antioxidant activity was reported by Castro et al. (2019), where the antioxidant activity of GTE had a strong effect compared to other antioxidant extracts. Based on previous research, GTE is perfect for combining with fibrous protein. Therefore, this study added GTE to edible film formulation to increase antioxidant and antimicrobial activity.

Table 2. Antioxidant activity (DPPH method) and inhibition range of edible film in varying GTE concentration

GTE (%v/w)	*Dilution*	Antioxidant Akt. (%)	Inhibition (mm)	
			E. coli	*S. aureus*
0	1	0	0	1.73
2	1	31.96	0.80	3.55
4	1	37.88	3.59	5.03
6	1	58.04	4.54	5.55
8	1	63.07	6.01	7.93

Table 2 shows the antioxidant activity of edible film. This analysis was carried out using the DPPH method to evaluate the antioxidant capacity of the film. The addition of GTE to edible velvet bean film increases antioxidant activity. This uplift indicates that the addition of GTE will increase polyphenol levels. Antioxidant activity is closely related to the film's polyphenols levels (Dou et al., 2018).

Escherichia coli		*Staphylococcus aureus*

Figure 1. Antibacterial inhibition test for *edible film*

The antimicrobial activity of edible velvet bean film with variations of GTE against *E. coli* and *S. aureus* is indicated in Figure 1. At concentration of 0% GTE, there was no result in inhibition of *E. coli* cultures. However, there are inhibition results in *S. aureus* cultures. This suggests that the film of the protein matrix has potential as an antimicrobial substance. The increasing GTE levels from the analysis results show that the distance between the inhibition of bacteria and the film is getting farther away. The edible film of velvet bean with the addition of GTE shows selective inhibition of food pathogens. This phenomenon is consistent with previous study (Seydim et al. 2020). Xu et al. (2020) reported that antioxidant compounds from GTE inhibit glucosyltransferase activity responsible for an enzyme that forms chromosomal chains in bacteria. Based on this analysis, the edible film made of velvet bean can be considered as feasible and functional edible film.

Effect of antioxidant (GTE) concentration on mechanical properties

Figure 2 and figure 3 show the thickness, tensile strength (TS), elongation (Elongation at Break or EAB), and WVTr values of edible film in varying GTE concentration (0%, 2%, 6%, and 8%v/w). The result shows that there is no significant different of edible film thickness due to the increase of GTE concentration. It was in good agreement with the previous study (Dou et al. 2018).

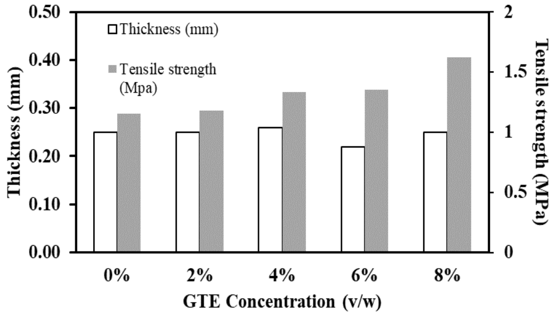

Figure 2. Thickness and TS properties of edible film in varying GTE concentration

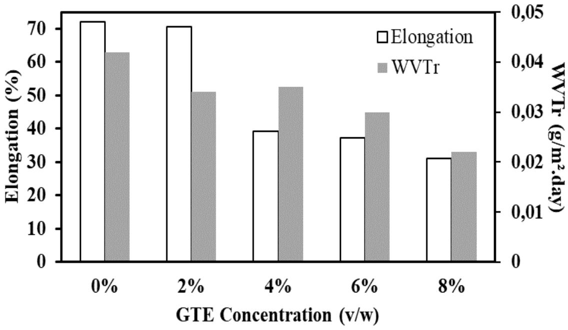

Figure 3. Elongation and WVTr properties of edible film in varying GTE concentration

Figure 2 shows that the higher GTE concentration added would increase TS value. Commonly, the GTE addition can increase the stability of the matrix. The interaction between phenolic compounds (GTE) and protein molecules (velvet beans) would lead the film stiffer and the energy required to break existing interaction is higher than film without GTE addition. The specific interactions are due to positive charge of hydrogen bonds with negative charges of amino compounds (nitrogen and oxygen elements) (Pluta-Kubica et al., 2021). Amino compounds in gelatin and pea protein provide groups with a reverse charge by hydrogen bonds or are also called hydrogen acceptors (Chen et al., 2019). In accordance to Figure 2, TS values of GTE variations meets JIS standard, where the minimum TS is 0.39 MPa.

Figure 3 shows the decrease value of EAB due to GTE addition. It can be caused by the presence of intermolecular crosslinking of polyphenols due to hydrogen bond interactions. This result similar with the previous studies (Chen et al. 2019; Olszewska et al. 2020). In addition, only EAB value in the variation of the 2%v/w GTE that meets JIS standard, where the minimum value of EAB is 70%.

Commonly, the addition of GTE could decrease WVTr properties (Figure 3). The addition of GTE provided a compact formation on the structure of the edible film. This result was in good agreement with the previous work, where the addition of tea polyphenols on edible films from a mixture of gelatin and sodium alginate has an effect as a barrier to air and humidity (Dou et al. 2018). In addition, the values of WVTr in all GTE variations can meet JIS requirement, where the maximum WVTr is 10 g/m^2.day.

Effect of Gelatin Concentration on Mechanical Properties of Edible Film

Figures 4 and 5 show the thickness-TS and elongation-WVTr of edible film in varying gelatin concentration (5, 10, 15, & 15%w/w). Generally, the higher gelatin added then the thicker film obtained (Figure 4). It is caused by the presence of gelatin that increase the viscosity. Furthermore, the differences of amino acid structure, intramolecular distribution, and hydrophobic protein properties can also play a role for this result (Wulandari et al., 2017). The thickness of all gelatin variations successfully meets JIS standard, where the maximum thickness is 0.25 mm.

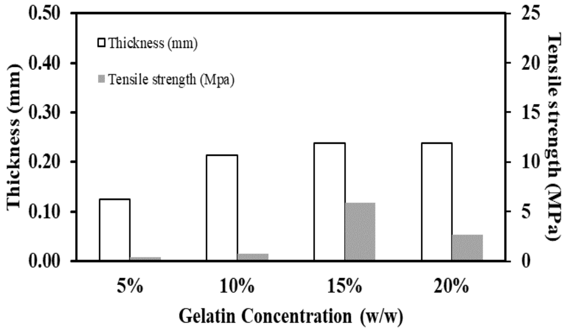

Figure 4. Thickness and TS of edible film in varying gelatin concentration

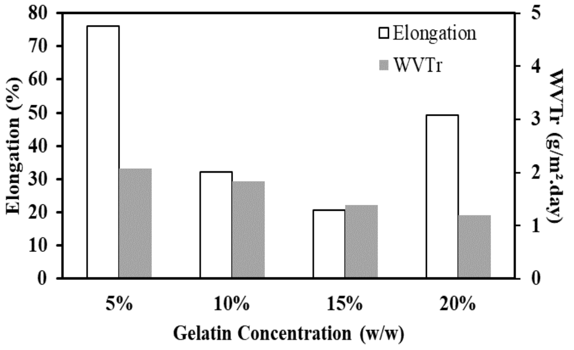

Figure 5. Elongation and WVTr of edible film in varying gelatin concentration

Figure 4 shows that the use of 5% to 15%w/w gelatin concentration will lead the TS value increase. However, the TS value decreased at 20% w/w gelatin concentration. The increase in TS is related to the interaction of protein contained in the velvet bean and gelatin with hydrogen bonded to form a micro-network structure. Similar result was obtained by Robles-Flores et al. (2018) and Córdoba et al. (2017). Meanwhile, the decrease in TS can be caused by an excess of gelatin composition, which can reduce intermolecular interactions and increase biopolymer chain mobility (Nata et al., 2018). The TS of all gelatin variations meets JIS standard, where the minimum TS is 0.39 MPa.

Figure 5 represents EAB value in varying gelatin concentration. Commonly, the use of 5% to 15%w/w gelatin would increase the EAB value. It might be due to the stronger bonds formed between protein of velvet bean and gelatin, thereby reducing the flexibility of the film (Nazmi et al., 2017). Another cause was the polymerization reaction that is not optimal (Nata et al., 2018). However, the use of 20%w/w gelatin concentration increased the elongation of edible film. It can be caused by the imperfect dispersion of gelatin in the protein-to-protein bonds (Robles-Flores et al., 2018). The result depicts that only the use of 5%w/w gelatin could produce edible film which met JIS requirement, where the minimum EAB is 70%.

Figure 5 also shows the decrease value of WVTr in varying gelatin concentration. It means that water and steam are difficult to penetrate the film. The decrease of WVTr could be due to protein interactions between velvet beans with protein from gelatin which can form hydrogen bonds between two polymers. Further, it formed a microtissue structure that inhibited water and vapor (Pérez Córdoba & Sobral, 2017). The WVTr value of all gelatin variations fulfilled JIS standard, where the maximum WVTr is 10 g/m^2.day.

Effect of Alginate Concentration on Mechanical Properties of Edible Film

Figure 6 and 7 show the thickness-TS and elongation-WVTr properties of edible film in varying sodium alginate (SA) concentration (2, 3, 4, and 5%w/w). Commonly, the higher SA used, then the thicker edible film will be obtained (Figure 6). It was caused by the higher concentration of alginate that increase the viscosity of film solution. This result also reported by previous research (Kadzinska et al. 2020). The thickness and structure of the edible film might vary due to the influence of differential film drying kinetics (Chakravartula et al., 2019). The thickness of alginate variation (2%, 3%, and 4%w/w) can meets the JIS standard, where the maximum thickness is 0.25 mm.

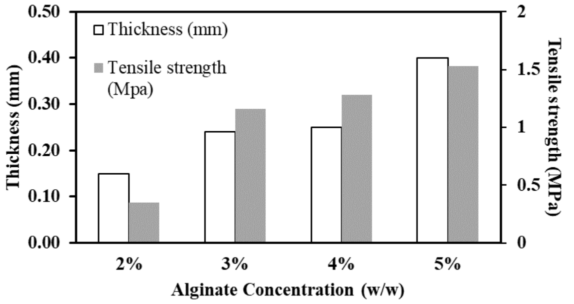

Figure 6. Thickness and TS of edible film in varying alginate concentration

The TS value increased along with the increase of alginate used (Figure 6). It caused by a linear alginate structure that forms a stronger bond with protein. This result was also reported by previous work (Chakravartula et al. 2019). The used of pectin, alginate, and whey protein composites will increase the TS value. Further, the increase of TS was also associated with the $CaCl_2$ crosslinking agent. Divalent cation (Ca^{2+}) causes conformational changes in alginate, such as an increase in the number of G-blocks resulting in the production of a rigid and dense film (Parreidt et al., 2018). TS value of SA variations (3%, 4%, and 5%w/w) could meet JIS standard, where the minimum TS is 0.39 MPa.

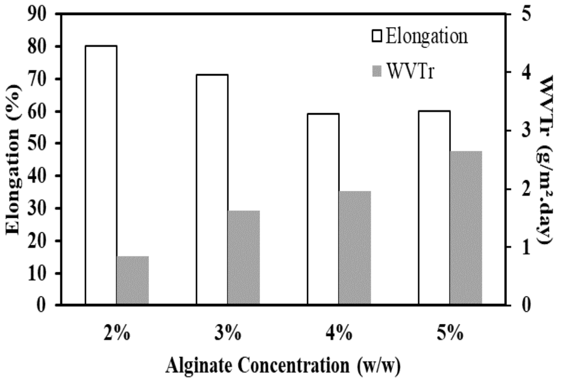

Figure 7. Elongation and WVTr of edible film in varying alginate concentration

Figure 7 shows that the value of EAB decreased along with the higher SA used. It was caused by the linear structure of alginate, which forms a stronger bond with protein from velvet beans (Giyatmi et al., 2020). This result was in good agreement with other work which reported that an edible film made from pectin, alginate, and whey protein composites could decrease the EAB value (Chakravartula et al. 2019).

The higher SA used also increased the value of WVTr as shown in Figure 7. It might be due to the natural hydrophilic nature of SA, which absorbs water and humidity (Wenwen et al., 2019). WVTr in all SA variations fulfilled JIS standard, where the maximum WVTr is 10 g/m^2.day.

Scanning Electron Microscopy **(SEM)**

The microstructure of edible film at the optimum variation of GTE (8%), gelatin (20%), and alginate (5%) is shown in Figure 8. In general, the edible film's structure looks compact and not perforated. In the gelatin variation, the surface is flat and smooth. Then, in the GTE and alginate variations, it seems rough. The addition of GTE did not significantly affect the film's surface structure. The evenly distributed surface is because the green tea extract molecules were homogenous to the film matrix (Dou et al., 2018). On the gelatin variety is a visible non-hollow surface of the film. This structure shows that the corporation of gelatin and the protein of the velvet bean gives a denser structure so that the holes in the film are smaller (Lin et al*.*, 2020).

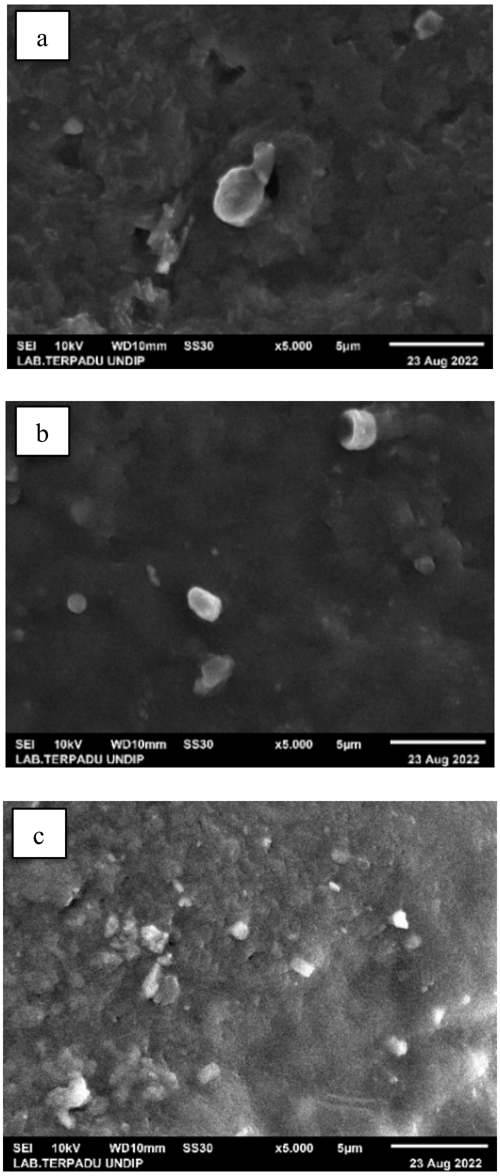

Figure 8. Surface morphology of edible film on maximum variation of (a) GTE; (b) Gelatin; (c)Alginate

In the GTE and gelatin variations, it can be seen that there are large white particles, but in the alginate variation, there are more white particles. This structure shows that the presence of these particles is due to differences in surface tension during drying (Kadzinska et al*.,* 2019). The phenomenon can also be caused by the hydrophilic properties of SA, which affect the low solubility of SA in water (Prasetyaningrum et al*.,* 2021).

The Chemical Properties of Edible Film Fortified with GTE

The infrared spectra of velvet bean edible film with GTE (8%v/w) were showed in Figure 9. There are four peaks represents the functional group of edible film with the presence of GTE (8%v/w). The four peaks consist of OH stretch (3300-3200 cm^{-1}), Aromatic C-H sp^2 bend (3100-3000 cm^{-1}), C=O stretch (1700-1600 cm^{-1}), and Alkoxy C-O stretch (1100-1000 cm^{-1}) (Beauchamp, 2017). A particular compound that have abundant antioxidant activity in GTE is flavonoid monomers which is known as catechins in the form of epigallocatechin-3-gallate and epicatechin-3-gallate compounds (Theansungnoen et al., 2022). The catechin compound is a polyphenol that contains an OH- group, C-H sp^2 aromatic group, carbonyl group (C=O), an alkoxy group (C-O). All four groups of catechin compounds have been found in FTIR spectra results. The same results were reported by the previous research (Dou et al. 2018). Therefore, this result indicated that edible film contained antioxidant compounds.

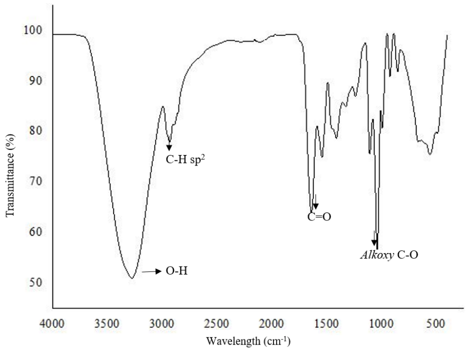

Figure 9. FTIR spectra of edible film in the presence of GTE

CONCLUSIONS

Edible film derived from velvet beans (*Mucuna pruriens*) with modified gelatin and alginate as a plasticizer and green tea leaves have been successfully prepared. In general, the mechanical properties of edible film met the JIS standards. The addition of gelatin, alginate, and green tea extract was found to change mechanical properties of the edible film (TS, EAB, WVTr, elongation). The SEM result also showed a compact edible film. In addition, the edible film also had antioxidant activity and antimicrobial properties due to the presence of green tea extract and this compound was indicated by FTIR spectra. The optimum composition of gelatin, alginate, and green tea leaves, which resulted in JIS standard-edible film are 5%w/w, 3%w/w, and 2%v/w, respectively.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Education, Culture, Research and Technology Republik of Indonesia and Diponegoro University through "Program Kreativitas Mahasiswa" PKM 2022.

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