Development of Alginate-based Antibacterial Edible Films by Incoporating Green Betel Leaf Extract

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Keywords: Antibacterial, Edible Film, Green Betel Leaf Extract, *Staphylococcus aureus*, *Escherichia coli*.

Abstract: The aim of this research was to develop an environmentally friendly packaging of alginate-based antibacterial edible films. The antibacterial sources used was green betel leaf extract. Concentration levels of green betel leaf extract which was studied were 0,5%, 1,0% and 1,5%. The edible film obtained was evaluated physically, mechanically, chemically and microbiologically including parameters of Water Vapor Transmission Rate (WVTR), water solubility, tensile strength, elongation, brightness, thickness, moisture content, and antibacterial clear zone test. Experiment was conducted two replicates. The results showed that concentration level of green betel leaf extract affected on the quality and antibacterial properties of alginate-based edible films. Overall, the best alginate-based antibacterial edible film is produced by incorporating green betel leaf extract at concentration level of 1,5%.

1 INTRODUCTION

Edible films are types of biodegradable plastic that attract scientists around the world to explore. Edible films have been employed to pack a variety of food products, such as meat, sausages, fresh fruits and vegetables. Edible films can maintain the quality of packaged food because edible film can withstand carbon dioxide, oxygen diffusion, water evaporation, and flavor contamination with other products. Another benefit of edible films is that it can extend the shelf life and is environmentally friendly. Edible films can be consumed together with the product. (Junianto *et al*. 2012). The main components of edible film can be grouped into three categories, namely hydrocolloids, lipids, and composites. Composites are a combination of hydrocolloids and lipids. The hydrocolloids that can be used to make edible films are proteins and carbohydrates. Meanwhile, the lipids used are wax and fatty acids (Donhowe and Fema, 2994). Alginate is one of hydrocolloids polysaccharides which are available in nature derived from seaweed and can be applied as bio polymeric film. Film forming and binding ability of alginate makes it suitable for the use of packaging material (Parveen *et al*, 2019).

Production of edible films from alginate have been explored by Murdinah *et al*. (2007), Khairunnisa *et al.* (2018), Koushki *et al*. (2015) and others. Alginate based edible films are found strong, but poor water resistance due to hydrophilic nature. One way to increase the performance of alginate based edible film is by developing it as an active packaging. The film can be used as a carrier of antioxidants, flavoring and/or coloring agents and antimicrobials to improve food safety and quality (Siracusa *et al*, 2018). Incorporating antimicrobial into alginate film can increase the resistance of the film to bacteria so that the film has good mechanical quality and can inhibit bacteria that potentially contaminate food.

One of the natural ingredients that contain antimicrobials is green betel leaf. The active antibacterial components found in green betel are tannins, flavonoids and essential oils (Baskaran *et al*., 2011). These compounds are able to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*. These bacteria produce enterotoxins which can cause diarrhea. The antimicrobial activity of betel leaf active compounds is by denaturing proteins and damaging bacterial cell walls, as well as destructing lipids in cell membranes by reducing the surface tension of the cell membranes.

This study was aimed to develop an environmentally friendly packaging of alginate-based

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antibacterial edible films by using green betel leaf extract as antimicrobial agent. added during the process. Meanwhile, physical, mechanical, chemical and antibacterial properties of the edible film were also observed.

2 MATERIALS AND METHODS

2.1 Material

Alginate flour used was extracted from *Sargassum* sp. by employing an extraction method developed by Yunizal (2000). The modified cassava (mocaf) flour used was the "Ladang Lima" brand, while sorbitol and beeswax were purchased from PT Geochem Globalindo, Jakarta. Green betel leaves were obtained from the Ciracas market, East Jakarta.

2.2 Method

2.2.1 Extraction of Antibacterial Compounds from Green Betel Leaves

Antibacterial from green betel leaves were extracted through modifying a method previously employed by Sitorus (2018) for extracting antibacterial from betel leaves as well. After washing, the betel leaves were chopped so that they were exposed to more ethanol. The mixing was carried out in a large and closed container so that the maceration process of the material run perfectly. During mixing, the mixture was stirred periodically. After that, the soaking process was conducted for 3 days with stirring every 24 hours. Then the mixture was filtered with a 100 mesh filter cloth and the obtained filtrate was evaporated using a rotary vacuum evaporator until a thick extract was obtained (Sitorus, 2018).

2.2.2 Preparation of Edible Film

Edible film was prepared according to the experiment conducted by Murdinah *et al* (2007) with modification. About 97 mL of distilled water in a beaker glass was heated to 50ºC, then 1,5 g of alginate powder was gradually added while stirring with a magnetic stirrer. After that, 0,3 g of mocaf starch was added with stirring for 30 minutes. After mixing completely, the antibacterial green betel leaf extract was added into the solution according to the treatment (0,5%; 1,0%; 1,5%) and stirred for 30 minutes until evenly mixed at 75ºC. Furthermore, the solution is casted by pouring into a square acrylic plate, where one edible film solution recipe can be casted on two acrylic plates. After that the acrylic plate was left to stand at room temperature for approximately 24 hours and then the dried edible film was removed.

2.2.3 Experiment

Experiment was executed in two stages, i.e. preliminary and main experiments. Preliminary experiment was conducted to investigate antibacterial activity of green betel leaf extract to *S. aureus* and *E. coli*. The results of this preliminary experiment will be used in the main experiment. The main research was performed to determine the optimum addition level of green betel leaf extract as antibacterial agent on alginate-based edible film. The concentration level of betel leaf extract tested consisted of 3 levels (0,5%, 1,0%, 1,5%) with 2 replications.

2.2.4 Analyses

Alginate based antibacterial edible films obtained were assessed their performance in terms of physical, mechanical, chemical and antibacterial properties including thickness using digital micrometer (Mitutoyo, Japan), tensile strength and elongation using Texture Analyzer (TAXT Plus, Stable Micro System, UK) (Balqis *et al*., 2017), water vapor transmission rate (WVTR) based on ASTM E96 using PERME® W3 / 031 Water Vapor Transmission Rate Tester (Labthink, China), brightness using Hunter Lab, water solubility (Murni *et al,* 2013), moisture content (Farhan and Hani, 2017), as well as antibacterial activity for *S. aureus* and *E.coli* (da Silva et al, 2013) and surface morphological analysis using SEM (*Scanning Electron Microscopy*) JEOL JSM-6360LA, Japan (Setiani et al, 2013).

3 RESULT AND DISCUSSION

3.1 Antibacterial Activity of Green Betel Leaf Extract

Antibacterial compounds extracted from green betel leaves using alcohol as the extracting solvent showed antibacterial activity on *S. aureus* and *E. coli* as shown in Table 1. The highest antibacterial activity of green betel extract against *S. aureus* based on the largest inhibition zone shown at the extract concentration of 1% was 14 mm, and the lowest activity exhibited by the extract concentration of 0,5% was 12,50 mm. While, the highest inhibition zone of green betel leaf extract against *E.* coli occurred at a concentration of 1,0% extract was 14 mm, and the lowest zone of inhibition shown at a concentration of 0,5% extract was 12,50 mm.

Table 1: Antibacterial activity of green betel leaf extract (Clear Zone, mm).

Microorganism	Concentration of Green Betel Leaf Extract		
	0,5%	1,0%	1,5%
Staphylococcus aureus	14,00	13,50	14,00
Escherichia coli	12,50	14,00	13,00

The antibacterial activity of betel leaf extract is suspected to be produced by the chemical compounds of the betel leaves, namely saponins, flavonoids, polyphenols, and essential oils. Saponin compounds can work as antimicrobials by damaging the cytoplasmic membrane and killing cells. Flavonoid compounds are thought to denaturate bacterial cell proteins and irreparably damage cell membranes. Natural phenols contained in essential oils have antiseptic properties 5 times stronger than ordinary phenols (bactericides and fungicides) but are not sporacid. The mechanism of phenol as an antibacterial agent acts as a toxin in the protoplasm, damages and penetrates the walls and precipitates bacterial cell proteins (Carolia and Noventi, 2016).

The main components of essential oil are betel phenol and some of its derivatives including eugenol allypyrocatechin 26,8 – 42,5%, cineol 2,4 – 4,8%, mehyl eugenol 4,2 – 15,8%, caryophyllen 3,0 -9,8 %, kavikol hydroxy, kavikol 7,2-16,7%, kabivetol 2,7 6,2%, estragol, ilypryrocatekol 9,6%, carvacol 2,2 – 5,6%, alkaloids, flavonoids, triterpenoids or steroids, saponins, terpenes, phenylpropane, terpinen, diastase from 0,8 - 1,8%, and tannins 1,0 – 1,3% (Damayanti and Mulyono, 2003). Phenol at a concentration of 0,1 – 1,0% is bacteriostatic, while it is as bactericidal at a concentration of 1 - 2% (Fuadi, 2014),

3.2 Characteristics of Alginate-based Antibacterial Edible Film

The moisture content of the edible film increased with the increase in the concentration of antibacterial extract of green betel leaves in the film (Table 2). Suryaningrum *et al* (2005) noted that edible films being biodegradable with high moisture content will be easily grown by microorganisms, due to the presence of nutritional components in the film such as protein. On the otherhand, edible films with low moisture content will be more resistant to microbiological destruction.

WVTR value of alginate edible film with different antibacterial concentration treatment resulted in different WVTR values with a tendency to decrease with increasing concentration. WVTR test results for edible film made with various concentrations of betel leaf extract yielded WVTR values ranging from 1640,65 to 1871,79 g/m^2.24h. The lowest WVTR value of edible film was obtained at the addition of 1,5% antibacterial extract of betel leaves. Irianto *et al* (2006) reported that the lowest WVTR was resulted from a combination of 2,0% carrageenan, 0,7% tapioca and 0,3% beeswax, i.e. 746,2g/m^2/day, which was lower than the value from this study.

The WVTR value in this study showed that the edible film with the addition of green betel leaf extract had a WVTR value which increased in line with the increase in the addition level of betel leaf extract. The higher the WVTR value, the higher the permeability of the edible film. When edible film is made into packaging, it will cause more moisture to come out and enter the package. A good edible film must not be easily passed by moisture or have a low WVTR. The compact edible film structure can inhibit water vapor diffusion through the edible film. Betel leaf extract contains oil and water. Oil components have high protective properties against water vapor which will reduce the hydrophilic properties of the film. Guilbert & Biquet (1996) informed that the fatty components such as wax, emulsifier and fatty acids in composite edible films have an effect on reducing WVTR because fat has low polarity and a dense crystal structure.

Table 2: Physical and Chemical properties of alginate based antibacterial edible film.

Parameter	Concentration of Green Betel Leaf Extract		
	0,5%	1,0%	1,5%
Moisture Content (%)	11,09±0,40	11,53±0,04	12,42±0,10
WVTR (g/m^2.24h)	1871,79±68,19	1790,28±4,15	1640,65±57,05
Water Solubility (%)	57,98±10,22	70,59±1,68	84,95±11,90
Brightness (%)	14,2±1,27	12,1±0,27	11,5±1,70
Thickness (mm)	0,114±0,014	0,104±0,013	0,108±0,008

The water solubility value of alginate edible film with the addition of antibacterial extract of betel leaves at different concentrations resulted in film solubility which increased with increasing concentration of betel leaf extract. The more betel leaf extract is added, the more easily the edible film will dissolve. The lowest solubility value indicates that the edible film is the best because it plays a very important role when the film is used for edible products. This is also in accordance with the opinion of Stuchell and Krochta (1994) that if the application of a film is expected as an edible packaging, high solubility is desired. Likewise, if the application of edible film on foods with high water content, a film that is not water-soluble is used. Imeson (1999) adds that water resistance is an important property for films applied as food protection.

The addition level of green betel leaf extract affected the brightness of alginate edible film, in which increasing addition level of betel leaf extract resulted in edible film with lower brightness percentage values. Ningsih (2015) mentioned that the basic material used will affect the color of the edible film. Therefore, the edible film using different antibacterial concentrations will bring about different brightness of edible films.

The use of betel leaf extract with different concentrations in the production of alginate edible film did not significantly affect the thickness of the edible film, in which the thickness of edible film obtained in this study was in the range of 0,104 – 0,114 mm. Those values are thicker compared to thickness values of edible film made of composite consisting of carrageenan, tapioca flour and beeswax, i.e. 0,050 – 0,074 mm (Irianto *et al*, 2006). Meanwhile, Saputro *et al* (2017) produced edible films with a thickness of 0,03 – 0,08 mm which was carrageenan, tapioca flour and glycerol used as raw materials. According to Wahyu (2009) that a good edible film has a thickness of 0,15 – 0,20 mm which can withstand external influences and reduce WVTR. Thickness is an important parameter that needs to be considered in the use of edible film as a packaging. The thickness of the film will affect the gas permeability. The thicker the edible film, the lower the gas permeability, which can protect the product.

The tensile strength values of alginate edible film were insignificantly influenced by the addition levels of green betel leaf extract. The higher addition of the betel leaf extract did not result in a pronounced difference in tensile strength of the edible film. The tensile strength of the edible film in this study was in the range of 1,31±0,84 – 2,67±0,51 MPa (Table 3). Those tensile strength values are not in the range of ASTM polypropylene standard values, i.e. 24,7-302 MPa. In addition, the best tensile strength of edible films is between 10 - 100 MPa while edible films with a tensile strength range of 1 - 10 MPa are classified as marginal (Han and Gennadios, 2005). The increase in the forces of attraction between the molecules making up the edible film induces an increase in the strength of its structure. The greater the tensile value, the stiffness of a material will increase or be inelastic. Sara (2015) noted that tensile strength is a mechanical property related to the strength of the edible film to withstand physical damage when applied as food packaging. Edible film with high tensile strength value is highly expected in order to minimize packaged product damage due to mechanical interferences.

Table 3: Mechanical properties of alginate based antibacterial edible film.

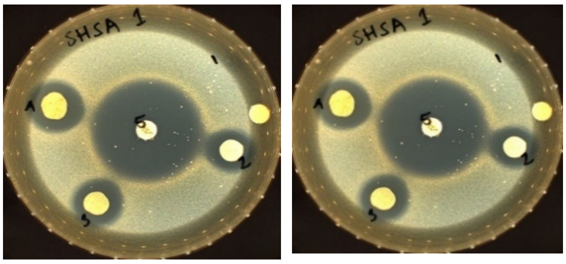
Parameter	Concentration of Green Betel Leaf Extract		
	0,5%	1,0%	1,5%
Tensile Strength (Mpa)	2,52±0,75	2,67±0,51	1,31±0,84
Elongation (%)	47,05±18,88	38,30±4,95	24,40±13,58

The elongation test results showed that the more the betel leaf extract on the alginate edible film, the lower the elongation value. The elongation percentage of the film is categorized as poor if the value is less than 10% and good if the elongation percentage exceeds 50%. Elongation percentage of alginate based edible film added with various concentrations of betel leaf extract in this study was in the range of 24,40±13,5 – 47,05±18,88 %. The elongation value of the edible film from this study was lower than the elongation value of edible film made of bligon goat skin gelatin, namely 70,97 – 95,33% (Said *et al.*, 2013). Edible films from whey and agar have comparable elongation, i.e. 26,06 – 34,52% (Hakim, 2015). Elongation of edible films made from avocado seed starch and agar is smaller, i.e. 0,17 – 0,45% (Coniwanti *et al*, 2016).

The antibacterial test showed that the alginate-based edible film incorporated with green betel leaf extract had antibacterial activity against *S. aureus* and *E. coli* (Table 4) as indicated by the formation of a clear zone on the test medium (Figure 1). The antibacterial activity was getting more significant with the higher concentration level of antibacterial betel leaf extract, both for *S. aureus* and *E. coli* which showed the same trend. Thus, if greater antibacterial activity is expected, the addition level of betel leaf extract must be increased. The development of antibacterial edible films based on various types of starch has also been carried out using several antibacterial sources from the extracts of beluntas leaves (Mulyadi *et al*, 2016), garlic (Anggraini *et al*, 2018), red galangal/*Alpinia purpurata* (Sholehah *et al*, 2016) and areca nut (Ningsih, 2018).

Table 4: Antibacterial properties (Clear Zone, mm) of alginate based edible film added with green betel leaf extract.

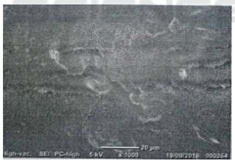
Parameter	Concentration of Green Betel Leaf Extract		
	0,5%	1,0%	1,5%
Staphylococcus aureus	14,25	17,85	18,15
Escherichia coli	12,40	14,00	13,40



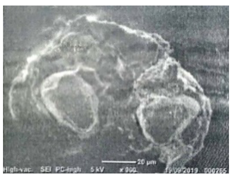
(1) *Staphylococcus aureus* (2) *Escherichia coli*

Figure 1: Clear zone as antibacterial effects of green betel leaf extract of alginate based edible film on *S. aureus* and *E coli*.

The molecular structure surface of the alginate edible film is dense at 500 times magnification.



The molecular structure surface of the alginate edible film is dense at 1000 times magnification.



The molecular structure surface of the alginate edible film is not dense at 2000 times magnification

Figure 2: SEM images of alginate edible film added with 1.5% betel leaf extract with three times magnification.

Surface morphological analysis using SEM was carried out on alginate based edible film added with green betel leaf extract at a concentration of 1.5% (Figure 2), generally showing the best physical, mechanical and chemical properties. Analyses revealed that the molecular structure surface of the alginate edible film at 500 and 1000 times magnification was dense. The higher the magnification used, the more obvious the surface morphology of the edible film, and it turned out that it showed the lack of molecular density in the resulting edible film. The less dense structure of these molecules brought about more water to be absorbed. The image also showed a less smooth and porous surface. The non-smooth surface indicated that the film was less homogeneous. This occurrence was probably due to a too short stirring period, so that it resulted in inhomogeneous edible film solution inducing a non-dense molecular structure surface of the alginate edible film. Therefore, further study to explore this phenomenon are highly recommended.

4 CONCLUSION

1) Green betel leaf extract has antibacterial activity against *S. aureus* and *E. coli*
2) Alginate-based edible film added with green betel leaf extract has relatively good physical and mechanical characteristics, especially in terms of thickness, tensile strength, elongation and WVTR.
3) Edible film added with betel leaf extract as antibacterial showed antibacterial activity against *S. aureus* and *E, coli*; so it can be called as an antibacterial edible film
4) Antibacterial edible film is recommended for performance testing by being used as a packaging for food products

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AUTHORS' CONTRIBUTIONS

Giyatmi Giyatmi and Hari Eko Irianto are the main contributors in conducting experiment and preparing the draft of manuscript, while Mohammad Sabariman and Bintang Anggoro are as supporting contributors. All authors read and approved the final manuscript.

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