Use of basil leaf ethanol extract in alginate base edible film

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Abstract. Alginate is usually obtained by extracting from brown seaweed, mainly Sargassum sp which grows almost along the coasts of the islands in Indonesia. One of the efforts to utilize alginate was by employing as raw material in the production of edible film. The disadvantage of films made from carbohydrates is that they are not very good at regulating water vapor migration. One way to improve performance of edible film in inhibiting water vapor transmission is to add vegetable oil. In this study, the effect of adding basil leaf ethanol extract was observed to improve the performance of alginate-based edible film. Basil leaf extract was added at concentrations of 0.5%, 1% and 1.5%. The resulting edible films were observed for chemical characteristics (moisture content), physical characteristics (thickness, tensile strength, water vapor transmission rate, elongation, brightness, solubility) and antibacterial performance. The results showed that the concentration of basil leaf extract had a significant effect on moisture content, thickness, tensile strength, water vapor transmission rate (WVTR), and elongation values. Edible films do not have antibacterial activity against Staphylococcus aureus and Escherichia coli. The addition of basil leaf extract at a higher concentration resulted in an alginate-based edible film with lower moisture content, higher water solubility, higher thickness, higher tensile strength, lower WVTR, and lower elongation percentage.

1. Introduction
Alginate is a heteropolysaccharide compound which is composed of mannuronic acid monomer chains (Poly-D mannuronic acid) and guluronic acid (Poly-L guluronic acid) from cell walls which are commonly found in brown algae (Phaeophyta) [1]. Considering to the availability of brown seaweed as a raw material growing wild in nature, alginate is usually extracted from Sargassum sp. This seaweed grows almost along the coasts of the islands in Indonesia, especially on the coast consisting of dead coral slabs [2].

Alginate is an important ingredient as a thickener or emulsifier that is needed in various food, non-food and medical or pharmaceutical industries, but alginate to meet industrial demand is still imported from abroad. Alginate based on its quality is divided into 3 quality groups, namely industrial grade, food grade and pharmaceutical grade, in which the extraction process requires different raw material quality and processing according to the expected alginate quality [3]. Alginate is widely used in various industries such as food, beverage, textile, printing, and pharmaceutical as a thickening agent, stabilizer, emulsifier, chelating agent, encapsulation, swelling, a suspending agent, or used to form gels, films, and membrane. Sodium alginate is the most common salt of alginate [4].
Alginate is able to form insoluble gels or polymers being resistant in the presence of polyvalent metal cations such as calcium. The gelling mechanism is based on the interaction between calcium ions and carboxylate groups, forming a three-dimensional cross-linking network. This interaction occurs when the components (alginate-calcium) are mixed to form a film or by pouring a solution of calcium onto a previously dried alginate layer [5]. The performance of edible films made from hydrocolloids such as alginate is not as good as a moisture barrier to hydrophilic in nature, because the transfer of water vapor through the film is not linear with the partial pressure gradient of water vapor [6].

This study investigates the possible use of an ethanol extract of basil leaves to improve the properties of edible film. Basil leaf ethanol extract (BLEE) contained secondary metabolite compounds of essential oils, flavonoids alkaloid, saponin, phenol and tannins [7,8]. Basil essential oil can improve physical and mechanical properties such as tensile and elongation strength as well as water vapor and oxygen transfer restriction of edible films, in which other essential oils have also demonstrated. Lemongrass essential oil improved the tensile strength and elongation of the bioplastic of chitosan and sorbitol mixture [9]. The addition of garlic essential oils to the carrageenan edible film reduced the tensile strength and water vapor transmission rate, as well as increased the elongation percentage [10]. The addition of bergamot essential oil improved the quality of bio cellulose edible film, especially in reducing the water vapor permeability value and increasing elasticity [11].

BLEE has possibility to be employed as antibacterial agent, as has been demonstrated by some studies. BLEE showed antibacterial activity against Streptococcus mutants [12], Staphylococcus aureus and Salmonella typhii [13]. Staphylococcus epidermidis [14,15], Pseudomonas aeruginosa [14] and Escherichia coli [7]. With regard to the above explanation, this study was carried out with the aim of exploring the use of BLEE to improve the characteristics of alginate-based edible films, particularly the physical, mechanical and antibacterial properties.

2. Methodology

2.1. Materials
Sargassum used to extract alginate was purchased from a seaweed trader in Gunungkidul, Jogjakarta Province. Fresh green basil leaves were obtained purposively from the Ciracas market, Jakarta Province. “Ladang Lima” brand - Mocaf (modified cassava) starch was used. Sorbitol and beeswax were supplied by PT Geochem Globalindo, Jakarta.

2.1.1. Extraction of Alginate
Dried Sargassum was soaked in NaOH solution for 2 hours and neutralized with fresh water. Alginate extraction was carried out by boiling Sargassum in Na2CO3 solution for 2 hours. The seaweed was filtered and the filtrate was blanched with NaOCl. Furthermore, the filtrate was added with HCl solution until pH 2.5-3 to obtain alginic acid. The alginic acid was washed with water and added with NaOH solution until pH 9-10 to obtain sodium alginate. The sodium alginate was precipitated with isopropanol to obtain alginate fibers. The alginate fibers were sun dried and then milled to obtain alginate flour.

2.1.2. Extraction of Antibacterial Compounds
Two kilograms’ basil leaves were soaked in 20 liters of 96% ethanol using a large closed container so that the extraction process run perfectly. Soaking was carried out for 3x24 hours with occasional stirring every 24 hours. After that, the mixture was filtered using a 100 mesh filter cloth to separate the filtrate and the soaking solution. The filtrate was evaporated using a rotary vacuum evaporator to obtain a viscous anti-bacterial extract or BLEE.

2.1.3. Edible Film Preparation
The modification of method used by Murdinah [18] was the addition of mocaf starch, sorbitol and antibacterial agents (BLEE). Firstly, 97 mL of distilled water was put into a beaker glass and heated to 50oC. After that, 1.5 g of alginate powder was put slowly into the beaker glass to avoid lumps. Gradually, 0.3 g mocaf starch, 3 mL sorbitol and 0.25 g beeswax were added until homogeneous.
Antibacterial agent of BLEE act was then added and leave it with stirring for about 30 minutes at 75oC. The amount of BLEE added varied according to the concentration levels being tested. Each added ingredients is preheated to 80oC to avoid clumping. Homogenization for each ingredient added was carried out for approximately 15-30 minutes. The solution obtained was casted by pouring 50 ml edible film solution on a square acrylic glass plate, and left it to dry at room temperature for 24 hours. After drying, the edible film was peeled off.

2.2. Experiment
The addition levels of BLEE to the alginate-based edible film being tested for their effects were 0.5%, 1.0% and 1.5%. Those concentration levels referred to the results of the research conducted by Sholehah et al [19], in which the optimum addition level of red galangal essential oil as antibacterial on carrageenan-based edible film was 1.0%. Before being added to the alginate edible film, the BLEE was tested for antibacterial activity against Staphylococcus aureus and Escherichia coli at concentrations of 0.5%, 1.0% and 1.5%.

2.3. Analyses
Edible films obtained were analyzed for moisture content, water vapor transmission rate (WVTR), thickness, tensile strength, elongation, brightness, water solubility and antibacterial activity. Moisture content is determined by drying in an oven at 105oC for 2 hours [20]. WVTR was assessed according to the ASTM standard method E 96. Thickness was measured using a Mitutoyo digital micrometer - Japan with an accuracy level of 0.001 mm and measurement was carried out at least at ten different random points [21]. Tensile strength and elongation were determined using Texture Analyzer (TAXT Plus, Stable Micro System, UK), in which sample was prepared by cutting to a 15 x 1.5 cm square and clamped in the grip on Texture Analyzer at 2 mm / s speed and stopped when the sample was cut off [22]. Hunter Lab was used to determine brightness value of edible films. A method used by Murni et al. [23] in their experiment was employed to measure water solubility of edible film. Antibacterial activity of BLEE was assessed using a disc diffusion method with chloramphenicol as positive control and 2% acetic acid as negative control, while that of alginate based edible film was evaluated with chloramphenicol as a positive control and a blank disk as a negative control [24].

3. Result and Discussion

3.1. Chemical and Physical Properties
The chemical parameter of alginate-based edible film analyzed in this study was moisture content, while the physical parameter included water vapor transmission rate (WVTR), water solubility and thickness of the film (Figure 1).

3.1.1. Moisture Content
The moisture content of edible films plays an important role in maintaining the quality of the packaged products, so that edible films with low moisture content might have a little chance of affecting the moisture content of the packaged products.
Figure 1. Chemical and Physical Properties of Alginate Based Antibacterial Edible Film

The results showed that the addition level of BLEE had an effect on the moisture content of the alginate edible film, where the higher concentration of BLEE resulted in the edible film with lower moisture content. This implies that the addition of BLEE to the film matrix reduced the hygroscopicity of the films, because the hydrophobic BLEE reduced the water-retention capacity of the films. The similar occurrence was also found in the addition of sunflower seed oil into mung bean edible film [25] and the incorporation soybean oil into edible films made of thermoplastic starch and poly (butylene adipate co-terephthalate) mixture [26].

Edible films with a BLEE concentration of 0.5% had a higher moisture content. Increasing the BLEE concentration in edible films showed a trend to reduce the moisture content of the obtained edible films. The phenomenon of decreasing moisture content with an increase in the concentration of BLEE in edible films is caused by BLEE as one of the base materials carrying dissolved solids in the solution for producing edible films which caused hydrogen bonds to form between the molecules making up the edible film. This resulted in reduced free water content in the resulting edible film.

3.1.2. Water Vapor Transmission Rate (WVTR)

The results of the WVTR determination on alginate edible film with the addition of BLEE as much as 0.5%, 1.0% and 1.5% were 2730.15 g/m2.24h, 2093.12 g/m2.24h and 1961.446 g/m2.24h respectively, in which the higher addition levels of BLEE, the lower WVTR values. A good edible film is indicated by a low WVTR value [27]. Edible films with low WVTR can inhibit the loss of water from the product so that the product quality is maintained. In addition, the low WVTR can inhibit product deterioration due to hydrolysis and microorganism activities in the presence of water.

The WVTR values are influenced by several factors. The addition of essential oils is one of the factors that can affect the WVTR values. The more solids contained in the edible film, the smaller WVTR values. This occurrence brings about water vapor from outside the package not easily absorbed by the packaging material and not make the product to easily come into contact with outside air generating oxidation [28]. Essential oil is one of components contained in BLEE [7].

3.1.3. Water Solubility

The water solubility of the edible film is an important factor that determines the biodegradability of the film as a packaging. Measurement of the solubility of edible film aims to determine the ability of edible film to dissolve in water and to hold water. High solubility causes the edible film to dissolve easily in water and its ability to hold water is reduced. Edible film with high solubility is expected for use in ready-to-eat food products because it dissolves easily when consumed [29]. High solubility is also related to the biodegradation properties of edible films. On the other hand, low solubility is one of the important requirements for edible films, especially for use as food packaging which generally has a high moisture content and water activity or in the use of edible films that come into contact with water and act as a protective food product [30,31].
Water solubility values of alginate based edible film added with BLEE at the concentration levels of 0.50%, 1.00% and 1.59% were in the range of 60.75% – 75.23%, in which edible film with 1.5% BLEE had the highest solubility value. The water solubility values of the film were comparable to the values of arrowroot starch – carrageenan based biofilms [32]. The water solubility of alginate based edible film tended to increase with increasing BLEE concentration. A similar trend was reported that water solubility of the taro starch edible film increased with the increasing concentration of galangal essential oil [33]. This occurrence is presumably because the addition of BLEE may cause damage to the polymer and hydrogen bond structure of the alginate, thereby increasing the solubility in water. Essential oil as one of the components of BLEE has a hydroxyl group so that the greater the BLEE concentration causes an increase in the hydroxyl group in the alginate-based edible film. The more hydroxyl groups contained in the edible film matrix, the higher the water solubility of the film [34].

3.1.4. Thickness
Film thickness is an important characteristic in determining the feasibility of edible films as food packaging because thickness greatly affects the other physical and WVTR. A thick edible film will provide better protection for packaged food products, but the WVTR will be higher. Thick edible films will increase tensile strength, but the elongation value will decrease [35].

Results showed that the thickness of the edible film tended to increase with the increase in BLEE concentration. The increase in the concentration of BLEE in making edible films caused an increase in dissolved solids in the edible film solution so that the thickness of the edible film produced was higher. The thickness of alginate based edible films incorporated with BLEE at the concentration of 0.50%, 1.00% and 1.50% was 0.084 mm, 0.082 mm and 0.112 mm correspondingly. Those thickness values are classified as good because they are below the maximum standard of edible film thickness according to the Japanese Industrial Standard, which is 0.25 mm [35]. The increase in the thickness of the edible film was also related to the unique properties of colloid compounds as a thickener and suspension, and the interaction between the constituent components of the edible film [36].

3.2. Mechanical Properties
The important mechanical parameters used in this study to assess the feasibility of alginate based edible films to be employed as food packaging material to replace non-biodegradable plastics were tensile strength and elongation (Figure 2).

3.2.1. Tensile Strength
Tensile strength is one of the most important mechanical properties in determining the characteristics of edible film because high tensile strength will be able to protect the product to be packaged from mechanical disturbances. Tensile strength values of alginate based edible films incorporated with BLEE at the concentration of 0.50%, 1.00% and 1.50% were 2.56 Mpa, 2.75 Mpa and 3.13 Mpa respectively. Another study revealed that tensile strength of alginate based edible film with polyethylene glycol as plasticizer varied between 2.83±0.16 to 43.13±0.33 MPa [37]. The highest tensile strength was shown by edible film added with the highest level of BLEE addition, i.e. 1.50%. Thus, the addition of BLEE could have an effect on the tensile strength of the edible film and showed that the edible film had a better strength to withstand pressure.

The results clearly revealed that the addition of BLEE to the edible film packaging with a higher concentration will affect the physical properties of the packaging material. The higher the BLEE concentration, the higher the tensile strength of edible film will be. This occurrence was due to that BLEE interfered with the formation of the film matrix by the alginate polymer and plasticizer, so that the film formation was not optimal. BLEE contained solute components entering the film matrix network so that it disrupted and weakened the bonds between polymers.
The high tensile strength value of the edible film is expected, since edible film with this characteristic is suitable to be used as a packaging material. The higher the tensile strength of edible film, the better its strength to withstand the applied pressure [38]. Likewise, conversely the lower the tensile strength value, the worse its ability to withstand the applied pressure.

3.2.2. Elongation
Elongation is the percentage increase in the length of the film when it is pulled until breakage. The elongation value is inversely proportional to the tensile strength value. Elongation analysis is carried out to determine the stretch ability of the edible film, the higher the elongation value, the more flexible and plastic the edible film packaging will be [28]. Results showed that the elongation values of edible film in this study were in the range of 45.50% – 60.40%. The highest elongation value was exhibited by alginate based edible film with 0.50% BLEE addition, while the lowest one was shown by edible film with 1.50% BLEE addition. The Japanese Industrial Standard mentioned that elongation percentage categorized as good if the value was more than 50% and bad if the value was less than 10% [35]. Therefore, the elongation value of the edible film obtained in this study was classified as good, particularly edible films made by the addition of 0.50% and 1.00% BLEE. Based on the tensile strength and elongation values, the edible film produced in this study can be applied as primary packaging for food products.

3.3. Antibacterial Properties
The antimicrobial activity test of edible film was carried out to determine the concentration of BLEE which can inhibit the growth of microorganisms, at least providing evidence through this study that BLEE added to edible film has antimicrobial activity. In preliminary research, it was proven that BLEE has antimicrobial activity against S. aureus and E. coli. BLEE showed antimicrobial activities on S. aureus at all tested concentration levels (Table 3), in which the higher concentration, the more antimicrobial activity will be. BLEE did not demonstrate antibacterial activity on E. coli at 0.50% addition level, meanwhile the activities were found at the BLEE concentration of 1.00% and 1.50%.

| Table 1. Antibacterial Activity of the Basil Leaf Ethanol Extract against Staphylococcus aureus and Escherichia coli |
|-----------------------------------------------|
| Concentration of Basil Leaf Ethanol Extract | Inhibition Zone (mm) |
|                                          | Staphylococcus aureus | Escherichia coli |
| 0.50%                                      | 3.50                  | 0.00            |
| 1.00%                                      | 7.50                  | 3.00            |
| 1.50%                                      | 8.50                  | 8.00            |

Table 3 shows that the more BLEE concentration, the wider inhibition zone indicating the higher antibacterial activity against S. aureus and E. coli will be. Those results exhibited that BLEE had a potential to be used as antimicrobial agent, particularly on S. aureus and E. coli. A similar result was
obtained by Angelina et al [7] reporting that ethanol extract of basil leaves was observed to have antibacterial activity inhibiting the growth of E. coli and S. aureus. Phytochemical test revealed that secondary metabolite compounds identified from the ethanol extract of Ocimum sanctum were flavonoid, tannin, and essential oil that act as antibacterial. Antibacterial activities of alginate based edible films incorporated with BLEE on S. aureus and E. coli can be seen in Figure 1. Surprisingly that no antibacterial activities were shown by edible films added with all tested concentration levels of BLEE indicated by no clear zone on the media for both E. coli and S. aureus. Thus, even though BLEE exhibited antibacterial activity, but its activity disappeared after incorporated into the solution of alginate based edible film. This means that the basil leaf ethanol extract used in the edible film formulation cannot function as antibacterial.

Figure 3. Images of Inhibition Zones of BLEE-Alginate Films Against (A) S. Aureus and (B) E. Coli.

4. Conclusion
The use of basil leaf ethanol extract (BLEE) could improve physical and mechanical properties of alginate based edible film. BLEE addition increased water solubility, tensile strength and thickness values of edible film. Meanwhile, incorporation of BLEE reduced water vapor transmission rate (WVTR) and elongation values. Even though basil leaf ethanol extract showed antibacterial activity on S. aureus and E. coli, but alginate based edible film incorporated with BLEE had no antibacterial activity on both bacteria. Therefore, alginate based edible film added with BLEE could not act as an antibacterial food packaging.

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