Characterization of alginate-chitosan membrane as potential edible film

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Abstract. Alginate-chitosan based biopolymer for possible application as edible film coating has been studied. Alginate hydrosol and chitosan hydrosol with mass ratio of 1:1 were mixed to form a thin membrane and then dried. The obtained alginate-chitosan membrane was confirmed using FTIR spectrophotometers. Characterization of the membrane, which includes thickness, tensile strength, water vapor sorption, resistance to pH change and antimicrobials properties, were conducted. It was showed that the interaction of alginate and chitosan in the membrane occurred through the electrostatic interaction of the carboxylic group of alginate and ammonium groups of chitosan. At the same thickness, the alginate-chitosan membrane tensile strength was higher and more resistant to pH changes than both native alginate and chitosan membranes. Furthermore, the alginate-chitosan membrane has good antibacterial potential against Gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli). It is expected that the alginate-chitosan membrane has the potential application for safe and efficient fruit coating.

1. Introduction

Recently, there has been a shift in the use of petrochemical based synthetic food packaging to biodegradable packaging due to their serious ecological problems [1]. The most promising and versatile biodegradable polymers, chitosan, has been used as food coatings in terms of their ability to form strong, elastic, flexible and difficult to tear edible films [2]. Chitosan is a good barrier for gas and water vapor because of its matrix structure and antimicrobial properties that inhibit the growth of food-destroying microbes [3, 4]. Chitosan coating inhibits maturation by reducing the production of ethylene and carbon dioxide on fresh lychee, star fruit, and tomatoes [5]. Alginate membranes are also used as wrappers for fish, fruit [6], and meat to preserve them [7]. However, both of them have their own weaknesses, chitosan polymers undergo leaching under acidic conditions and alginate polymers undergo swelling when interacting with water. This weakness can be overcome through the formation of alginate-chitosan membranes.

Alginate-chitosan membranes are formed from polycationic chitosan and polyanionic alginate through electrostatic interactions [8]. With its dense matrix structure, the membrane inhibits the decay process by blocking oxygen inhalation. The formation of ethylene in fruit can be stimulated by mechanical damage and infection. By coating the alginate-chitosan membrane, the fruit is protected.
from physical impact because the dense matrix structure increases the tensile strength of the membrane. The use of alginate-chitosan membrane has fulfilled food safety and price worthiness because the membrane only consists of alginate and chitosan without other toxic materials, in accordance with the safe edible film criteria [9]. The simple preparation technique makes it easy for large scale application both for home and large industries in creating smart bioplastic and environmentally friendly food packaging. In this study, alginate-chitosan film will be synthesized, and the physical, chemical, and antibacterial properties of formed film will be examined.

2. Materials and methods

2.1. Materials

Chitosan (deacetylation > 90%, Mol. wt. 300 KDa-400 KDa), sodium alginate (Mol. wt. 12Kda-40 KDa), S. aureus (ATCC 25923) and E. coli (ATCC 25922) were obtained from Sigma-Aldrich (USA). Hydrochloride acid (37%), glacial acetic acid (98%), sodium hydroxide (40%), and sodium alginate (12 KDa) were synthesized. Sodium hydroxide and glacial acetic acid were obtained from Merck (Germany).

2.2. Method

2.2.1 Film preparation. The alginate-chitosan film (1:1 in mass) was prepared based on the procedure described by Hermanto et al. [10]. Briefly, hydrosol of chitosan at 1% (w/v) were prepared in 20 mL glacial acetic acid under stirring at 400 rpm using a magnetic stirrer (IKA Combimag REO Drehzahl Magnetic Stirrer Electronic, Germany), while alginate was dissolved in deionized water until hydrosol at concentration of 4% (w/v) was formed. Then, both alginate and chitosan hydrosols were left out overnight to remove air bubbles. The chitosan hydrosol (100 mL) was added to the alginate solution and added 8 mL of 32% HCl (100 mL) at 25 °C, homogenized using a homogenizer (IKA T18 Ultra Turrax, Germany) for 90 seconds. Following, NaOH 10% (w/v) was added till the pH of the suspension was elevated to 5.28 and homogenized again for 90 seconds. The mixture was then transferred in the glass plate and dried at room temperature for 72 h.

2.2.2 Membrane characterization regarding physical, chemical and antibacterial properties. The film thickness was determined with a digital micrometer (MDC-25S, Mitutoyo) at 5 random points. The film tensile strength and elongation at break were determined with Instron Universal test Machine (Model 4210, Canton MA). Film resistance to pH change was evaluated by comparing both tensile strength and elongation film immersing at different pH solution. Functional groups of films were characterized using the KBr pellet method at a compression pressure of 2500 Ib/m² on an FTIR spectrophotometer (FTIR 1600 Perkin Elmer Co, Japan). Water vapor sorption was determined using gravimetry method, according to Bueno et al. [11]. The experiment was performed in triplicate by measuring the weight gradual increase 1 cm x 1 cm film (in 95% relative humidity at 32 °C). Vapor sorption capacity ($V_c$) was calculated based on the following Equation 1 below,

$$V_c = \frac{(W_t-W_o)}{W_o} \times 100$$ (1)

$W_0$ is the initial weight of the film and $W_t$ is the weight at t time of the film.

The disk diffusion method was used to determine the antibacterial activity of the alginate-chitosan film against both E. coli and S. aureus as the one implemented by Kaya et al. [12]. As a reference standard 0.5 McFarland was used to adjust bacterial susceptibility. Suspension of bacterial culture was wiped onto a plate inside agar of Müller-Hinton. On agar medium that has been inoculated with bacteria, paper discs were placed, then loaded with 20 mL hydrosol film. It was incubated for 24 hours at 37 °C. As positive control, it was used 10 mg gentamicin per disc and deionized water as a negative control. Inhibition zone diameter on a clear area of the paper disc was measured. All experiments were done in triplicate.
3. Result and discussion

3.1. Formation of the alginate-chitosan film
Finding out molecular interactions in the film between chitosan and alginate can be done by FTIR spectrophotometry. FTIR spectra interpretation of chitosan, alginate and alginate-chitosan films are described in Table 1.

| Chitosan spectra (cm\(^{-1}\)) | Alginate spectra (cm\(^{-1}\)) | Alginate-chitosan spectra (cm\(^{-1}\)) | Vibration |
|-------------------------------|-------------------------------|--------------------------------------|----------|
| 3459                          | 3459                          | 3459                                 | N-H and O-H vibrations |
| 2924                          | 2930                          | 2924                                 | symmetric C-H vibration |
| 2852                          | 2864                          | 2870                                 | asymmetric C-H vibration |
| 1589                          | 1628                          |                                     | Primary amine N-H bending |
| -                             | -                             | 1578                                 | –COO\(^-\) group vibrations |
| -                             | -                             | 1550                                 | presence of NH\(^3\) ions |
| 1426                          | 1420                          | -                                    | CH\(_2\) bending |
| -                             | -                             | 1398                                 | Electronic interaction of alginate-chitosan |
| 1375                          | -                             | -                                    | CH\(_3\) symmetric deformation |
| 1325                          | -                             | -                                    | C-N vibration |
| -                             | 1305                          | -                                    | O-C-H vibration |
| 1260                          | -                             | -                                    | hydroxyl groups |
| 1157                          | -                             | -                                    | Amino groups |
| 1081 and 1028                 | -                             | 1081                                 | C-O vibration |
| -                             | 1028                          | 1028                                 | C-O-C vibration |
| -                             | 950                           | -                                    | uronic acid residues |
| 897                           | -                             | 897                                  | CH bending |
| -                             | 891                           | -                                    | manuronic acid residues |

There is absorption in the wave number 3459 cm\(^{-1}\) which indicates the presence of the -OH group of alginate and -NH\(^3\) chitosan, 2924 and 2870 cm\(^{-1}\) are the absorption of CH (sp\(^3\)), 1578 cm\(^{-1}\) is the vibration of the -COO\(^-\) group. The loss of amine group absorption in 1157 cm\(^{-1}\) indicates that the chitosan amine group has been protonated and interacts with the carboxylate group of alginate [13]. This is strengthened by the appearance of peaks in the area of 1550 cm\(^{-1}\) which shows the presence of protonated amine (–NH\(^3\) ion) [14]. Peak intensity at wave number of 1398 cm\(^{-1}\) also shows electrostatic interactions on alginate-chitosan film [15], with alginate and chitosan mass ratio of 1:1. The presence of the absorption band mentioned above shows the existence of ionic bonds of protonated amine and the carboxylate groups.

Chitosan consists of amine groups which have intrinsic pKa value of 6.5, while alginites consist of carboxyl groups that undergo ionization at pH of the solution above its pKa of 4.7 [10, 16]. Therefore, at pH 5.28 chitosan presence at protonated form while alginate is in carboxylate form which is negatively charged. When both hydrosols are mixed in a 1:1 ratio, electrostatic interaction of protonated amine and carboxylate has occurred [17].

3.2. Physical, chemical, and antibacterial properties
The film mechanical properties i.e. tensile strength, elongation and Modulus Young are important parameters for understanding material behavior during functional application. The Young Modulus describes the stiffness of material. The greater the Young modulus values of the material, the higher the level of stiffness. As part of mechanical strength, tensile strength is defined as the maximum tension achieved when the test of tension was carried out [12]. Meanwhile, elongation at break describes the
elasticity of the film. In this study, for alginate-chitosan film, Young Modulus and tensile strength value was observed higher but its elongation was lower than both chitosan and alginate films, as described in Figure 1. It is indicated that alginate-chitosan film stiffer than its native single films. It is possible that the alginate-chitosan film has a tighter structure since protonated amine and carboxylates interaction. The better mechanical properties of chitosan-alginate films can minimize pressure damage of food coated during packaging and transportation. Meanwhile, film resistance to pH change is shown in Figure 2. Alginate-chitosan film shows higher stability to pH change than alginate or chitosan films, in line with previous study [11].

![Figure 1. The mechanical properties of films.](image)

![Figure 2. The pH effect on the mechanical properties of films.](image)

The water vapor sorption capacity reached the maximum amount after its saturation which is 8 hours, as shown in Table 2. The value shows that the alginate-chitosan films have greater water vapor sorption.
and resistance than single films (alginate and chitosan). It is influenced by the hydrophilic and hydrophobic balance of the film, crosslinking, the degree of ionization and its interaction with the counter ion. Sorption on alginate-chitosan films is probably due to the presence of hydrophilic COO⁻ ions in the film. The film's hydrophobicity increases due to the formation of amine and carboxylate interaction and it is expected to have greater resistance to water-insoluble resistance.

The antibacterial activity of films is compared in Figure 3. Alginate-chitosan films shows the highest antibacterial activity resist \( E. \text{coli} \) and \( S. \text{aureus} \) compare to other films, except gentamicin as a positive control. The polymer structure of alginate-chitosan which has a positively charged of amine group can interact with the negative charge of a molecule such as proteins from microbes that cause leakage of proteins and intracellular structure of microbes. The interaction of protonated amine and carboxylate in alginate-chitosan is ionic bonds that are not as strong as covalent bonds. It causes the availability of protonated amino group in order to react with the negatively charged of bacterial protein.

### Table 2. Water vapor sorption capacity of films.

| Film            | % sorption | % resistance |
|-----------------|------------|--------------|
| Alginate        | 94         | 95           |
| Chitosan        | 98         | 99           |
| Alginate-Chitosan| 104       | 105          |

![Figure 3. The antibacterial activity of films.](image)

### 4. Conclusion

The interaction of alginate and chitosan in the film occur through ionic interaction of protonated amine and carboxylate. The alginate-chitosan film has better mechanical strength and antibacterial activity resist \( E. \text{coli} \) and \( S. \text{aureus} \) than both alginate and chitosan film as the native membrane. Therefore, alginate-chitosan film has the potential application for safe and efficient fruit coating.

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