Synthesis and Characterization of Chitosan with Addition of Patchouli Oil to Improve Mechanical Properties Biofilm

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Abstract. In this study, The mechanical properties and antimicrobial activity of chitosan films added with patchouli oil were investigated. Film chitosan with the addition of essential oils (EO) have shown great promise for application in food preservation. Patchouli oil have contains essential oil that can inhibit Echerchia Staphylococcus aureus and E.coli bacteria. Characterization tensile strenght increasing at composition ratio patchouli oil 7 g, chitosan 45 g and glycerol 1,6 ml is 16 Mpa while moisture content ability 31.47% and thermal stability 521.18°C. In otherhands, patchouli oil could improve mechanical properties of the film. The resulting film can be suggested as a promising application for food packaging.

1. Introduction

Consumer demand for high quality food without chemical preservatives is a challenge for the food industry. This prompted an increase in business for the discovery of new natural preservatives and antimicrobials. One of the ways that can be done by the producer to extend the life of product is by using the natural material of packaging (bio-film).

Biofilm is thin layer of edible material, formed lining product (coating) or placed between components of a product that serves as a barrier (inhibiting migration) to the mass transfer (eg, water vapor, gas, grease, solutes, light) and to improve the handling of a food as well as inhibiting the growth of bacteria in food [1]. It is made from renewable material, its come from the compounds of the plants, for example starch, cellulose, collagen, kassein, proteins or lipids contained in the animal.

Recently, food packaging based on biopolymers has received significant attention on environmental issues. Moreover, the addition of antimicrobial agents into biodegradable food packaging system to prevent the growth of microbes on the surface of the food has been the focus of research in recent decades [2]. One of the biopolymers that are used to make natural packaging is chitosan.

Chitosan is a type of polymer chains that are not linear the general formula \((C_6H_{11}O_4)_n\). Chitosan is an amino polysaccharide with a high molecular weight, deacetylation of chitin derivative which is one of the many abundant polysaccharide in nature [3]. Chitosan is a cationic polysaccharide with the best film-forming properties. It is derived from chitin deacetylation with alkali process. These
polysaccharides are widely used not only for the film forming ability but also their antimicrobial properties, thereby encouraging its use as a potential packaging material [4].

Chitosan is polymer non-toxic and very similar to the plant fiber (cellulose). The only difference between chitosan and cellulose are the amine group (-NH₂) on the position of atoms C-2 and the hydroxyl group (OH) found in cellulose [5]. However, unlike plant fiber, chitosan has a positive ionic charge, which gives the ability to bind chemically with the negative fats such as lipids, cholesterol, metal ions, proteins and macromolecules [6].

The use of chitosan as the packaging is an innovation by using the concept of active packaging biodegradable. Where packaging developed has the ability to reduce or inhibit microbial growth on the surface of the food. The packaging of chitosan is potentially inhibit the growth of microbes, bacteria and fungi. This is because the chitosan-containing amino polysaccharides [7].To enhance the antimicrobial activity of chitosan, has been studied by adding other materials such as biopolymers, polysaccharides, lipids or mixtures and the addition of fatty acid and essential oil.

Moreover to improve the properties of chitosan as an antibacterial agent can be carried out with the addition of fatty acids and essential oils, where the addition of fatty acid and essential oil will also improve the physical and mechanical properties of chitosan films [8]. Essential oils and active ingredients such as antimicrobial and anti-oxidant diffuses from the film into the food to control the growth of microorganisms [9].

Based on research that has been done before, the addition of curcumin on the film material can inactivate UV light to prevent contamination L. Inocua on the sausage [10]. The addition of turmeric oil into chitosan is able to enhance the antimicrobial activity of the film, reducing the amount of bacteria Staphylococcus aureus and Salmonella significantly compared to pure chitosan during the exposure period of 3 hours [3].

Utilization of essential oils in packaging systems is the potential, including patchouli oil that is native to Indonesia many produced in various parts ranging Aceh, Java and Borneo. The factor that makes this oil demand is a compound of essential oils, one of which is antibacterial and antioxidant properties, which is very influential of consumers.

Patchouli oil (Pogostemon cablin benth) contain bioactive compounds like anti-allergic, bacterial, oxid effects and inflammatory. Patchouli oil contains patchouli alcohol (Patchouli alcohol), patchouli camphor (Patchouli camphor), cadinene, benzaldehyde, eugenolAnd cinnamic aldehyde. Inside this patchouli oil, patchouli substance contained alcohol (abbreviated as PA) are very useful in the world. The best patchouli oil is containing PA containing 30% [11].

Based on previous studies, it is known that the essential oil contains compounds that can inhibit the growth of antimicrobials in food surfaces so that in this study used an additional antimicrobial agent into chitosan films in the form of essential oil of patchouli. This study is aims to produce biofilms and characterize the mechanical and antimicrobial properties of chitosan films added with patchouli oil.

2. Material and Methods

2.1 Material

This study used chitosan from shrimp skin with deacetylation level of 75%. Tween 80, glacial acetic acid 98% were purchased from Sigma Chemical Co. Patchouli oil (PO) derived from Laboratory Process in Chemical Engineering Department Politeknik Negeri Lhokseumawe and Yayasan Puga Aceh Riset (YPAR) 30%.

2.2 Preparation Chitosan

Shrimp shells from the fishing industry Lhokseumawe city was dried, ground and polished until it reaches the form of a powder of 100 mesh. Shrimp shell powder was mixed with HCL 1.5 N with a ratio of 1:10 (w / v), then stirred using for 60 minutes to remove minerals. Then washed with distilled water until a neutral pH. The next step is the removal of protein content with NaOH (2 M) at a ratio of 1:10 (w / v) and stirred for 2 hours. Then washed with distilled water until a neutral pH. Finally adding with NaOCl 0.315% 1:10 (w / v) for 5 minutes to remove the color.
2.3. *Preparation patchouli oil*

This study in order to help farmers of patchouli in Aceh Regional, redistilled products patchouli done by farmers. Patchouli oil consist a mixture of terpene compounds are mixed with alcohol, aldehydes and esters that give a distinctive and specific aroma. Such compounds include, sinamaldehyde, benzaldehyde, patchoulen, patchouli alcohol and eugenol benzoate. Patchouli alcohol is the main component of patchouli oil. Oil which contains terpenes will lower the value of its solubility. Terpenes in the oil will be susceptible to the polymerization process, oxidation or hydrolysis because of their light, and water. For patchouli oil purification can be performed by using a chelating compound and the removal of terpenes (terpeless). Oil purification using Na-EDTA (in Sodium Ethylene diamine tetra acetic acid) 0.05 M with a ratio of 1: 1 and stirring for 5 minutes will eliminate the content of Fe (iron). Then it will be done redistilled to produce a purer product.

2.4. *Characterization*

2.4.1 *Gel Permeation Chromatography for Molecular weight of polyl*

GPC is used to chromatographically separate polymer molecules by size (or hydrodynamic volume) and through various calibration methods, determine the molecular weight averages (molecular weight, viscosity, and average molecular number) and molecular weight distribution of a sample. The technique is generally applicable in the molecular weight range of 500 to 5,000,000 amu. Gel Permeation Chromatography (HLC-8020 TOSOH, Japan) calibrates with PS standard and THF is being used to determine of the molecular weight.

2.4.2 *Film Thickness*

The film thickness is determined by using a handheld digital micrometer (hand-held digital micrometer) (Mitutoyo No. 293-766, Tokyo, Japan) with a precision of about 0.0001 mm measurements carried out at least five random locations, and an average thickness value used to calculate the permeability and mechanical properties of the film.

2.4.3 *Thermogravimetric Analysis (TGA)*

Thermogravimetric Analysis (TGA) was performed to evaluate the thermal stability of the composite film using DTG-60A (Shimadzu). About 5 mg of sample was heated from 50 to 500 °C at a heating rate of 10°C / min under nitrogen flow of 70 mL / min. A decrease in the sample weight measured as a function of temperature TGA.

2.4.4 *Fourier-Transform-Infrared Spectroscopy (FT-IR)*

Spectrofotometry FTIR is a method to observing the interaction of molecules with electromagnetic radiation in the wavelength range from 0.75 to 1000 μm or at wave number 13000-10 cm⁻¹, which in this study used a model FTIR instrument IR Prestige-21.

2.4.5 *Morphological Structure Test Tool Material by Scanning Electron Microscope (SEM)*

SEM (Scanning Electron Microscope) is one type of electron microscop namely the loupe to see small objects nano-sized structure that uses an electron beam to describe the shape of the surface of the material being analyzed. The working principle of this SEM is to describe the object surface or material with electron beam that is reflected by the high energy.

3  *Results and Discussion*

3.1  *Patchouli oil compositions*

In this study, analysis of patchouli oil quality standards compliant Ernest Guenther with samples from farmers in North Aceh and continuous purified in Laboratory Process of Chemical Engineering, Politeknik Negeri Lhokseumawe. From the analysis, Patchouli oil has reached the standard color, but
the product will be the best if it saving in long time. According to specific gravity analysis the heavier weight of patchouli oil will be affect to density. Also the lower the temperature the weight of its kind will be smaller. Further to the analysis of the refractive index of the sample has reached the standard as well as the analysis of the solubility test in 90% ethanol, it appears that the sample can be dissolved in ethanol 90% (Table 1).

Table 1. Patchouli oil composition’s North Aceh

| Name Test                     | Standard Ernest Guenther | Result                          |
|-------------------------------|---------------------------|---------------------------------|
| Color                         | Light yellow to dark brown| Reddish yellow                  |
| A specific gravity of 25°C / 2°C | From 0.943 to 0.983       | .9584                           |
| The refractive index of 20°C   | 1.506 to 1.516            | 1.5088                          |
| Solubility in 90% ethanol at 25°C | Clear in vol. 10 ml       | Clear in vol. 10 ml (separate)  |
| Acid number                   | Max. 5.0                  | 5.61                            |
| Ester numbers                 | Max. 10.0                 | 14.02                           |

3.2. Chitosan molecular weight

The molecular weight (MW) of a polyol obtained by using gel permeation chromatography (GPC). GPC is also often referred to by size exclusion chromatography (SEC). The separation mechanism in GPC / SEC is based on the size difference seen among components of the sample contained in the solution. There are many methods for testing the molecular weight of the sample average as Number average (Mn), can be obtained by using the membrane osmometry, molecular weight average (MW) of the tested light scattering, where the average Z and Z + 1 (Mz and Mz + 1) is tested by ultracentrifugation. However, GPC is a unique tool which can calculate overall in all analyzes. The molecular weight of chitosan obtained was 185,000 g / mol with a number average (Mn) of 141,000 g / mol. Research conducted by Shun, et.al (2018) obtained different results about 160,000 g / mol which is caused by different methods and materials.

3.3. Mechanical testing material

Mechanical tests of material can be seen the results in the table below. Based on table 2 show the best values of tensile strength and % Elongation in composition patchouli oil 7 g; chitosan 45 g; and glycerol 1.6 ml. It is based on a reference, where that is getting a lot of essential oils are added in the manufacture of edible film will be increasing the value of tensile strength and elongation [12], while increasing plasticizers will lower the hydrogen bonds in edible film, thereby increasing the flexibility of film, which will make the value of tensile strength of edible film will be smaller [12].
Table 2. Result of mechanical tensile strength, elongation and moisture content ability

| Weight Patchouli oil (gram) | Mass of Chitosan (gram) | Glycerol (mL) | Characterization Analysis | Appearance |
|-----------------------------|-------------------------|--------------|---------------------------|------------|
|                             |                         |              | Tensile Strength (MPa)    |            |
|                             |                         |              | Elongation (%)             |            |
|                             |                         |              | Moisture Content Ability (%)|            |
|                             |                         |              |                           |            |
| 5                           | 35                      | 1.2          | 1.10                      | 127        | 20.07       |
|                             |                         |              |                           |            |            |
| 6                           | 40                      | 1.4          | 8.5                       | 437        | 15.67       |
|                             |                         |              |                           |            |            |
| 7                           | 45                      | 1.6          | 16                        | 775        | 31.47       |

However in Table 2 also shows that the highest moisture content ability properties in the sample No. 3, the absorption of water will be 31.47%, while the composition consist a mixture of patchouli oil 7 g; chitosan 45 g; and glycerol 1.6 ml. This is according to research [13] which states that the plasticizer glycerol has a high moisture content ability, where nature can be reduced by the addition of chitosan which have amine functional groups, primary and secondary hydroxyl groups, in the presence of functional groups the resulting chitosan has a high chemical reactivity because it can form hydrogen bonds, so that chitosan is an ideal mixing ingredients. Besides chitosan is a derivative of chit in, a polysaccharide most on Earth after cellulose.

3.4. Degradation test

Study of thermal stability, determined by thermogravimetric analysis (TGA) was conducted to understand the degradation characteristics of the specimen using the TGA. Heat materials analysis provides some basic information on the thermal stability of materials. All measurements were carried out under a stream of nitrogen (30 ml / min), maintaining a constant rate of heating of 10 °C / min and using alumina container. Thermogravimetric analysis (TGA) was used to characterize the decomposition and thermal stability of various material conditions. Especially in the TGA analysis examined changes in thermal stability in terms of the percentage of the sample weight loss as a function of temperature.
In this study, the results of thermogravimetric analysis (TGA) matrix composite that has been done and investigated that the percentage of the sample weight with an increase in temperature related. Mass loss with increasing temperature, for process heating increased 10 °C / min, can be seen on the graph shows the results of the analysis at the transition temperature ranging from 278.02 to 310.26 °C and range for the final transition at the start of 600.86 to 601.58 °C. From room temperature to 600 °C.

From the above chart shows that the best temperature values in the matrix of Chitosan and essential oil is resistant to heat upon mixing 70% Chitosan and 30% of patchouli oil, starting at 360.55 °C with the first temperature at the beginning of degradation ranging from 446.63 - 492.20 and ended at 521.18 temperature °C until get a mid-point value of 467.22 °C with a value of -6.730 mg weight loss (Figure 1a). The graph analysis results Thermogravimetric (TGA) upon mixing patchouli oil 7 g; chitosan 45 g; and glycerol 1.6 ml can be seen in Figure 1b.

3.5. Structure morphology analysis
The effect of the treatment on the surface structure of the material is done by using scanning electron microscopy. The surface structure was observed by using a microscope JEOL-T220. Analysis Scanning Electron performed at a voltage of 5-20 kV.

Scanning Electron Microscopy (SEM) was used as a morphological analysis technique that can identify the surface of a material with a higher resolution than conventional optical microscopes are inclined. This method is easier for researchers to be able to observe sample and around the interface between materials with an oxide layer in detail or even In-Situ [14]. The result morphology sample analysis present by Figure 2.

Figure 1a. TGA graph analysis results Figure 1b. Best Value TGA

Figure 2. Structure morphology of Chitosan / patchouli oil at 430 times magnification.
In Figure 2, shown the surface of structure samples Chitosan / patchouli oil that has better mechanical characteristics, chitosan 45 g and 7 grams of essential oil. The samples was taken at a magnification of 430 times. From these images can be seen the surface of the sample that is not too uniform by the presence of Chitosan clot in some parts. Based in figure, it appears that the spread of patchouli oil look evenly. It can conclude, homogeneity between Chitosan and Patchouli Oil has achieved well.

4. Conclusion

In this research, the author has investigation about adding chitosan and patchouli oil to improving mechanical properties biofilm. Based in testing characteristic, it’s shown at composition patchouli oil 7 g; chitosan 45 g; and glycerol 1.6 ml has good characteristic tensile strength 16 Mpa, moisture content ability 3147% and thermal stability 521.18°C.

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