ALKIMIA : Jurnal Ilmu Kimia dan Terapan

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Aplication Chitosan Modified Carboxymethyl as Antibacterial Agent of Paper Packaging

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Abstract. Chitosan and chitosan modified carboxymethyl were used to enhance performances of paper packaging. Chitosan 1.0 wt% and chitosan modified carboxymethyl solutions with concentrations of 0.25; 0.5 and 1.0 wt% were manually applied on paper surface using dip coating method. Effects of chitosan coatings on antibacterial properties of the paper were investigated. The antimicrobial activity of chitosan against gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*) was found to be enhanced when chitosan and chitosan modified carboxymethyl solution were entrapped within paper. Further paper coated chitosan and chitosan modified carboxymethyl has higher antibacterial activity againt *Staphylococcus aureus* rather than *Escherichia coli*.

Keywords : Chitosan carboxymethyl, antibacterial agent, paper packaging

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Introduction

Packaging is a container (wrapper) that can help prevent or reduce damage to the packaged material. However the material used in plastic food packaging will have a bad impact and be harmful to health. Plastics consist of various chemicals (monomers) where under certain conditions, contact between plastics and food can cause the transfer of hazardous chemicals from the container to the food which is called migration. The migration (transfer) of hazardous chemicals occurs due to the influence of food temperature, storage or processing [1]. The higher the temperature, the more likely migration will occur.

The self life of stored food will also have an effect. The longer the contact time between food and plastic packaging, the higher the amount of hazardous chemicals which can migrate. If it consumed continuously over a long period of time, it can endanger health such as triggering cancer. In addition, some types of plastic are not heat-resistant and take tens to hundreds of years to decompose naturally (non-biodegradable) [2]. This can trigger various problems for the environment, especially for human survival.

Therefore, now people are starting to switch to using paper-based food wrappers. Paper is a material that has several superior properties, including: economical, recycle and flexible in designing forms that are difficult to imitate [3]. The latest development in the field of packaging technology is the development of an active packaging that is antibacterial (Antibacterial packaging) and biodegradable [4]. An alternative that can be done is the manufacture of biocomposites with the addition of materials that have antibacterial and biodegradable properties. One of the ingredients that can be used as an antibacterial agent is chitosan.

Isolated chitosan from crab shell waste has potential antimicrobial properties on various bacteria such as *Escherichia coli*, *Straphylococccuc aureus* etc [5]. It also stated that chitosan derived from shrimp has antibacterial activity against both gram-positive and gram-negative bacteria [6]. Chitosan as an edible film has also been studied by previous researcher [7] where chitosan effectively inhibits bacterial growth and effectively reduces water evaporation and product oxidation.

In this study, chitosan derivative compounds will be used, namely carboxymethyl chitosan from crab shell waste. The compound obatined is coated into paper packaging material and tested for its antibacterial activity against gram-positive and gram-negative bacteria.

Experimental

Materials

The materials to be used are crab shells waste (collected from several restaurants at Bandar Lampung), NaOH, HCl, Acetic Acid, Methanol, Monochloroacetic Acid, Wrapping Paper, Aquades, Filter Paper, gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*), distilled water, Whatman 42 filter paper ashless circles 110 mm, universal indicator.

Synthesis of Carboxymethil Chitosan

Carboxymethyl chitosan was synthesized according to the method of [8] in the following way: five grams of chitosan was dissolved in 100 mL of 20% NaOH solution and stirred for 15 minutes. 15 grams of monochloroacetic acid solution was added to the chitosan solution and stirred for 2 hours at 40 ^{o}C. Then the mixture of chitosan-monochloro acetate solution was neutralized with 10% acetic acid solution, poured into 70% excess methanol solution, filtered and washed with methanol. The modified carboxymethyl chitosan product obtained was then dried at 55^{o}C for 8 hours.

Preparation of modified carboxymethyl chitosan solution and coating of modified carboxymethyl chitosan on wrapping paper

Preparation of modified carboxymethyl chitosan solution

Chitosan solution was prepared by adding chitosan powder in 3% acetic acid solution and then stirring using a magnetic stirrer for 24 hours at room temperature to obtain a homogeneous chitosan solution. The chitosan solution obtained was then used as the mother liquor. This mother liquor was then further divided into several parts and each part was diluted again by adding 3% acetic acid solution to obtain a chitosan solution with a concentration of 0.25; 0.5 and 1.0 wt%.

Coating of modified carboxymethyl chitosan on paper substrate

The process of coating chitosan on paper is carried out using a dip-coating technique. Paper measuring 20 x 20 cm^2 was dipped in the chitosan solution until all parts of the paper were submerged for 3 minutes, then the paper was removed and left for 3 minutes with the aim that the paper was slightly dry and the dripping chitosan solution had run out. After 3 minutes had passed, the paper was then dipped again into the chitosan solution until it was completely submerged and then removed again and then left for 3 minutes.

After immersing the paper into the chitosan solution twice, the paper was then aerated in the open air for at least 30 minutes until the paper was slightly dry and the chitosan solution did not drip anymore. The next stage is drying, which is drying the paper in an oven at a temperature of 60 ^{o}C for 10 minutes with the aim of evaporating the water.

Characterization of Modified Carboxymethyl Chitosan Coated Paper

FTIR and SEM Analysis

Paper coated with modified carboxymethyl chitosan were characterized using a FTIR spectrophotometer to determine the characteristic functional groups of chitosan. While the topography and morphology of the particles that make up paper coated with modified carboxymethyl chitosan were seen through image results using SEM.

Antibacterial Test of Modified Carboximethyl Chitosan Coated Paper

An antibacterial test was carried out on paper coated with modified chitosan carboxymethyl to determine whether antibacterial chitosan had actually adhered to the paper surface. The parameters of the sticking of chitosan on the paper were monitored by the magnitude of the inhibition of the growth of gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*) on food wrapped in chitosan-coated paper compared to paper without chitosan -coated and control media.

Results and Discussion

Synthesis of Chitosan modified carboxymethyl

Chitosan modified carboxymethyl which is a chitosan derivative compound, was synthesized from chitosan raw material obtained through the preparation process from crab shell powder. Chitosan modified carboxymethyl was synthesized by reacting chitosan with monochloroacetic acid. The reaction for the conversion of chitosan to modified carboxymethyl chitosan is estimated as follows:

$$\underset{\text{Chitosan}}{\text{R-OH}} + \text{Cl-CH}_2\text{-COOH} \rightarrow \underset{\text{Chitosan modified carboxymethyl}}{\text{R-O-CH}_2\text{-COOH}}$$

In this study, the yield of modified carboxymethyl chitosan was 72.9%. This shows that chitosan which is the raw material for the synthesis of chitosan modified carboxymethyl has decreased in mass because according to the theory, the mass of chitosan modified carboxymethyl should be greater than the mass of chitosan due to the substitution of hydroxyl groups with carboxyl groups. The phenomenon of decreasing the mass of chitosan may occur when mixing chitosan with 20% NaOH solution which causes the dissolution of protein residues and the possibility of breaking the acetyl group into an amine group.

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Characterization of carboxymethyl modified chitosan coated paper

The FT-IR spectra of carboxymethyl modified chitosan-coated cellulose paper are presented in Fig1. a-c. A similar pattern of FT-IR peaks was observed for chitosan types with various concentrations. The broad bands located at 3332 and 2892 cm^{-1} contributed to the O−H and C−H strains

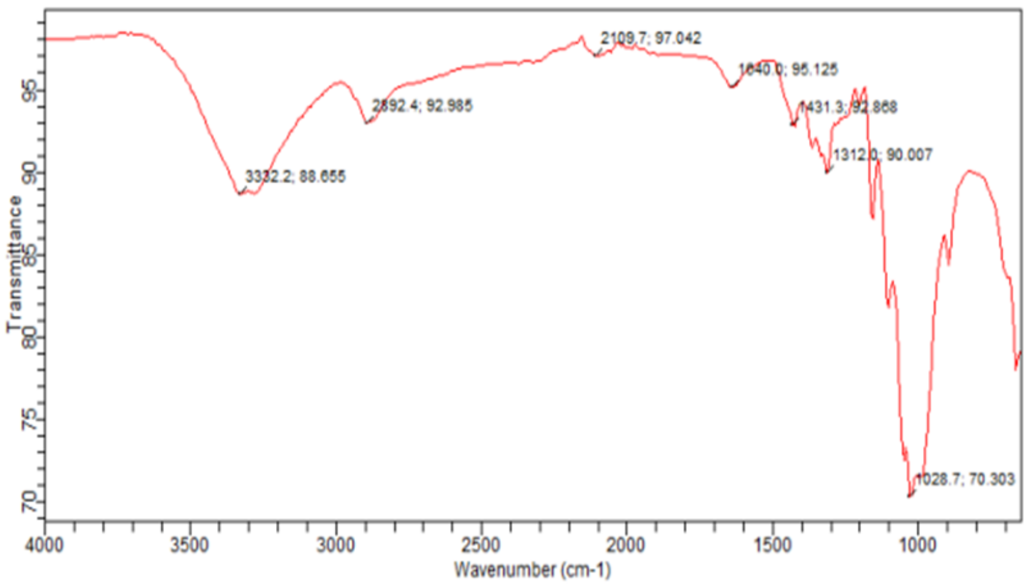

Fig 1a. FTIR spectra of 0.25% carboxymethyl modified chitosan coated paper

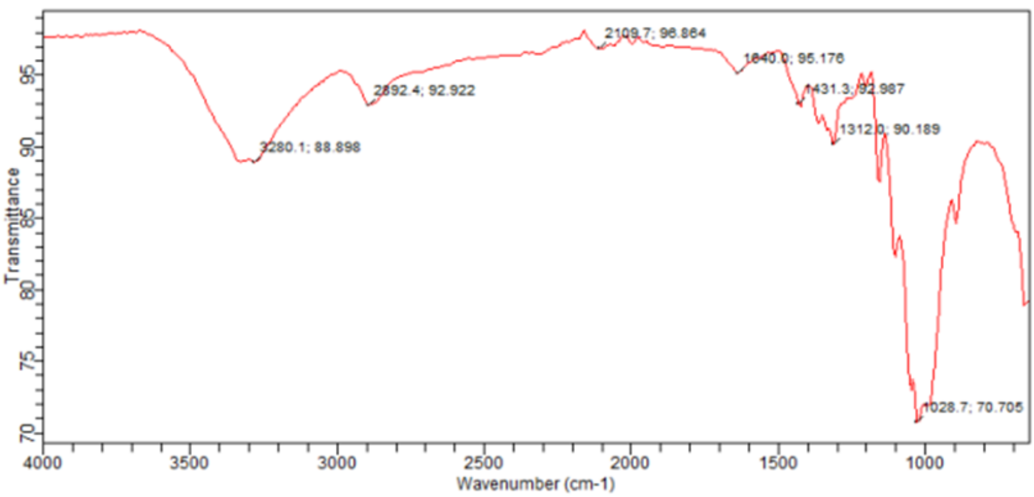

Fig 1b. FTIR spectra of 0.5% carboxymethyl modified chitosan coated paper

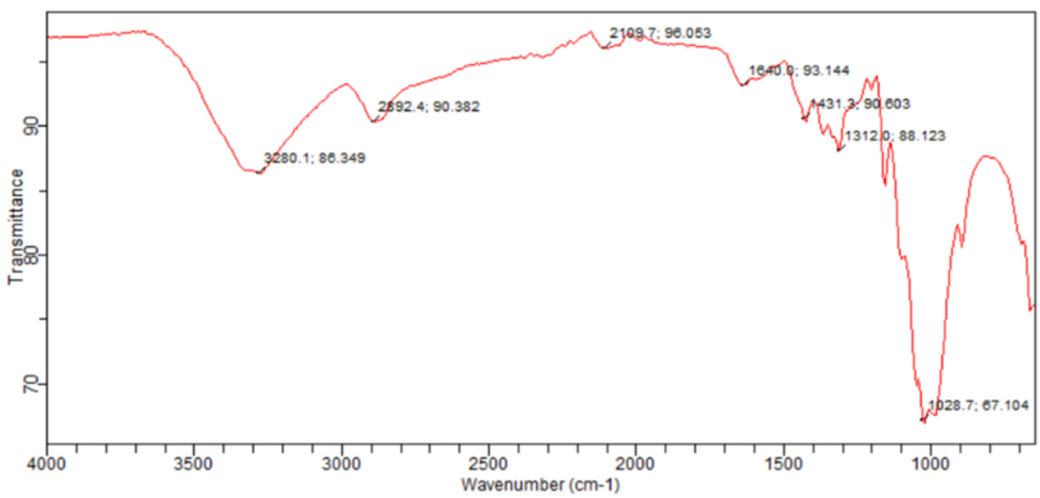

Fig 1c. FTIR spectra of 1% carboxymethyl modified chitosan coated paper

of chitosan, bending vibrations occurred at 1640 and 1431 cm^{-1}. The absorption bands at 1150 and 1028 cm^{-1} represent C−O−C and pyranose groups in the chitosan structure. Because the chemical composition of cellulose and chitosan are almost the same, the appearance of different peaks at 1431 cm^{-1} is due to stretching of the amide group, which is an indication of the presence of chitosan in the paper [10]. The increasing in absorption peaks of the amide band (stretching C=O) which appeared at wave numbers 1640 cm^{-1} indicated an increase in the C=O group due to the addition of a carboxylic group (-COOH) in chitosan, indicating that carboxymethyl modified chitosan was successfully coated on the paper. In addition to evidence of the appearance of C=O absorption peaks, the formation of carboxymethyl chitosan from chitosan can be strengthened by the widening of the absorption peaks of the C-O stretching vibrations, which appear at wave numbers 1028 cm^{-1} and indicate that there has been addition of a carboxylic group. The shift of the OH band at 3332 cm^{-1} (Fig 1. a) to lower wavenumber 3280 cm^{-1} (Fig1.b-c) corresponded to an increase in carboxymethyl modified chitosan concentration coated on paper,

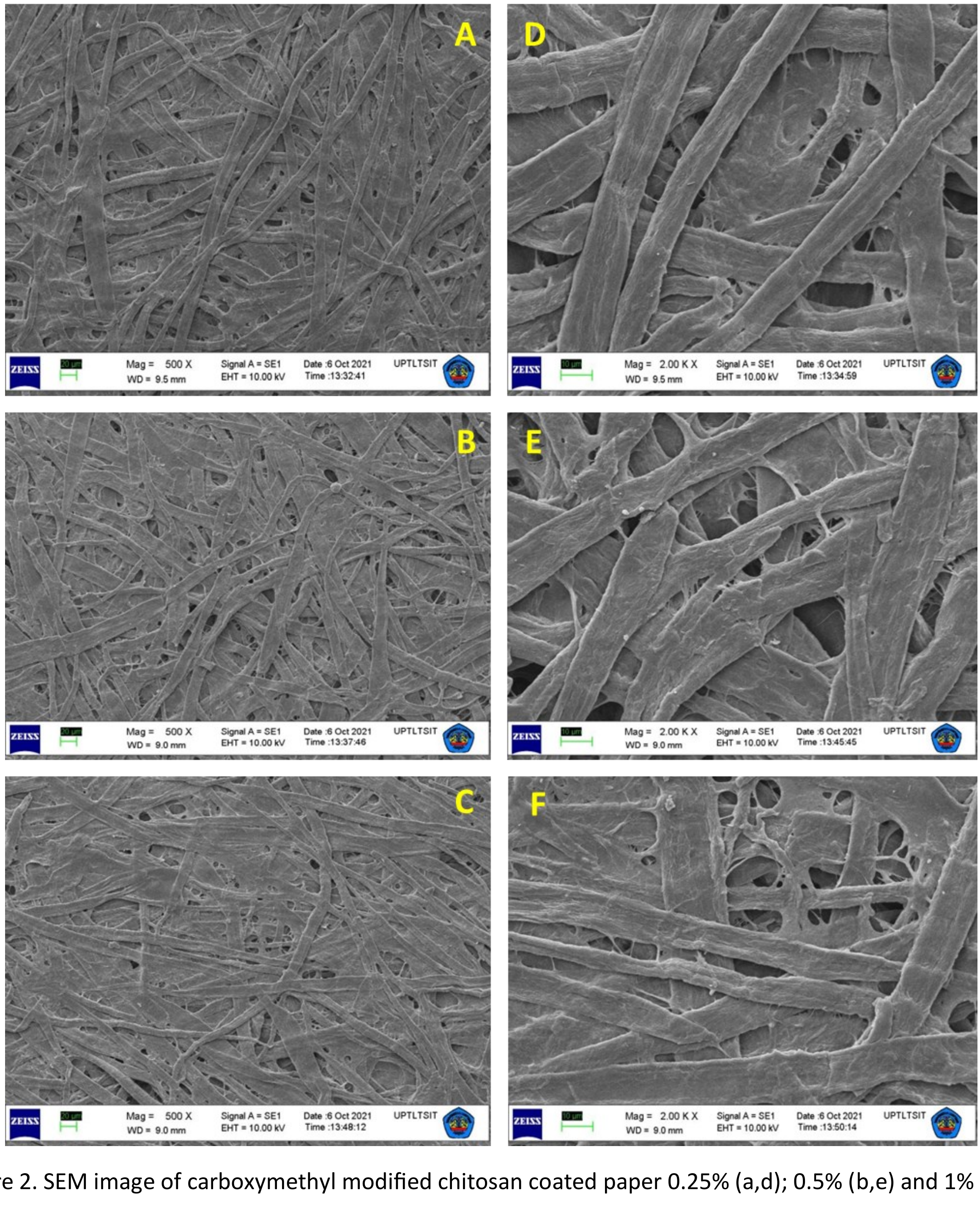

Figure 2. SEM image of carboxymethyl modified chitosan coated paper 0.25% (a,d); 0.5% (b,e) and 1% (c,f).

which correlated to an increase in hydrogen bond intensity of chitosan. This is due to the carboxymethyl chitosan concentration added to paper increased from 0,25% to 0,5% and 1 %.

Morphological structure analysis using SEM

The surface morphology of the paper coated with several concentrations of carboxymethyl modified chitosan is presented in Figure 2 (a-f). After incorporation of the carboxymethyl modified chitosan solution, the pores in the paper tissue were filled with chitosan, and a matrix phase was formed. This phenomenon is increasingly visible as the concentration of the carboxymethyl modified chitosan increases. This happens because the higher concentration of chitosan can interact better with the hydroxyl groups of cellulose fibers, and fill the empty pores in the cellulose network because of its longer chain.

Fernandes et al. [11] found a relationship between changes in the morphology of paper-infused chitosan and increasing the deposited chitosan layer. Chitosan penetration in the cellulose pores occurs with the first layer of chitosan layer. When more than three layers of chitosan are applied, the layers of chitosan can form a film on the paper surface. As a result, the surface roughness decreases (the paper surface is smoother) and the paper thickness increases.

On the other hand, the results of Gatto et al. [12] even showed that when three layers of chitosan were applied, the chitosan only filled the spaces in the porous structured network, not forming a film on the paper surface. It should also be noted that the paper coated with carboxymethyl modified chitosan in this study did not show a significant increase in paper thickness. This is because chitosan is only coated twice so it does not really have a significant effect on the thickness of the paper.

Antibacterial activity of Chitosan modified carboxymethyl coated wrapping paper

Carboxymethyl chitosan with a concentration of 1% has an inhibition zone of 11.50 mm against E.coli and has an inhibition zone of 12 mm against *S. aureus*. While paper coated chitosan modified carboxymethyl with concentration of 1% has inhibition zone of 10.60 mm while other concentration has no activity againts E.coli bacteria (gram negative bacteria). Paper coated chitosan modified carboxymethyl with concentration of 0.25; 0.5% and 1% has inhibition zone of 9.30; 9.70; 10,70 mm respectively againts Staphylococcus aureus bacteria (gram positive bacteria). As is known, chitosan has an amino group (NH_2) which will become ammonium (NH_4^+) in an acidic environment [13]. The positive charge of this ion will interact with the bacterial cell wall which is negatively charged, so that it can inhibit the growth of bacteria, both gram-positive and gram-negative [10].

When viewed from the antibacterial activity of carboxymethyl chitosan compared to paper coated with carboxymethyl modified chitosan, it decreased. However, the inhibition zone of carboxymethyl-modified chitosan and chitosan-modified chitosan-coated paper was still classified as having strong inhibitory activity except for the inhibition zone of chitosan-modified chitosan-coated paper with a concentration of 0.25 and 0.5% against *E.coli* bacteria. This is in accordance with the provisions of the inhibitory activity criteria [14], the inhibition zone formed 20 mm is considered to have very strong inhibitory activity, 10-20 mm is declared to have strong inhibitory activity, 5 -10 mm was declared to have moderate inhibitory activity and 5 mm was declared to have weak inhibitory activity.

The zone of inhibition against *Escherichia colibacteria* is smaller than that of *Staphylococcus aureus* bacteria compared to *Staphylococcus aureus* bacteria, this is because the cell wall structure of *Escherichia colibacteria* has a more complex cell wall structure (three layers). This inhibits antibacterial agents from penetrating the cell wall of *Escherichia colibacteria* compared to the cell wall of *Staphylococcus aureus* bacteria.

Conclusion

Based on data analysis and discussion, it can be concluded that chitosan modified carboxymethyl can be synthesized by reacting chitosan with monochloroacetic acid in 20% NaOH solution. Moreover, chitosan modified carboxymethyl can be coated on cellulose paper using the dip-coating method and acts as an antibacterial agent that has an inhibitory zone against Escherichia colibacteria of 10.60 mm and Staphylococcus aureus bacteria as much as 10,70 mm with 1% concentration of 1% chitosan modified carboxymethyl.

Acknowledgements

The authors are grateful to the DIPA Politeknik Negeri Lampung for the funding support so that the research can be conducted and this article is published.

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