A preliminary study on modified chitosan-curcuminoids as material active food packaging with antioxidant and antibacterial activities

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Abstract. The compound of inorganic nanoparticles is commonly mixed with polymeric materials for food packaging that has antibacterial function. However, the inorganic nanoparticles are toxic to human and the polymeric materials used are not easily biodegraded. The curcuminoids compound from turmeric extract has antioxidant and antibacterial activity which can be a candidate as polymer combination. The polymer used is chitosan which isolated from crab shell with the yield of 4.54 %. In this study, the modified chitosan-curcuminoids was synthesized by in-situ loading method and moulded into a film. Interaction between chitosan and curcuminoids is hydrogen interaction which determined using FTIR. Then, antioxidant activity was tested using radical DPPH scavenger. Antioxidant test results showed that modified chitosan with 20, 40, 60 80 and 100 ppm of ascorbic acid have inhibition levels of 15.49, 16.20, 16.90, 23.94 and 27.47 %, respectively. Modified chitosan with 25 ppm of curcuminoids have a comparable antioxidant activity to chitosan modified ascorbic acid 80 ppm with 24.65 % inhibition. Meanwhile, in the antibacterial test, chitosan modified with curcuminoids showed antibacterial activity toward *E. coli* and *S. aureus* bacteria with inhibition zone of 9 and 6 mm, respectively.

Keyword: Food packaging, chitosan-curcuminoids, antioxidant, antibacterial

1. Introduction
In the food packaging field, development of polymer material with antioxidant and antibacterial activity is an important aspect. Materials with antioxidant activity are able to increase the lifespan of the food by preventing the oxidation process in the food [1]. Inorganic nanoparticle materials such as copper [2], zinc oxide [3] and silver [4], are often used as packaging material additives to enhance the antibacterial activities of polymeric materials. However, inorganic nanoparticles materials are heavy metal, which can be toxic to humans’ body and the polymer material used are non-biodegradable [5]. Modified natural polymer materials have a potential to replace the function of inorganic nanoparticles used in the synthetic polymer blends. The ability of the natural polymer, which is to be naturally degradable, makes it a highly potential biomaterial to be developed and modified in various sectors [6]. The example of natural polymers is chitin and its derivatives, such as chitosan, which has a potential for packaging materials. Chitin can be converted into chitosan by deacetylation process [7].

Chitosan isolation from crab shells waste has biocompatibility property, antibacterial, antioxidant activity and also good to form film. Thus, chitosan can be used as a coating material, packaging and wound cover in the fields of food, health and chemical industries [8]. Curcuminoids are natural...
compounds from turmeric (Curcuma domestica Val.) extract. Curcuminoids are known for their antibacterial and antioxidant activities. Curcuminoids has potential as an antibacterial agent towards foodborne pathogens namely S. aureus and E. coli which represent gram-positive and gram-negative bacteria, respectively [9]. Curcuminoids can be a strong candidate to suppress oxidative propagation chains and minimize lipid oxidation in food systems, as well as maintaining the nutritional quality and extending the age of food by controlling the process of decaying microorganisms. The aim of this study is to make the material active food packaging from the modified chitosan-curcuminoids with antioxidant and antibacterial activities.

2. Methods

2.1. Preparation of crab shells
Crab shells were washed with clean water to remove physical impurities. Then, dried at 60 °C and mashed using a mortar

2.2. Demineralization of crab shells
Powder of crab shell was dissolved in HCl 1 M with ratio of 1:15 and stirred for 30 min. The residue was washed with mineral-free water until neutral (pH 6–7), then dried at room temperature [10].

2.3. Deproteination of crab shells
Powder from demineralization process was dissolved in NaOH 3.5 % with ratio of 1:10. The solution was refluxed for 2 h at 65 °C while being stirred. Then, the solution was filtered and washed with mineral-free water until the pH was neutral and dried at 60 °C for 4 h [10].

2.4. Conversion of chitin into chitosan
Chitosan was acquired by deacetylation of chitin by adding 50 % NaOH with ratio of 1:15 and then heated to 70–75 °C for 1 h. The obtained solid was washed with water until neutral pH. Then, chitosan was dried at 80 °C for 24 h. The isolated chitosan was weighed and stored at room temperature. Chitosan was analyzed using Fourier transform infrared (FTIR) [11].

2.5. Isolation of curcuminoids
Turmeric extract was obtained by using maceration extraction method. Turmeric powder was made by drying turmeric and made it into 80 mesh powder. Furthermore, 100 g of turmeric powder was dissolved in 400 mL of acetone for 3×24 h. Then, the solvent was evaporated until the extract of turmeric remained. Turmeric extracts were tested using silica thin layer chromatography with eluent of chloroform and methanol (95:5) [9]. The isolation results were also analyzed by mass spectrometry instrumentation to confirm the structure.

2.6. Preparation of chitosan-curcuminoids film
Chitosan film control was obtained by dissolving 2 g of chitosan in 90 mL glacial acetate 1 % and stirred at room temperature until became homogeneous. Then, propylene glycol of 0.5 mL was added to the chitosan gel. Followed by the addition of 2 mL additives compounds to obtain the modified chitosan film. The additives compound used in this study were curcuminoids 25 ppm from turmeric extract and commercial antioxidant as comparison was ascorbic acid with variation of 20, 40, 60, 80, 100 ppm. The gel solution of films was dried at 60 °C and stored in a desiccator before test. Chitosan and modified chitosan film were analyzed by FTIR.

2.7. In vitro antioxidant assay
The antioxidant activities of film were obtained by putting 0.14 g of film into 5 mL of DPPH radical solution (10 ppm). Then, the mixture was shaken and incubated in dark condition for 1 h. The absorbance
of the DPPH radical solution was analyzed with UV-visible spectroscopy instrument at 515 nm. The antioxidant capacity was measured on the basis of the following equation [6].

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\text{DPPH Scavenging ratio} = \frac{\text{ABS}_c - \text{ABS}_e}{\text{ABS}_e} \times 100
\]

2.8. Antibacterial activity assay
The antibacterial activity of the chitosan-curcuminoids films was investigated by the inhibition zone method. \textit{E. coli} as gram-negative bacteria and \textit{S. Aureus} as gram-positive bacteria were used in this study. Bacterial suspensions were activated in the nutrient broth medium. The diluted bacterial suspension mentioned above was pipetted as much 200 μL onto a sterilized plate, then 15 mL of nutrient agar was added and coated uniformly. After the NA medium became solid, the chitosan film sample with diameter 6 mm was placed on top of the medium and incubated at 32–37 °C for 24 h. Antibacterial activity was indicated by the inhibition zones of bacterial growth.

3. Results and discussion
3.1. Isolation chitosan from crab shells
Isolation chitin from crab shell was done by deproteination and demineralization. At the demineralization stage, mineral compound such as calcium that contained in the crab shells would react with water-soluble hydrochloric acid. After the demineralization stage, the precipitate obtained was washed with distilled water to neutral pH, universal pH was used as indicator. This washing was intended to prevent the product degradation during the drying process. Then, the deproteination aimed to break the bond between protein and chitin, by adding NaOH. During immersion, the protein would be extracted in the form of sodium-fatty acid (Sodium-proteinate) due to saponification reaction between the fat that contained in the crab shells with NaOH solution, where the Na’ ions bonded to the end of the negatively charged protein chain and then settled. Chitosan was produced through the deacetylation step by adding NaOH. Deacetylation aimed to break the acetyl group (COCH₃) that present in chitin. Based on this research, 4.54 % of chitosan was obtained and then analyzed using FTIR. The FTIR spectrum of chitosan is presented in figure 1.

Based on the spectrum in figure 1, the functional group can be identified by the absorption of the wave number 3250–3500 cm⁻¹ which is the absorption bands of the -OH group. The -OH group is used as a standard in chitosan [12], while the spectrum of chitosan compound is seen in the wave number

![Figure 1. Chitosan Powder FT-IR spectrum.](image-url)
1600–1700 cm$^{-1}$ which is the N-H bending swell that indicates the presence of primary amine (NH$_2$). The wave number 1690 cm$^{-1}$ is the absorption bands of the vibration that remaining C=O acetyl group on the chitosan chain. The wave number 1160 cm$^{-1}$ shows the vibration of the C-O-C bridge which is the typical absorption of the saccharide structure.

3.2. Isolation and characterization of curcuminoinds
Isolation of curcuminoinds was done by maceration method, because the tool that was used is simple and can be used for substances that resistant and not resistant to heating. Maceration was carried out with acetone, because it was obtained more curcuminoinds compounds than hexane, chloroform, ethyl acetate, and methanol [13]. After obtaining that crude extract, the curcuminoinds was tested by thin layer chromatography in chloroform and methanol (95:5), and then compared with standard curcumin. The result of TLC for the separation of standard curcumin and isolation result are shown in figure 2. Based on TLC spot, retention factor (Rf) were obtained for each spot contained in table 1.

Order of the spots appeared were determined by its polarity. Compound with more polar characteristic will appear earlier than others. The order of the spots from high to low are: curcumin, demethoxy curcumin, and bis-demethoxy curcumin. Data in the table 1 has a similar trend with the data reported before [13].

Analyses were also performed by using the MS instrument to confirm the structure of the compounds. These compounds have $[M+1]^+$ peak at MS spectra (figure 3). The bisdemethoxy curcumin spectra appear as positive ion mode with m/z 309.11, demethoxy curcumin m/z 339.12, and curcumin m/z 369.13 [14].

3.3. Control chitosan film and modified chitosan-curcuminoinds film
The film was produced by mixing chitosan in 1 % acetic acid and added with propylene glycol as plasticizer. In general, the addition of propylene glycol aims to form hydrogen bonds

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**Table 1. Comparison of Rf from curcumin standard and curcuminoinds extract.**

|                | Curcumin | Demethoxy curcumin | Bis-demethoxy curcumin |
|----------------|----------|---------------------|------------------------|
| Standard       | 0.66     | 0.45                | 0.34                   |
| Turmeric extract | 0.58     | 0.40                | 0.31                   |
| Ref no.13      | 0.75     | 0.55                | 0.27                   |

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![Figure 2. The result of thin layer chromatography between a) standard and b) turmeric extract.](image)
in the bonding chain between the polymers so that causing the interaction between the biopolymer molecules to be reduced. With the reducing bonding between the polymers, the resulting properties of polymer will be more elastic. Modifications were also made by adding curcuminoids and ascorbic acid in various concentrations as comparison. The interaction between compounds for this modification with the polymer is by hydrogen bonding. Furthermore, the addition was analyzed by FT-IR and the spectrum is shown in figure 4.

**Figure 3.** MS spectra of curcuminoids.

**Figure 4.** Comparison of FT-IR spectrum (a) Modified chitosan-curcuminoids film and (b) Control chitosan film.
Based on the measurement results it can be seen that chitosan-curcuminoids has broader absorbing band around 3300–3600 cm⁻¹ than chitosan. It is indicating that there is a hydrogen interaction between chitosan and curcuminoids. In addition, there is a shift in C=O stretch at the wave number 1600–1750 cm⁻¹ indicating that curcuminoid additive has been successfully mixed [5].

3.4. Scanning electron microscope test of film

Modified chitosan was further analyzed using SEM to see the effect of curcuminoid addition to its morphological structure. The SEM results are presented in figure 5.

Based on the observation of morphology using SEM, it can be seen that the surface of chitosan modified curcuminoids looks rougher than the control chitosan. This suggests that the presence of additional additives into chitosan may decrease the homogeneity of chitosan and increases the roughness.

3.5. In vitro antioxidant assay

Control and modified chitosan polymers were assayed with 10 ppm radical DPPH to see the magnitude of inhibition ability of the sample. DPPH measured in the range of 600–400 nm to see the maximum wavelength of DPPH and obtained maximum wave length absorption at 515 nm (figure 6). The control and sample absorption were measured with UV-Vis at 515 nm after stored an hour in dark condition (figure 7).

Figure 5. Structure morphology of film using SEM with 30,000 times magnification, (a) control chitosan film and (b) chitosan modified with curcuminoids.

Figure 6. Graph of the absorption spectrum of DPPH.
Figure 7. Comparison of inhibitory DPPH radicals on control chitosan, modified chitosan-curcuminoids 25 ppm and modified chitosan-ascorbic acid at various concentrations.

Figure 8. Antibacterial test result of chitosan modified with curcuminoids (DCA: Chitosan modified curcuminoids against E. coli bacteria. KA: Control chitosan against E. coli. DCB: Chitosan modified curcuminoids against S. aureus. KB: Control chitosan against S. aureus bacteria.

Based on the results, it can be seen that chitosan modified with curcuminoids have activity that approach ascorbic acid 80 ppm. The antioxidant activity occurs because the modifying compound is simply bonded by a fairly weak hydrogen bond. So that, when added into the radical DPPH solution, the modifying compound can release and inhibit the DPPH radical activity.

3.6. Antibacterial activity assay
Chitosan film modified with curcuminoids tested its antibacterial properties by performing antibacterial test against E. coli and S. aureus bacteria. The results of the antibacterial test can be seen in figure 8.

In figure 8, the clear zone was appeared which is indicating the presence of antibacterial activity of chitosan modified with curcuminoid. The inhibition zone of chitosan-curcuminoids against E.coli and S.aureus is 9 and 6 mm, respectively. This is due to the presence of curcuminoids that located on the surface of chitosan film so that it can make the bacteria unable to grow because of the diffusion of curcuminoids on the chitosan film modified.

4. Conclusion
Curcuminoids makes hydrogen interaction with chitosan. Basically, chitosan film has antioxidant and antibacterial activity. Modification of chitosan film with curcuminoids that isolated from turmeric can
enhance the antioxidant and antibacterial activities. The curcuminoids can easily diffuse from chitosan to inhibit DPPH radicals and also the growth of *S. aureus* and *E. coli* bacteria.

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