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# Preparation of Polyelectrolyte Complex Films of Chitosan-Alginate Incorporated by Eugenol and its Potency as an Antioxidant Packaging

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## Abstract

Preparation of PEC chitosan-alginate films incorporated by eugenol has been investigated. Incorporation of eugenol in chitosan-alginate films was conducted by using the different concentration of eugenol including 0.25% 0.5%, and 1% (% w/v). The effect of eugenol incorporation in chitosan-alginate films was investigated through some properties of the films such as tensile strength, elongation at break, transparency value, and water vapor permeability. Meanwhile, the effectiveness of eugenol incorporation as an active compound of the films was investigated from antioxidant activity of chitosan-alginate films incorporated eugenol. Polyelectrolyte complex (PEC) films of chitosan-alginate was occurred through molecular interaction between polycationic groups of chitosan and polyanionic groups of alginate. The formation of chitosan-alginate PEC films was synthesized at pH ± 4.0. Based on FTIR analysis, the ionic interaction between amine groups ($–NH_3^+$) and carboxylate groups ($–COO^-$) formed strongly. Characterization of films also indicated that PEC films of chitosan-alginate incorporated of eugenol was formed. Study showed that PEC chitosan-alginate films had good mechanical properties. Antioxidant activity assay through fixed reaction time method using DPPH radical (α,α-difenil-β- pikrilhidrazil) resulted in good percentage of radical scavenging activity (%RSA) from the films. The E3 films which contain 1% eugenol has 55.99% of RSA value in 96 hours.

## INTRODUCTION

Active food packaging is one of novel packaging techniques which is developed in nowdays. The development of active packaging is a promising packaging system because it can maintain the quality, the safety, and the shelf-life of food. Active food packaging is a packaging which carry an active compound that has antioxidant or antimicrobial properties. These compounds are incorporated into the matrix films and their release will be controlled from the films (Li et al., 2014; Peng et al., 2013; Ramos et al., 2012).

Eugenol (Figure 1) is one of phenolic compounds which is found as a major essential oil of clove (*Sygizium aromaticum*). Eugenol has known as a great antioxidant agent. It also has antibacterial and antifungal activities so it is used in many industries such as food, cosmetics, and pharmaceuticals. Development of packaging using eugenol had been investigated by Woranuch & Yoksan (2013). They investigated that the effect of eugenol incorporation in thermoplastic flour (TPF) has a weak tensile strength but it exhibit a great antioxidant activity compared to TPF without eugenol. Muratore et al. (2019) also reported eugenol as an active component in LLDPE films. Impregnation of eugenol in LLDPE showed a good antioxidant activity. They also reported that the eugenol-loaded films had fumigant toxicity against species *R. dominica* and *T. castaneum*, pests of stored grain, derivatives, and flour.

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Chitosan is a polysaccharide which can be obtained from chitin deacetylation, the main component of invertebrate exoskeleton. Chitosan is known as a friendly material in environment because it has biodegradable, biocompatible, and nontoxic properties (Peng et al., 2013). Alginate is a natural polysaccharide, found from marine brown algae by extraction method. Same as chitosan, this polymer is also known as a biocompatible and a biodegradable material. Polyelectrolyte complex (PEC) films made of chitosan-alginate is known has better mechanical properties than films made of one type polymer individually. Based on previous studies by Kulig and Zimoch-korzycka (2017) and Yan et al., (2000), polyelectrolyte complex films from chitosan and alginate have good mechanical properties, transparent, and flexible. Therefore, they can be used for the development of food packaging (Riyandari et al., 2018).

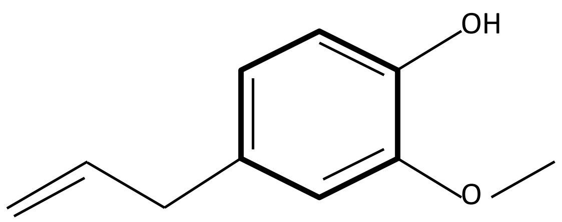

Figure 1. Structure of eugenol.

The objective of this research is to study about the effect of eugenol incorporation in to the properties of chitosan-alginate polyelectrolyte complex (PEC) films, therefore the potency of these films as antioxidant packaging can be detected. The effect of eugenol in chitosan-alginate PEC films investigated from mechanical properties of films, water vapor permeability, and transparency value. Furthermore, the antioxidant activity of PEC also investigated using DPPH method. Based on the investigation, it expects that eugenol incorporation in chitosan-alginate film can be develop as an antioxidant packaging.

## MATERIALS AND METHODS

### Materials

Chitosan was purchased from Bogor (CV Ocean Fresh), alginate (PA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, eugenol compound was obtained from the Indesso factory in Bogor, and distilled water.

### Methods

***Preparation of Films***

- ***Preparation of films with variation on the ratio of chitosan to alginate***

Alginate powder was dispersed in distilled water (A solution). Chitosan powder was poured into A solution (B solution). Then, glacial acetic acid (2%, v/v) was added into B solution. After dissolved, eugenol (0.5%, %w/v) was added into the solution and stirred using magnetic stirrer for 24 h. The films (A1, A2, and A3) was prepared from 10 mL solution (chitosan-alginate-eugenol solution) which was poured into petri dish. After that, the films were dried for 3 days at 25 °C. The films were kept in a box containing silica.

- ***Preparation of films with variation of eugenol concentration***

Alginate powder (0.25%, %w/v) was dispersed in distilled water and it was stirred with a magnetic stirrer at 25 °C. Chitosan powder (1%, %w/v) was added into alginate solution and stirred using magnetic stirrer for 20 minutes. After dissolved completely, glacial acetic acid (2%, v/v) was poured into the solution. Eugenol was added into the solution and stirred using magnetic stirrer for 24 h. The films (E1, E2, and E3) was prepared from 10 mL solution (chitosan-alginate-eugenol solution) was poured into petri dish and dried it for 3 days at 25 °C. The films were kept in a box containing silica. The detail composition of PEC chitosan-alginate films incorporated by eugenol is shown in Table 1.

Table 1. Composition of PEC chitosan-alginate films incorporated by eugenol.

| Code of films | The ratio of chitosan to alginate | Chitosan (%w/v) | Alginate (%w/v) | Eugenol (%w/v) |
|---|---|---|---|---|
| A1 | 9:1 | 1.125 | 0.125 | 0.500 |
| A2 | 8:2 | 1.000 | 0.250 | 0.500 |
| A3 | 7:3 | 0.875 | 0.375 | 0.500 |
| E1 | 8:2 | 1.000 | 0.250 | 0.250 |
| E2 | 8:2 | 1.000 | 0.250 | 0.500 |
| E3 | 8:2 | 1.000 | 0.250 | 1.000 |

***Characterization of the Films***

The films incorporated by eugenol were observed using FTIR. Dried films were kept in desiccator for 14 days at 25 °C before analysis. The films were scanned from 400 $cm^{-1}$ to 4000 $cm^{-1}$.

### *Mechanical Properties*

Mechanical properties of films (tensile strength and elongation at break) were measured by using Universal Testing Machine. The film sample was cut into 100 mm × 20 mm and put into the machine. The test speed was set as 10 mm/min.

### *Water Vapor Permeability (WVP)*

Distilled water was put into petri dish and the petri dish was then covered using aluminum foil. Aluminum foil was perforated with area 1.1 × 1.1 $cm^2$. Each sample film (1.2 × 1.2 $cm^2$) was put on the hole. All petri dishes were put into the oven at temperature of 37 ± 0.5 °C for 5 hours. Measurement of petri dish weight was conducted every hour. Water vapor transmission rate (WVTR) was used to determine water vapor permeability (WVP). WVP values were calculated using Eqs. (1) and (2).

$$WVTR = \frac{\text{mass water lost}}{time \times area} \quad (1)$$

$$WVP = \frac{WVTR}{S \times (R_1 - R_2)} \times d \quad (2)$$

Where S is saturated vapor pressure at temperature of 37 °C, $R_1$ is relative humidity in petri dish, $R_2$ is relative humidity at temperature 37 °C and $d$ is film thickness (m).

### *Transparency Value*

Transmittance of the films was measured using UV-Vis spectrophotometer at wavelength range from 200 to 800 nm. The transparency value of films was calculated using Eq. (3).

$$\text{Transparency value} = -\log T_{600} / x \quad (3)$$

Where $T_{600}$ is transmittance of the films at 600 nm and x is the film thickness (mm), the lower transparency value indicates the greater transparency of films.

### *Assessment of Antioxidant Activity*

The films incorporated by eugenol were immersed into ethanol 96% (v/v). The testing solution was added DPPH radical solution (75 µM) and it was incubated in the dark at room temperature for 30 min. DPPH radical solution without eugenol was used as a control. The absorbance of solution was measured at 517 nm using UV-Vis spectrophotometer. Radical scavenging activity (%RSA) was calculated using Eq. (4).

$$\%RSA = (1 - \frac{Abs\ sample}{Abs\ control}) \times 100\% \quad (4)$$

## RESULTS AND DISCUSSION

### Preparation of Films

Polyelectrolyte complex (PEC) films of chitosan-alginate occurred through molecular interaction between polycationic groups of chitosan and polyanionic groups of alginate. The formation of PEC films was affected by pH and ionic strength. Chitosan-alginate PEC film was synthesized at pH ± 4.0 because of PKa value of chitosan is 6.3 while alginate's pKa is 3.4-3.7 (Lawrie et al., 2007). When pH is optimum, amino groups of chitosan and carboxylate groups of alginate were ionized largely (Yan et al., 2000). Thus, the ionic interaction between amine groups ($-NH_3^+$) and carboxylate groups ($-COO^-$) will be formed strongly. The illustration of PEC formation is shown in Figure 2 and the product of PEC films are shown in Figures 3 and 4.

### Characterization of the Films

Characterization of films using FTIR is used to analysis the molecular interaction. The infrared spectrum of eugenol, PEC chitosan-alginate films and PEC chitosan-alginate films incorporated by eugenol are shown in Figure 5. Based on characterization of films, it indicated that PEC films of chitosan-alginate incorporated of eugenol was formed (spectrum c). It was shown from the wavenumber shift of PEC films after eugenol incorporation. The summary of FTIR analysis was explained in Table 2.

### Mechanical Properties

Mechanical test was performed to identify the effect of eugenol incorporation in PEC chitosan-alginate films by evaluating different parameters such as tensile strength (TS) and elongation at break (EAB). The result of tensile strength and elongation at break (EAB) are shown in Table 3. As shown in Table 3, higher alginate strength (TS) of films

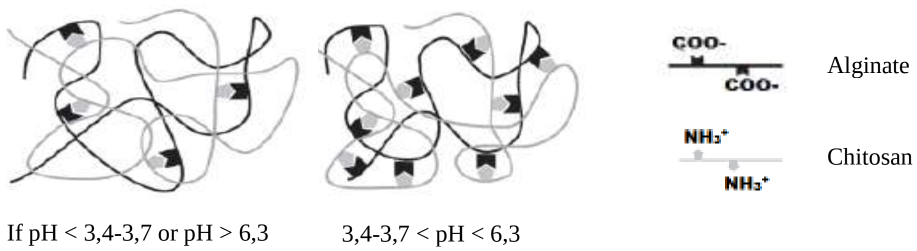


Figure 2. Illustration of ionic interaction between chitosan and alginate in some pH (Lawrie et al. 2007).


Figure 3. The products of PEC films incorporated by eugenol with variation on the ratio of chitosan to alginate a) A1 film (9:1), b) A2 film (8:2), c) A3 film (7:3).

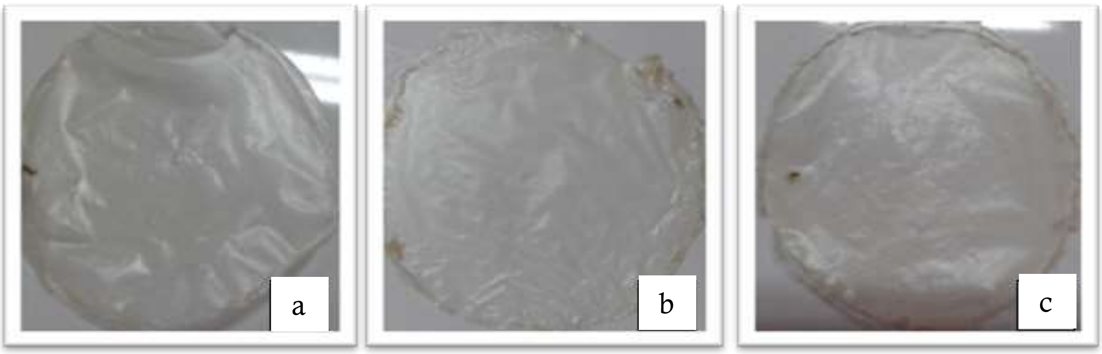


Figure 4. The products of PEC films incorporated by eugenol with variation of eugenol concentration a) E1 film (0.25%), b) E2 film (0.50%), c) E3 film (1.00% ).

(TS of A3 > A2 >A1). It reflected that molecular interaction between amino groups of chitosan and carboxylate groups of alginate became stronger. Tensile strength (TS) increased in E2 films which has final concentration of eugenol was 0.50%. This showed that E2 films have stronger interaction between chitosan-alginate than E1 films. Meanwhile, E3 films had the lowest tensile strength. It may be occurred because of the presence of hydrophobic agent. It will cause mobility of chain decrease and structure of films will be discontinued. Thus, the flexibility decreased and resistance to fracture. Rubilar et al. (2013) also found the same behavior when carvacrol mixed into fish skin gelatin films.

Based on this research, it showed that A1 higher EAB than both A2 films and A3 films. Increasing the composition of alginate resulted in decreasing of EAB on A2 and A3 films. This could be caused by weaker interaction between chitosan and alginate in A1 films. Incorporation of eugenol at higher concentration will increase EAB. As shown in Table 3, the highest EAB occurred in E3 films. It may be caused by eugenol which can act as a plasticizer same as glycerol, sorbitol, and polyethylene glycol (Bourtoom, 2008). Incorporation of eugenol as a plasticizer will increase free volume of chitosan. Thus, EAB will increase but it will decrease TS (E3 films).

Table 2. Results of film characterization using FTIR

| Material | Wavenumber ($cm^{-1}$) | Characteristics of group / explanation |
|---|---|---|
| Eugenol (Eu) | 3510 | O–H bonding |
| | 3070 | C–H bonding of aromatics |
| | 1604 | C=C of $H_2C$=CH– bonding in aromatics ring |
| | 1512 | C=C bonding of aromatics |
| | 1265 | C–O–C bonding of $CH_3$–O-$C_{aromatics}$ |
| | 1033 | C–O–H bonding of Ar–OH |
| CTS-ALG film | 3410 | O–H bonding |
| | 1566 | Ionic interaction of $NH_3^+$ of chitosan and $COO^-$ of alginate |
| | 1411 | COOH bonding |
| | 1072 | C–O–C bonding |
| CTS-ALG-Eu | 3371 | Peak shift after eugenol incorporation (3410 to 3371) |
| | 1558 | Peak shift from 1566 $cm^{-1}$ to 1558 $cm^{-1}$ |
| | 1327 | Shift of peak from 1342 $cm^{-1}$ (effect of benzene ring of eugenol in PEC films |
| | 1149 | C–O bonding (new peak) |
| | 1080 | Shift of peak from 1072 $cm^{-1}$ |
| | 896 | C–C bonding (new peak) |

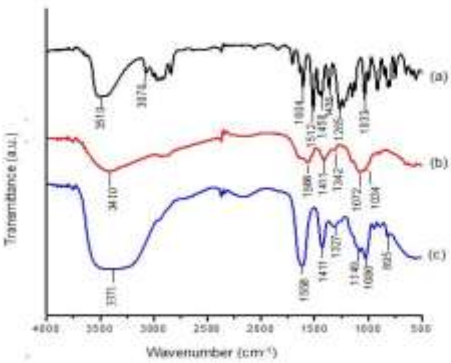


Figure 5. Spectrum FTIR of (a) eugenol, (b) PEC chitosan-alginate, (c) PEC-eugenol

**Water Vapor Permeability (WVP)**

Based on the calculation using Eq. (1) and Eq. (2), it showed that the addition of alginate composition will increase the WVP value. Higher alginate composition will increase free volume of chitosan so it allows more water to diffuse. The addition of alginate at higher levels concentration also will increase the content of hydrophilic groups, causing water can be transferred to the films easily. Thus, A3 film had the highest WVP value.

E3 films have the lowest WVP value compared with E1 and E2 films (Shown in Table 3). It was caused by ratio of hydrophilicity/hydrophobicity in E3 films decreased. Based on theory, migration of water vapor occurred on the hydrophilic site of films. When eugenol incorporation at higher level concentration, it could increase the hydrophobicity of films. It will decrease the migration ability of water vapor through the films because the hydrophilicity of films decrease. Rubilar et al. (2013) also reported that addition of hydrophobic agent such as carvacrol and grape seed extract into chitosan films reduced water vapor permeability values of films. Same behavior also occurred when the addition of root essential oil into gelatin films. It indicated decreasing WVP value (Tongnuanchan et al., 2013).

**Transparency Value**

Transparency value was obtained from Eq. (3). It can explain how particle size dispersed in PEC chitosan-alginate films. Big particle size at visible wavelength will inhibit light so it leads films transparent or not. Bigger transparency value indicated smaller transparency of films. The addition of alginate at higher level concentration into films caused the increasing of transparency value (Table 3). It indicated that bigger particle size was dispersed because of higher alginate composition. Consequently, the light which was passed through the films will be absorbed by particle, causing higher transparency value. Incorporation of eugenol at higher concentration resulted in higher transparency value. It means that E3 has the lowest transparency (Table 3). The addition of root essential oils from plai, ginger, and turmeric in higher concentration also decreased the transparency of films (Tongnuanchan et al., 2013). Hosseini et al. (2009) also reported the effect of

Table 3. Summary of PEC chitosan-alginate films properties

| Samples code | Tensile strength (MPa) | Elongation at break (%) | WVP ($\times 10^{-9}$ g det$^{-1}$ m$^{-1}$ Pa$^{-1}$) | Transparency value |
|---|---|---|---|---|
| A1 | 14.66 ± 0.11 | 4.31 ± 0.75 | 1.61 ± 0.02 | 1.86 ± 0.23 |
| A2 | 18.53 ± 1.97 | 3.65 ± 0.57 | 1.96 ± 0.01 | 2.57 ± 0.17 |
| A3 | 20.21 ± 7.11 | 3.66 ± 0.66 | 2.24 ± 0.03 | 3.58 ± 0.15 |
| E1 | 11.49 ± 1.33 | 3.52 ± 0.09 | 2.89 ± 0.03 | 2.01 ± 0.04 |
| E2 | 18.53 ± 1.97 | 3.65 ± 0.57 | 1.96 ± 0.01 | 2.57 ± 0.17 |
| E3 | 5.93 ± 0.04 | 4.81 ± 3.26 | 1.67 ± 0.01 | 4.08 ± 0.36 |

Table 4. Comparison of film properties from previous studies

| Materials of Films | Treatment | Tensile strength (MPa) | Elongation at break (%) | WVP (RH 50%, 32 ºC) ( g.mm. m$^{-2}$ 24h$^{-1}$ mmHg$^{-1}$) |
|---|---|---|---|---|
| PEC films of Chitosan- alginate **(Yan et al., 2000)** | Medium: NaOH<br>Medium: HCl<br>Medium: NaOH-HCl | 12.34 ± 1.20<br>7.53 ± 1.88<br>4.49 ± 0.76 | 7.75 ± 1.85<br>2.46 ± 0.57<br>2.32 ± 0.26 | 3.89 ± 0.12<br>2.37 ± 0.06<br>2.21 ± 0.10 |
| Thermoplastic flour (TPF) containing eugenol(E)-chitosan nanoparticles (C) **(Woranuch & Yoksan., 2013).** | TPF-E1<br>TPF-C1<br>TPF-E1-C1 | 1.51 ± 0.11<br>1.22 ± 0.09<br>1.53 ± 0.23 | 55.20 ± 6.97<br>61.33 ± 6.55<br>10.71 ± 2.38 | -<br>-<br>- |

incorporation of clove, thyme, and cinnamon essential oils would increase transparency value.

Table 4 showed comparison some properties of films which had been developed using chitosan, alginate, and eugenol. It indicated that PEC chitosan-alginate films incorporated by eugenol had better tensile strength(TS), elongation at break (EAB) and WVP value than PEC chitosan-alginate films without eugenol. Unfortunately, PEC chitosan-alginate films incorporated by eugenol had lower EAB than EAB of thermoplastic flour incorporated by eugenol.

**Assessment of Antioxidant Activity**

The DPPH method is known as a fast, easy, and reliable method to determine free radical scavenging activity of an antioxidant agent. The free radical scavenging activity of films leads to lose hydrogen and give an electron or hydrogen to DPPH free radical. As a phenolic compounds which is popular as antioxidant compounds, eugenol has really good ability to donor its electron for DPPH free radical (Gata et al., 2000). The mechanism of eugenol as antioxidant agent was showed in Figure 6.

PEC films incorporated by eugenol indicated good antioxidant activity. It was showed by RSA values of films using Equation 4. From Figure 7, it resulted in the incorporation of eugenol at higher level concentration caused increasing DPPH radical scavenging activity. E3 films have the greatest DPPH radical scavenging activity (55.99% in 96 h). Woranuch & Yoksan (2013) also reported similar results. They reported the highest percentage of radical scavenging activity (% RSA) from TPF-eugenol films was ± 50% in 72 h (Figure 8). Based on this result, it showed that eugenol incorporation in PEC chitosan-alginate film was effective to develop as an antioxidant packaging.

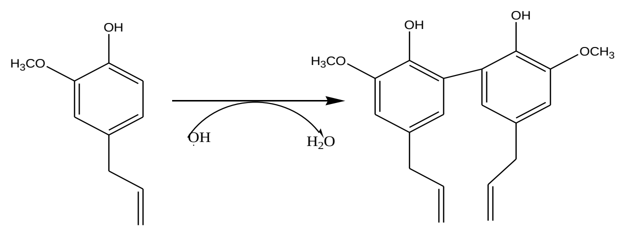


Figure 6. Antioxidant mechanism of eugenol (Gata et al., 2000)

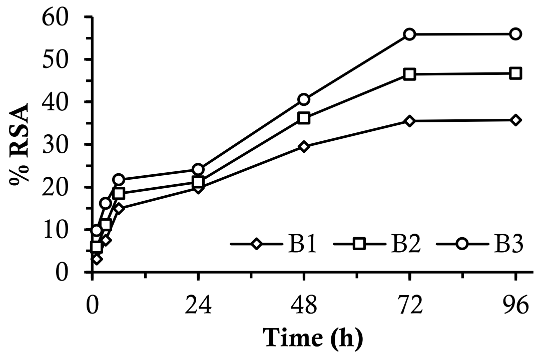


Figure 7. Antioxidant mechanism of eugenol (Gata et al., 2000)

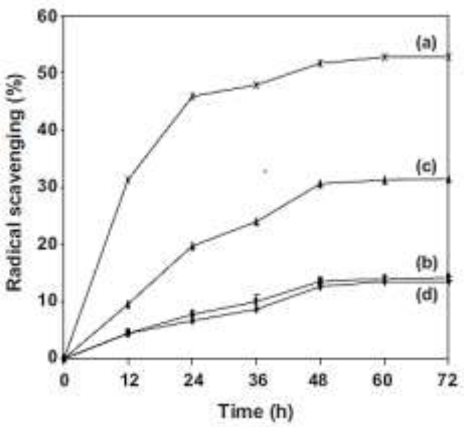


Figure 7. Profile of antioxidant activity of TPF-eugenol (Woranuch & Yoksan., 2013).

## CONCLUSION

Antioxidant activity of PEC chitosan-alginate films using DPPH method showed high RSA value of 55.99% in 96 h. Based on some properties of films including tensile strength, elongation at breaks, water vapor permeability, and transparency value, PEC chitosan-alginate films incorporated by eugenol has the potency to be developed as an antioxidant packaging.

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