The Physico-Mechanical Properties and Release Kinetics of Eugenol in Chitosan-Alginate Polyelectrolyte Complex Films as Active Food Packaging

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ABSTRACT

A study of eugenol release and its kinetics model from chitosan-alginate polyelectrolyte complex (PEC) films has been conducted. Some factors that affected the eugenol release were also studied, including the composition of chitosan-alginate PEC and the concentration of eugenol. The chitosan-alginate-eugenol PEC films were synthesized at pH ± 4.0, then the PEC films were characterized using a Fourier-transform infrared spectroscopy (FTIR) spectrophotometer. An investigation of the films' properties was also conducted, including morphology analysis using a scanning electron microscope (SEM), differential thermal analysis (DTA) / thermogravimetric analysis (TGA), mechanical strength, transparency testing, water absorption, and water vapor permeability. The release of eugenol was investigated through in vitro assay in ethanol 96% (v/v) for four days, and the concentration of eugenol was measured using an ultraviolet-visible (UV-Vis) spectrophotometer. The characterization of the films using FTIR showed that the formation of PEC occurred through ionic interaction between the amine groups ($–NH_3^+$) of the chitosan and the carboxylate groups ($–COO^–$) of the alginate. The result showed that the composition of chitosan-alginate PEC and the concentration of eugenol can affect the release of eugenol from PEC films. A higher concentration of alginate and eugenol could increase the concentration of eugenol that was released from the films. The mechanism for the release of eugenol from chitosan-alginate PEC films followed the Korsmeyer-Peppas model with an n value of < 0.5, which means the release mechanism for eugenol was controlled by a Fickian diffusion process. The antioxidant activity assay of the films using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method resulted in a high radical scavenging activity (RSA) value of 55.99% in four days.

Keywords: *PEC chitosan-alginate; eugenol; release; antioxidant; kinetics model*

ABSTRAK

Studi mengenai pelepasan eugenol dan model kinetika pelepasannya pada film kompleks polielektrolit kitosan-alginat telah dilakukan. Beberapa faktor yang memengaruhi pelepasan seperti komposisi KPE kitosan-alginat dan konsentrasi eugenol juga telah dipelajari pada penelitian ini. Pembuatan film kompleks polielektrolit kitosan-alginat yang terembani eugenol dilakukan pada pH ± 4,0. Film KPE kitosan-alginat-eugenol selanjutnya dikarakterisasi menggunakan spektrofotometer FTIR. Selain itu, dilakukan pula analisis morfologi menggunakan SEM, analisis termal DTA/TGA, uji mekanik film, uji transparansi, uji penyerapan air, dan permeabilitas uap air. Studi pelepasan eugenol dilakukan secara in vitro dalam etanol 96% (v/v) selama 4 hari dan konsentrasi eugenol yang terlepas diukur menggunakan spektrofotometer UV-Vis.Hasil karakterisasi dengan FTIR menunjukkan bahwa KPE kitosan-alginat terbentuk melalui interaksi ionik antara gugus amina ($–NH_3^+$) dari kitosan dan gugus karboksilat ($–COO^–$) dari alginat. Berdasarkan studi yang dilakukan, komposisi KPE kitosan-alginat dan konsentrasi eugenol akan memengaruhi pelepasan eugenol dari film. Peningkatan kandungan alginat dan peningkatan konsentrasi eugenol dalam film akan meningkatkan jumlah eugenol yang terlepas. Pelepasan eugenol dari film KPE kitosan-alginat mengikuti model kinetika Korsmeyer-Peppas dengan nilai n < 0,5. Hal ini menunjukkan mekanisme pelepasannya dikontrol oleh proses difusi Fickian. Hasil uji aktivitas antioksidan menggunakan metode DPPH menunjukkan bahwa film memiliki aktivitas antioksidan yang baik yaitu dengan nilai RSA sebesar 55,99% dalam waktu 4 hari.

Kata Kunci: *KPE kitosan-alginat; eugenol; pelepasan; antioksidan; model kinetika*

INTRODUCTION

Lipid oxidation is one of the factors that causes degradation and reduces the quality of food products. The oxidative reaction is caused by free radicals that attack the surface of the food so it can change the color, taste, or aroma of foodstuffs. In recent times, active food packaging has been developed, which is

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one of the most promising packaging systems created that has been found to be very effective in maintaining the food safety and its shelf life [1-3]. Active packaging is a food packaging system that contains an antioxidant or an antibacterial compound so it can inhibit free-radical activity, spoilage, or pathogenic microorganisms that contaminate foods. The technique of adding an antioxidant into active packaging films allows a controlled release of the antioxidant. This method is more efficient and effective than the addition of an antioxidant directly into the foods.

The addition of some food additives to food packaging, such as butylated hydroxytoluene (BHT) and butylated hydroxylamine (BHA), has been studied. The addition of BHT to low density polyethylene (LDPE) films could decrease the rate of lipid oxidation and extend the food shelf life [3]. However, BHT, which is a synthetic antioxidant, can be a toxic agent and has a carcinogenic potency for the human body [4-5]. Therefore, the development of active food packaging has been focused on the use of plant extracts, such as tea extract [6-7], grape extract [8-9], and ginger extract [8]; or active compounds, such as α-tocopherol [10], carvacrol [11-12], thymol [11], and eugenol [13].

Eugenol is a natural phenolic compound that is extracted from clove oil. Because of its great antibacterial, antifungal, and antioxidant activities, it has been used widely in some industries including food, pharmaceuticals, and cosmetics. A study on eugenol as an antioxidant in packaging films has been conducted by Woranuch and Yoksan [13]. They investigated the effect of incorporating eugenol-loaded chitosan nanoparticles in thermoplastic flour (TPF). These films exhibited a low tensile strength (1.53 MPa) and a high oxygen-permeability value. However, the result shows that TPF containing eugenol-loaded chitosan nanoparticles has significant radical scavenging activity compared with TPF without eugenol. That is why eugenol has the potential to be applied as an antioxidant for food packaging.

Chitosan is a natural polysaccharide that is obtained through the deacetylation of chitin, and is known as a biodegradable, cheap, renewable, and non-toxic polymer [6]. Chitosan is polycationic in an acid medium, so it has the ability to form a polyelectrolyte complex (PEC) through ionic interaction with a polyanionic substance. The formation of a PEC film is one of the techniques to employed to increase the mechanical properties of chitosan films [14]. Alginate is also a natural polysaccharide found in brown algae, and it consists of ß-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers [15]. Alginate is polyanionic so it can form a PEC with chitosan. Based on the previous studies by Yan et al. [16], and Kulig and Zimoch-Korzycka [17], PEC films from chitosan-alginate are transparent, flexible, and have good mechanical properties. Therefore, they can be used for the development of food packaging.

Although some previous studies have been conducted on food packaging using chitosan, alginate, and eugenol, no study exists regarding the incorporation of eugenol into chitosan-alginate films that focuses on the release mechanism for eugenol and its kinetics model. Hence, the objective of this research is to study the release of eugenol, including its kinetics model, release mechanism, and some factors that affect its release. Furthermore, the antioxidant activity of chitosan-alginate PEC films and their properties also will be studied in this research.

EXPERIMENTAL SECTION

Materials

Chitosan (with a degree of deacetylation of 0.87) was purchased from CV Ocean Fresh, Bogor; alginate (PA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich; eugenol was obtained from the Indesso factory in Bogor; and distilled water was also used.

Instrumentation

This work used a pH Meter Lab 680, magnetic stirrer, and hot plate. The characterization of films by Fourier-transform infrared spectroscopy (FTIR) was performed using a Shimadzu FTIR Prestige 21, and an ultraviolet-visible (UV-vis) spectrophotometer (Thermoscientific Evolution 201 UV-Vis Spectrophotometer) was used to analyze the concentration of the eugenol and DPPH radical solution. The morphology of the surface of the films was observed using a scanning electron microscope (SEM) (JEOL JSM-6510LA), and thermal analysis was performed using a Shimadzu DTG60 and a Shimadzu DSC-60.

Procedure

Synthesis of PEC chitosan-alginate films incorporating eugenol

Variation of PEC composition. Alginate solutions (0.125, 0.25, and 0.375%, w/v) were prepared by dispersing alginate powder in distilled water and stirring them with a magnetic stirrer at 25 °C. Chitosan (as shown in Table 1) was added to the alginate solution and it was stirred using magnetic stirrer for 20 min. After the chitosan had dissolved completely, glacial acetic acid (2%, v/v) was poured into the solution. Then, eugenol was added into the PEC solution to reach

Table 1. Variation of the PEC composition

Sample code	Chitosan to alginate ratio	Chitosan (% w/v)	Alginate (% bwv)
A1	9:1	1.125	0.125
A2	8:2	1.000	0.250
A3	7:3	0.875	0.375

Table 2. Variation of the concentration of eugenol

Sample code	Eugenol (% w/v)	Chitosan (% w/v)	Alginate (% w/v)
B1	0.05	1.000	0.250
B2	0.10	1.000	0.250
B3	0.20	1.000	0.250

a final concentration of 0.10% (w/v). The PEC-eugenol solution was stirred using a magnetic stirrer for 24 h. The PEC solution (10 mL) was poured onto 5.2 × 5.2 cm Petri dish and dried for 72 h at room temperature (25 °C). The dried films were kept in a box containing silica.

Variation of eugenol concentration. Alginate solution (0.25%, w/v) was prepared by dispersing alginate powder in distilled water and stirring it with a magnetic stirrer at 25 °C. Chitosan (1%, w/v) was added to the alginate solution and it was stirred using a magnetic stirrer for 20 min. After chitosan was dissolved completely, glacial acetic acid (2%, v/v) was poured into the solution. Then, eugenol was added into the PEC solution to reach final concentrations of 0.05, 0.10, and 0.20% (w/v). The PEC-eugenol solution was stirred using a magnetic stirrer for 24 h. The PEC solution (10 mL) was poured onto a 5.2 × 5.2 cm Petri dish and dried for 72 h at room temperature (25 °C). The dried films were kept in a box containing silica.

FTIR analysis. The molecular interaction of the PEC chitosan-alginate films incorporating eugenol was observed using FTIR. The film was dried in a desiccator containing silica for 2 weeks at room temperature (25 °C) before analysis. The films were scanned from 400 to 4000 cm^{-1}.

Film thickness. The thickness of the films was measured using a micrometer. Five locations on the films were chosen randomly to determine the average thickness of the films.

Mechanical properties

The tensile strength (TS) and percentage of elongation at break (EAB) were measured using a universal testing machine. The film samples were cut into pieces measuring 100 mm × 20 mm and put into the machine. The test speed was set at 10 mm/min and the measurements for the TS and EAB of each film were determined with three replications.

Water vapor permeability

The water vapor permeability (WVP) was determined using the wet cup method based on the American Society for Testing and Materials (ASTM) E96-95 1995 standard test method, with some modification. A volume of 10 mL of distilled water was put in a Petri dish and aluminum foil was used to cover the Petri dish. The aluminum foil covering the Petri dish was perforated and had an area of 1.1 × 1.1 cm. Then, each sample film (1.2 × 1.2 cm) was put in the hole and glue was used to stick in each film. The WVP of each film was determined three times. All Petri dishes were put into the oven at a temperature of 37 ± 0.5 °C for 5 h. Every hour, the weight of the Petri dish and its contents was measured. The water vapor transmission rate (WVTR) was used to determine the WVP. The WVTR and WVP values were calculated using the following equations:

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

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

where S is the saturated vapor pressure at a temperature of 37 °C, R_1 is the relative humidity in the Petri dish, R_2 is relative humidity at a temperature of 37 °C, and d is the film thickness (in m).

Water absorption

Water absorption is determined using the gravimetric method. The dried film samples were kept in a desiccator for one week. The dried films were then immersed in 10 mL of distilled water for 1 h. Next, the films were dried, and the films were weighed before and after immersing. The water absorption was calculated as follows:

$$\text{Water absorption}(\%) = \frac{Mo - M}{M} \times 100\% \quad (3)$$

where Mo is the mass of the film after immersing (in g), and M is the mass of the film before immersing (in g).

Film transparency value

The light transmittance of the films was measured using a UV-Vis spectrophotometer at a wavelength range from 200 to 800 nm. The transparency value of the films was calculated using the following equation:

$$\text{Tranparency value} = \frac{-\log T_{600}}{x} \quad (4)$$

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

Study of the release of eugenol from PEC chitosan-alginate films

The profile of the release of eugenol was investigated for 4 days through an in vitro assay in ethanol 96% (v/v) as a fatty food simulant. The films were immersed in 25 mL of ethanol 96%. At specific time intervals, the concentration of eugenol was measured using a UV-Vis spectrophotometer at a wavelength of 282 nm. The concentration of eugenol that was released from the films was calculated using the following equation:

$$\text{Eugenol release}(\%) = \frac{\text{Eugenol}(\text{released})}{\text{Eugenol}(\text{total})} \times 100\% \quad (5)$$

Kinetics model for the release of eugenol

The kinetics model for the release of eugenol was determined using four models, as follows:

$$\text{Zero-order kinetics model}: [A_t] = [A_0] + kt \quad (6)$$

$$\text{First-order kinetics model}: \ln[A_t] = -kt + [A_0] \quad (7)$$

$$\text{Higuchi kinetics model}: Q = kt^{1/2} \quad (8)$$

$$\text{Korsmeyer-Peppas kinetics model}: \frac{Mt}{M} = kt^n \quad (9)$$

where A_t is the concentration of the released active compound at t, A_0 is the earlier concentration of the active compound, Q is the release percentage (%), Mt/M is the cumulative fraction of the active compound at t, n is the release exponent indicating the chemical release mechanism, t is the release time, and k is the rate constant of the active compound.

Assessment of antioxidant activity

The antioxidant activity of the PEC chitosan-alginate films incorporating eugenol was determined using the fixed reaction time method. The films incorporating eugenol were immersed in 25 mL of ethanol 96% (v/v). A volume of 0.5 mL of the testing solution was added to a DPPH radical solution (75 µM, 3.5 mL) and then it was incubated in the dark at room temperature (25 °C) for 30 min. A DPPH radical solution with 0.5 mL ethanol solution (without eugenol) added to it was used as a control. At specific time intervals, the absorbance of the DPPH radical solution was measured at 517 nm using a UV-Vis spectrophotometer. Radical scavenging activity (RSA) was defined as the decreasing absorbance of the sample compared with the DPPH control solution. It was calculated using this equation:

$$\text{RSA}(\%) = \left(1 - \frac{\text{Abs sample}}{\text{Abs control}}\right) \times 100\% \quad (10)$$

Morphology analysis using an SEM

The films' surface morphology was examined using an SEM. Each film specimen was coated with gold and observed using an accelerating voltage of 20 kV.

Thermal analysis

The films were dried in a desiccator containing silica for 2 weeks at room temperature (25 °C) before the analysis. The dried films were scanned using a thermogravimetric analyzer from 30 to 300 °C at flow rate of 10 °C min^{-1} under a nitrogen atmosphere at rate of 30 mL min^{-1}.

RESULT AND DISCUSSION

Synthesis of PEC Chitosan-Alginate Films Incorporating Eugenol

The formation of the PEC films occurred through molecular interaction between polycationic groups and polyanionic groups. In this research, the chitosan-alginate PEC film was synthesized at pH ± 4.0, and it was formed through an ionic interaction between the amino groups of chitosan and the carboxylate groups of alginate. The pKa value of chitosan is 6.3, while the alginate pKa is 3.4–3.7 [18]. At pH ± 4.0, amino groups of chitosan and carboxylate groups of alginate are largely ionized [16]. Thus, the ionic interaction between the amine groups ($–NH_3^+$) and the carboxylate groups ($–COO^–$) are formed strongly. When the pH of the PEC solution is less than 3.4–3.7 or more than 6.3, the ionization of alginate is not optimal. When the pH is less than 3.4–3.7, the carboxylate groups are ionized slightly, and when the pH is more than 6.3, just a small amount of amine groups are ionized. However, if the pH of the PEC solution is in optimal circumstance (3.4–3.7 < pH < 6.3), both the amino groups of the chitosan and the carboxylate groups of the alginate are mostly ionized.

FTIR Analysis

Characterization by FTIR was used to analyze the molecular interaction, which relates to the physical properties of the films' matrices. The infrared spectrum of the PEC chitosan-alginate films and PEC chitosan-alginate films incorporating eugenol are shown in Fig. 1.

The spectra of the chitosan powder (spectrum a) shows characteristic peaks at wavenumbers of 1659 and 1597 cm^{-1}. The peak at 1659 cm^{-1} is due to the stretching vibration of C=O groups (amide I) and the peak at 1597 cm^{-1} corresponds to the stretching

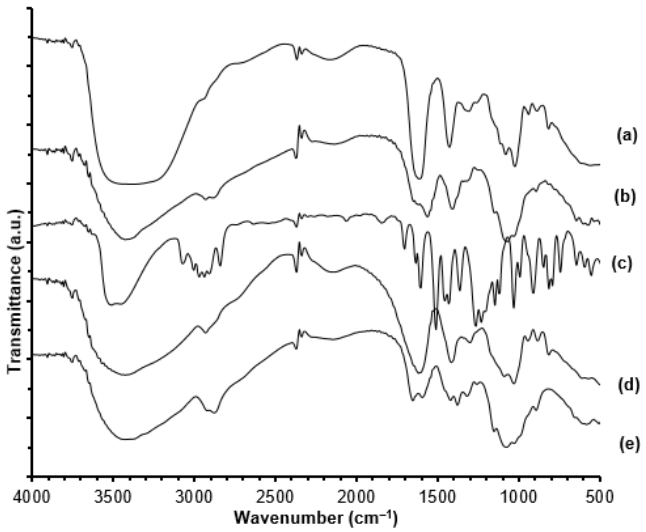

Fig 1. FTIR spectra for (a) A2, (b) PEC film, (c) eugenol, (d) alginate, and (e) chitosan

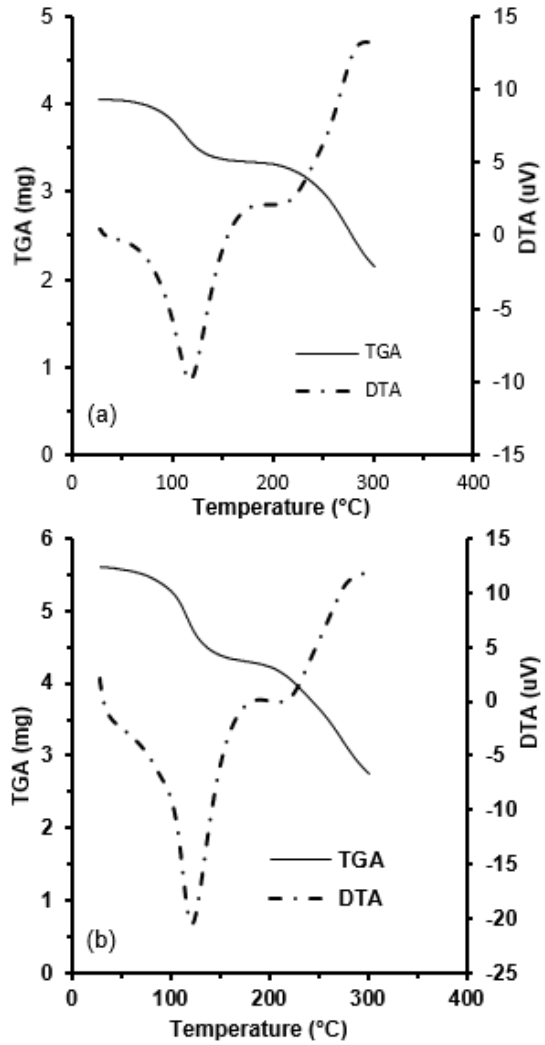

Fig 2. TGA and DTA curves for films A1 (a) and B3 (b)

vibration of the N−H bending from amine and amide II. These results are similar to the observation of Peng et al. [6]. For the alginate, there are strong peaks at 1605 cm^{-1}, representing the stretching vibration of antisymmetric $–COO^–$, and at 1420 cm^{-1}, representing symmetric $–COO^–$. Spectrum (c) shows that interaction occurred between the amino groups of the chitosan and the carboxylate groups of the alginate, so the characteristic bands at 1597 and 1605 cm^{-1} shifted to the lower wavenumber of 1566 cm^{-1}. This shift could be due to molecular interaction between the chitosan and alginate, which were under the different ionic circumstances.

When eugenol was added into the PEC chitosan-alginate films, the interaction between the eugenol and the PEC chitosan-alginate films is indicated by changes in PEC films' bands, which shifted to a lower wavenumber (from 1566 to1558 cm^{-1}). Other changes also occurred at a wavenumber between 1342 and 1327 cm^{-1}. This was possibly caused by the effect of the benzene ring in the eugenol structure. New spectra peaks appear at wavenumbers of 1149 cm^{-1} (C−O) and 896 cm^{-1} (C-C), which indicate that a strong interaction occurred between the PEC chitosan-alginate films and the eugenol. Similar results are also observed by Peng et al. [6], who report the effect of the addition of tea extract to the chitosan composite films.

Thermal Analysis

The thermal degradation behavior of films can be represented by a thermogravimetric analysis (TGA) thermogram, which can be used to investigate the loss of mass of films as a result of the decomposition process. As illustrated in Fig. 2, a single degradation step was observed for the A1 and B3 films. For the A1 film, a weight loss of 18.01% was obtained at a temperature (T_d) of 106.25 °C. A higher weight loss was noted for the B3 film when a higher level concentration of both alginate and eugenol was added to the films. The weight loss for the B3 film was 23.61% at a temperature (T_d) of 111.35 °C. The higher weight loss of the B3 film is possibly due to the higher content of water because of the presence of a higher concentration of alginate, corresponding to their hydrophilicity. The TGA reveals that the addition of eugenol did not significantly affect the thermal degradation profiles of the films. This means that eugenol could have been lost during the processing because the observed temperature was above the volatilization point of eugenol. A similar result was obtained by Ramos et al. [24].

The differential thermal analysis (DTA) technique was employed in this research; it is generally used to investigate the thermal transition, glass transition (T_g),

Table 3. Physico-mechanical properties of PEC chitosan-alginate films

Samples code	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	WVP (x 10^{-9} g det^{-1} m^{-1} Pa^{-1})	Transparency value
A1	0.0214	14.66 ± 0.11	4.31 ± 0.75	1.61 ± 0.02	1.86 ± 0.23
A2	0.0222	18.53 ± 1.97	3.65 ± 0.57	1.96 ± 0.01	2.57 ± 0.17
A3	0.0214	20.21 ± 7.11	3.66 ± 0.66	2.24 ± 0.03	3.58 ± 0.15
B1	0.0222	11.49 ± 1.33	3.52 ± 0.09	2.89 ± 0.03	2.01 ± 0.04
B2	0.0222	18.53 ± 1.97	3.65 ± 0.57	1.96 ± 0.01	2.57 ± 0.17
B3	0.0218	5.93 ± 0.04	4.81 ± 3.26	1.67 ± 0.01	4.08 ± 0.36

crystallization (T_c), and melting temperature of a polymer. From the curve achieved, the A1 and B3 films exhibited endothermic peaks at 117.14 °C (ΔH_m = 801.88 J g^{-1}) and 121.57 °C (ΔH_m = 861.44 J g^{-1}), respectively, corresponding to their melting temperatures (T_m). The higher alginate composition of the B3 film resulted in a higher melting temperature. This is due to the stronger molecular interaction that occurred between the chitosan and alginate.

Thickness and Mechanical Properties

The data on the thickness and mechanical properties of the PEC films incorporating eugenol are shown in Table 3. The films incorporating eugenol show similar values. This means that neither the composition of PEC nor the concentration of eugenol affected the thickness of the films.

The A3 film demonstrated the highest TS and the lowest EAB. As illustrated in Table 3, the addition of the alginate to the composition of PEC film increases the TS of the film (TS of A3 > A2 > A1). It implies that the higher the amount of alginate in the composition, the stronger the molecular interaction in the PEC chitosan-alginate, which is due to the amino groups of chitosan and carboxylate groups of alginate being mainly ionized. The incorporation of a higher amount of eugenol affected the PEC films in a different way. An increase in TS was observed in the B2 film when the final concentration of eugenol was 0.10%. This indicates that the ionic interaction between the chitosan and alginate in the B2 film is stronger than in the B1 film. A significant decrease in TS was observed in the B3 film. The addition of a hydrophobic agent into the film causes discontinuities in the film structure and less chain mobility, so it will decrease flexibility and resistance to fracture. The same behavior was also found when carvacrol was mixed into fish-skin gelatin films in a higher concentration [12].

The highest EAB value was detected for the A1 film. When the amount of alginate in the composition increased, the EAB of films A2 and A3 decreased. This could be due to the weaker interaction between the chitosan and alginate in the A1 film. Consequently, the incorporation of eugenol, which can be a plasticizer, will increase the free volume of chitosan, causing a reduced TS, but an increased EAB value. However, no significant difference was observed between the EAB values for A2 and A3. The effect of adding eugenol at a higher concentration also increased the EAB. The same factor could be used to explain this phenomenon. The effect of a plasticizer, such as glycerol, sorbitol, or polyethylene glycol, was investigated by Bourtoom [19]. A similar effect was found when this plasticizer was blended into rice-starch-chitosan films.

Water Vapor Permeability

As shown in Table 3, the addition of alginate into the composition of PEC films increases the WVP value. The A3 film shows the highest WVP value. A composition with a higher level of alginate increases the free volume of chitosan, allowing for more water diffusion. This could be due to the hydrophilicity of films. The addition of alginate at higher levels of concentration increases the amount of hydrophilic groups in the content of the film, meaning water can be transferred easily to the films. Similar results were obtained by Fakhoury et al. [20].

The WVPs of the PEC chitosan-alginate films, containing 0.05%, 0.10%, and 0.20% eugenol, are shown in Table 3. The WVP value of the B3 film is the lowest compared with the others. Migration of water vapor occurred on the hydrophilic site of the films. When eugenol was added into the films at higher level of concentration, it can increase the hydrophobicity of films, thereby decreasing the ability of water vapor to migrate through the film. Rubilar et al. [12] also report that the addition of a hydrophobic agent, such as carvacrol or grapeseed extract, into chitosan films reduces the WVP values of films. Similarly, the addition of a root essential oil into gelatin films also decreases the WVP because of the hydrophobicity of films [21].

Film Transparency Value

One of the important factors to be considered for food packaging film is film transparency. A transparency parameter is used to explain the size of particles dispersed in chitosan matrix films. The transparency value of each film is shown in Table 3. The greater the transparency value indicated, the lower the transparency of the film. The addition of alginate at

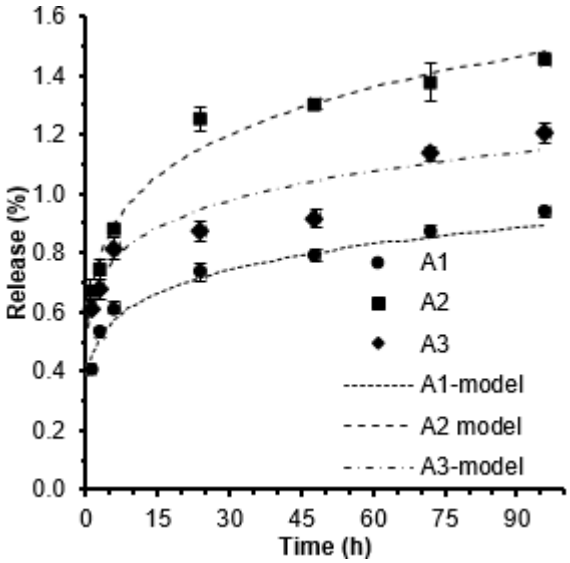

Fig 3. Profile of the release of eugenol from PEC films for different PEC compositions, with films' mass proportions of chitosan:alginate of 9:1 (A1), 8:2 (A2), and 7:3 (A3)

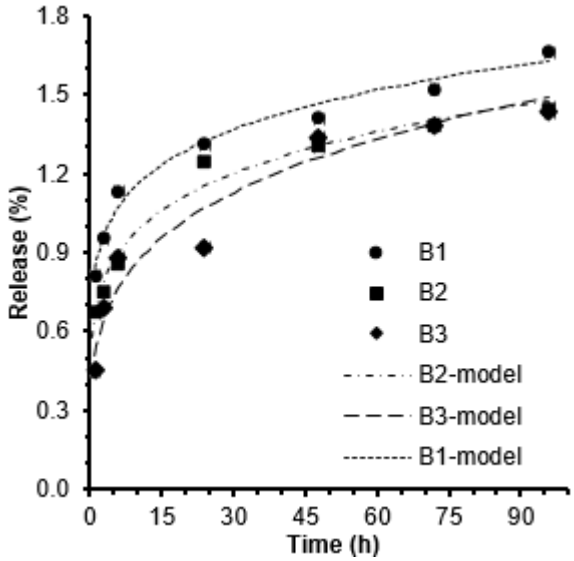

Fig 4. Profile of the release of eugenol from PEC films at different levels of eugenol concentration: B1 (0.05%), B2 (0.10%), and B3 (0.20%)

a higher level of concentration into a film leads to an increase in the transparency value. This implies that bigger particles were dispersed as a result of the higher alginate concentration, as the light that passes through the film matrix was absorbed by the particles, causing a higher transparency value. The B3 film has the highest transparency value. The results in Table 3 demonstrate that the incorporation of eugenol at a higher level concentration can decrease the transparency of films. A similar result was obtained by Tongnuanchan et al. [21], who state that the addition of essential oils from plai, ginger, and turmeric roots in higher concentrations decreases the transparency of films. Hosseini et al. [22] also describe the effect of incorporating clove, thyme, and cinnamon essential oils, which also increase the transparency value.

Study of the Release of Eugenol

Antioxidant packaging is one of the active packaging techniques, which allows a controlled release of an antioxidant from the packaging film to the surface area of the food. The release behavior of an antioxidant is related to factors such as solvent, polymer solubility, and swelling properties. A food simulant is generally used to investigate the release behavior of an antioxidant in packaging films. Eugenol is a hydrophobic active compound; therefore, it is suitable for high-fat foods. Ethanol 96% has a similar hydrophobicity to high-fat foods, so it can be used as a food simulant for eugenol.

The release profiles for eugenol from PEC chitosan films are shown in Fig. 3 and Fig. 4, which reveal that the percentage of eugenol release was very low. The A2 film had the highest percentage of eugenol release, which is 1.45% in four days (Fig. 3). Fig. 4 indicates the highest percentage of eugenol release occurred in the B1 film. Gargiulo et al. [10] explain that the release of an active compound from a polymer matrix consists of two steps. First, there is diffusion of the solvent from the outer solution into the film pores to dissolve the active compound. Second, there is the diffusion of the dissolved active compound out of the film pores. Gargiulo et al. [10] report that, when the hydrophobicity of the pore surface increases, it takes more time for an ethanol solvent to diffuse from the outer solution to the pores [10]. Thus, the release of α-tocopherol from a film pores depends on the hydrophilicity of the films. The release of eugenol from PEC chitosan-alginate films could also be related to the hydrophilicity of PEC films.

The hydrophilicity of the films can be reflected by their water absorption value. The water absorption test identifies that the addition of a higher level of alginate increased the percentage of water absorption. The water absorption percentages for A1, A2, and A3 are 46.25, 48.90, and 51.60%, respectively. Based on what is shown in Fig. 3, there was a decrease in eugenol release in the A3 film, although its water absorption value is higher than for the A2 film. This is due to the strong molecular interaction between the PEC chitosan-alginate film and the eugenol, and supported

Table 4. Kinetics data from various models for PEC chitosan-alginate films

Sample code	Zero-order		First-order		Higuchi		Korsmeyer-Peppas		
	k	r	k	r	k	r	k	R	n
A1	0.0134	0.9272	5×10^{-5}	0.9275	0.0532	0.9743	0.4307	0.9911	0.16
A2	0.0221	0.8993	8×10^{-5}	0.8996	0.0895	0.9656	0.6502	0.9902	0.18
A3	0.0160	0.9611	6×10^{-5}	0.9613	0.0607	0.9690	0.6070	0.9614	0.14
B1	0.0110	0.9360	8×10^{-5}	0.9364	0.0868	0.9777	0.8209	0.9931	0.15
B2	0.0221	0.9005	8×10^{-5}	0.9009	0.0902	0.9663	0.8209	0.9902	0.18
B3	0.0529	0.9129	9×10^{-5}	0.9133	0.1050	0.9595	0.4984	0.9718	0.24

by the higher TS of A3 compared to A2 (Table 3). Thus, it is more difficult for the eugenol in the A3 film to dissolve in the ethanol solution, which causes a reduction in the percentage of eugenol release. Fig. 4 reveals that the highest eugenol release occurred in the B1 sample. This can be explained by the hydrophilicity of each film. The water absorptions for B1, B2, and B3, are 51.48, 48.90, and 44.31%, respectively. The higher hydrophilicity of the films also resulted in a higher percentage of eugenol release.

The release rate of eugenol from the PEC chitosan-alginate films is very slow. This process is called sustained release. It indicates that the eugenol incorporated into the PEC films has a water-soluble character. The fast release of an active compound usually causes toxicity because of the very high concentration of the active compound released. For this reason, the sustained release of an active compound from the chemical matrix is more effective than fast release.

Kinetics Model for Eugenol Release

The kinetics model for eugenol release can be used to explain the mechanism for releasing eugenol from PEC chitosan-alginate films. For this research, he kinetics model for eugenol release is described using four models: the zero-order kinetics model, first-order kinetics model, Higuchi kinetics model, and Korsmeyer-Peppas kinetics model. The kinetics data for the eugenol release in the various models are shown in Table 4, and, based on this data, we can assume that the zero-order model, first-order model, and Higuchi model do not express the release mechanism for eugenol. Plotting the regression from the zero-order model, first-order model, and Higuchi model illustrate the correlation coefficient values (r) of the films are not good. A high linearity is expressed by a high value for the correlation coefficient (the r value is close to 1.0).

Among all the models, the correlation coefficient value for the Korsmeyer-Peppas model, for all films, is close to 1.0. This indicates that the mechanism for the release of eugenol from PEC chitosan-alginate films follows the Korsmeyer-Peppas model, and the n values of all films are less than 0.5. The release exponent value (n) indicates an active compound release mechanism.

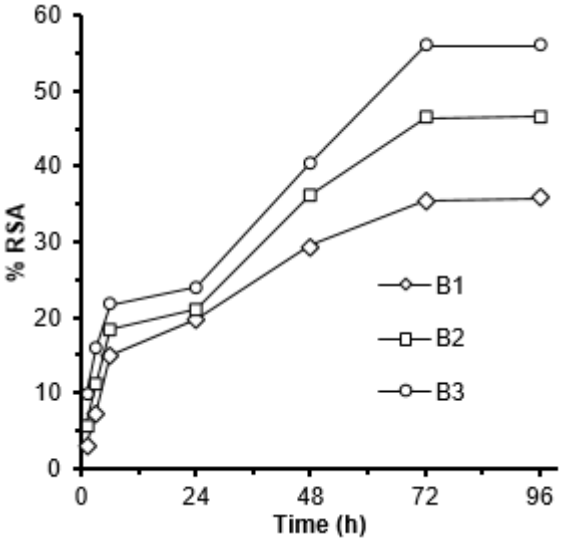

Fig 5. Antioxidant activity of PEC chitosan-alginate films incorporating eugenol

Because the n values of all films are less than 0.5, it is revealed that the release mechanism of eugenol is controlled by a Fickian diffusion process. This mechanism explains that the release of eugenol from PEC chitosan-alginate films occurs through a diffusion process without the disintegration of the PEC chitosan-alginate films.

Assessment of Antioxidant Activity

The antioxidant properties of films were analyzed and determined using a DPPH radical. This DPPH radical is one of the very stable nitrogen radicals, and measuring its scavenging activity is an important method for determining the antioxidant capacity of films [23]. Fig. 5 shows the presence of eugenol in PEC chitosan-alginate films increased the activity of DPPH radical scavenging. The addition of eugenol at a higher level of concentration caused increased DPPH radical scavenging activity. The greatest DPPH radical scavenging activity of the films is about 55.99% (Fig. 5).

The incorporation of eugenol into PEC chitosan-alginate films at a higher level of concentration causes a higher concentration of eugenol to be released from

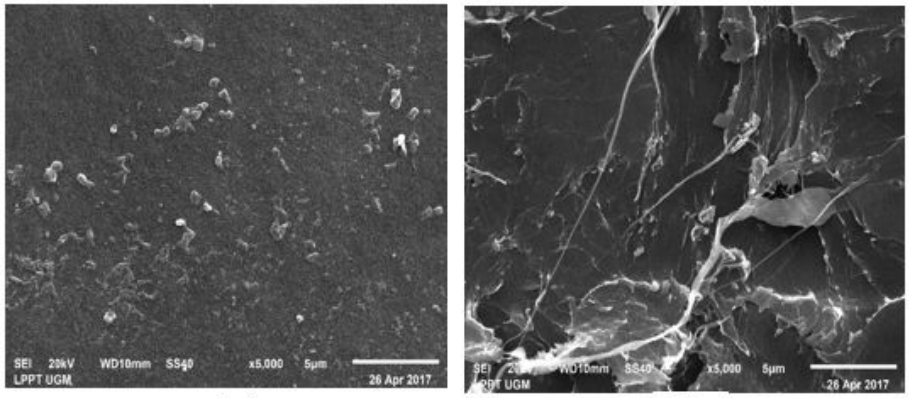

Fig 6. SEM micrograph of films A1 (a) and B3 (b)

films. Based on the data shown in Fig. 5, the DPPH radical scavenging activity values for the B1, B2, and B3 films are 35.72, 46.69, and 55.99%, respectively. The greatest DPPH radical scavenging activity appeared in the B3 film, which is due to the release of a higher concentration of eugenol from the B3 film than both the B1 and B2 films. From this release study, the concentration of eugenol released from the B3 film is about 8.18 μg mL^{-1} for 96 h (four days). Meanwhile, the concentrations of eugenol released from the B1 and B3 films are 2.38 and 4.15 μg mL^{-1}, respectively. A similar result was obtained by Woranuch and Yoksan [13]. The addition of eugenol-loaded chitosan nanoparticles into TPF resulted in a DPPH radical scavenging percentage of about 52.63% in 72 h (three days).

Morphology Analysis Using SEM

Fig. 6 shows the SEM micrograph of the PEC chitosan-alginate films incorporating eugenol. Observation of both A1 and B3 films shows heterogeneous surface morphologies after the addition of eugenol at a higher level of concentration and with a higher amount of alginate in the composition. The morphology of A1 film displays a porous formation in the surface the film. This is due to the presence of eugenol that was not dissolved completely in the aqueous phase of the chitosan and alginate. For the B3 film, the rougher surface and heterogeneous distribution are displayed on the SEM micrograph. This indicates that the addition of eugenol at a higher level of concentration caused eugenol segregation in the aqueous phase of the chitosan and alginate, so the surface of the B3 film became rougher [21].

CONCLUSION

The composition of the PEC chitosan-alginate films and the concentration of eugenol affected the release of eugenol from PEC films. Higher concentrations of alginate and eugenol increased the concentration of eugenol that was released from the films. The mechanism for the release of eugenol from PEC chitosan-alginate films followed the Korsmeyer-Peppas model and was controlled by a Fickian diffusion process. An assessment of the antioxidant activity of the films conducted using the DPPH method showed a high RSA value of 55.99% in 96 h. Based on the mechanical properties, water permeability, and transparency value, PEC chitosan-alginate films incorporating eugenol could be potentially used as antioxidant packaging.

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