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Antioxidant Release Profile from Chitosan/κ-Carrageenan-based Polyelectrolyte Complex Films as Active Packaging: A Preliminary Study

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ABSTRACT. Significant efforts are necessary to prevent the spoilage of fatty acid-rich foods as a result of lipid oxidation. Active packaging is a technology that employs packaging films containing antioxidants to increase the stability of perishable foods susceptible to oxidation. This preliminary study aimed to incorporate tannic acid (TA) into a chitosan/κ-carrageenan (Chit/κ-Car)-based polyelectrolyte complex (PEC) films and evaluate their antioxidant release profile. The composition of Chit/κ-Car, glycerol (gly), and TA in PEC films were also investigated in this preliminary study as factors influencing the mechanical properties of films and the antioxidant release profile. The prepared PEC films were identified by an FTIR spectrometer and examined through a mechanical property test. The DPPH method was used to assess the antioxidant property of the Chit/κ-Car-based PEC films. The infrared spectra revealed that PEC films were formed through the interaction between $-NH_3^+$ of Chit and $-OSO_3^-$ of κ-Car. Increasing the κ-Car and TA compositions in the PEC films increased the tensile strength (TS) and water absorption capacity (WAC), but decreased the elongation (E). The release profile of TA from PEC films followed the Korsmeyer-Peppas model through the Fickian diffusion mechanism with $n < 0.5$. The antioxidant activity test of PEC films using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method revealed high antioxidant activity with radical scavenging activity (RSA) values up to 95.04%. As a result, the Chit/κ-Car-based PEC films have the potential to be developed as active packaging.

Keywords: Active packaging, antioxidant, Chit/κ-Car-based PEC films, release profile, TA

INTRODUCTION

Lipid oxidation can lead to a decrease in food product quality, which is characterized by changes in taste, aroma, and color. In addition to spoiling fatty acid-rich foods, lipid oxidation also has detrimental effects on food quality by degrading polyunsaturated fatty acids (PUFA), forming toxic aldehydes, and reducing nutritional value (de Carvalho et al., 2021; Liu et al., 2015; López-de-Dicastillo et al., 2013). Various methods for preventing these issues included the incorporation of antioxidant compounds into food products and the development of active packaging (Gómez-Estaca et al., 2015; Woranuch et al., 2015). The process of lipid oxidation can be slowed down by adding antioxidant compounds to food products, but relatively high initial concentrations of them will have the opposite effect and act as pro-oxidants (Nikbakht-Jam et al., 2015). The alternative approach to address the issue is to limit the presence and amount of oxygen interacting with the food product by using active packaging.

Active packaging is a type of food product packaging system that has been altered to maintain or enhance food quality and safety (Ahmed et al., 2022; Domínguez et al., 2018). One of the active packaging modifications being developed is antioxidant packaging. Antioxidant packaging involves adding antioxidant substances to packaging films to enhance food stability and slow oxidation by releasing antioxidants onto the food's surface, thus extending its shelf life (Gómez-Estaca et al., 2015; López-de-Dicastillo et al., 2013). Studies on the development of antioxidant packaging have been widely reported, including the development of synthetic antioxidant compounds in the form of butylated hydroxytoluene (BHT) on low density polyethylene (LDPE) films (Torres-Arreola et al., 2007) and incorporation of green tea extract antioxidants into ethylene vinyl alcohol (EVOH) copolymer films (López-de-Dicastillo et al., 2011). However, because BHT antioxidants are carcinogenic and LDPE/EVOH-based films are less biodegradable, more environmentally friendly food packaging is required.

Many studies have been published on the fabrication of biodegradable composite materials for food packaging, including the use of starch

(Jayakumar et al., 2019), polyvinyl alcohol (Huang et al., 2017), collagen (Bhuimbar et al., 2019), and chitosan (Zhang et al., 2017). Chitosan (Chit), which is considered to have great potential for use as a packaging film, is the second most prevalent polysaccharide material found in nature after cellulose. Due to its non-toxicity, biodegradability, and biocompatibility, Chit is frequently used in the biomedical, chemical, food, cosmetic, agricultural, and pharmaceutical industries. Chit has a good ability to form films, and these films can be used for food packaging (Zhang et al., 2017), body tissue replacements (El-Meliegy et al., 2018), and dressings for wounds (Mukherjee et al., 2018). However, the use of Chit-based films is frequently limited due to their less stable mechanical properties, despite the fact that the mechanical properties of films are one of the most important aspects of food packaging. Several techniques, such as blending (Bhuimbar et al., 2019; Haghighi et al., 2019), cross-linking (Bhat et al., 2021; Tripathi et al., 2009), and the development of Chit-based PEC (Erceg et al., 2023; Lai et al., 2021), can be used to improve the physical properties of Chit-based films.

There was a lot of potential for food packaging with the development of Chit-based PEC. The presence of a protonated amine group $-NH_3^+$ in the structure of Chit tends to generate polycationic in an acidic medium and forms PEC with a polyanionic. Several polyanionics have been studied in the fabrication of Chit with PEC, including hypromellose/carmellose sodium, gelatine, pectin, and carrageenan. The preparation of chitosan/hypromellose/carmellose sodium-based PEC resulted in high flexibility and antibacterial properties of food packaging (Lai et al., 2021). The development of chitosan/gelatin-based PEC that have been modified with β-cyclodextrin/lemongrass essential oil resulted in edible films for food packaging (Erceg et al., 2023). The formation of ionic interactions between the anion groups ($-COO^-$) of pectin and the protonated cation groups of Chit during the synthesis of chitosan/pectin-based PEC resulted in a film that has a higher tensile strength than the Chit films (Chen et al., 2010). Chit modified with κ-carrageenan/gelatine resulted in PEC in the form of hydrogel and was used as tissue regeneration (Loukelis et al., 2022). κ-carrageenan (κ-Car), an anionic polysaccharide with one sulfate functional group derived from red seaweed species extracts, was used in this study to prepare PEC because of its biocompatible character and ability to form gels. The formation of Chit/κ-Car-based PEC increased the tensile strength (TS) of the undegraded film from 3.8 to 25.5 MPa due to the formation of hydrogen bonds in the mixtures (Shahbazi et al., 2016). Therefore, Chit/κ-Car films with high mechanical strength have the potential to be modified with antioxidant compounds to form active packaging.

Antioxidants are substances that can impede, postpone, or even stop the oxidation process. Antioxidants must also be effective at low concentrations, compatible with food substrates, non-toxic, and not affect the flavor, color, or aroma of food products. Tannic acid (TA) was one of the antioxidants with high antioxidant activity (Jing et al., 2019). TA, catechins (CAE), caffeic acid (CA), and ferulic acid (FA) were the four phenolic compounds that were investigated in a previous study (Maqsood & Benjakul, 2010). TA had the highest antioxidant activity due to its ability to donate hydrogen to free radicals against DPPH of the four phenolic compounds at the same concentration, followed by CAE, CA, and FA. As a result, this preliminary study prepared the way for the development of PEC based on Chit and κ-Car polymers that have been modified with TA-based antioxidant compounds. The compositions of Chit/κ-Car, gly, and TA in PEC films, followed by the release profile and bioactivity assay tests of TA, were also investigated in this preliminary study.

EXPERIMENTAL SECTION

Materials

The materials used in this study were produced by Merck with pro-analyte quality and included: gly ($HOCH_2CH(OH)CH_2OH$; molecular weight, Mw = 92.09 g/mol), TA ($C_{76}H_{52}O_{46}$; Mw = 1701.20 g/mol), ethanol (CH_3CH_2OH), and 1,1-diphenyl-2-picrylhydrazyl (DPPH; $2,4,6\text{-}(O_2N)_3C_6H_2NHN(C_6H_5)_2$; Mw = 395.33 g/mol). Chit (deacetylation degree = 87%; viscosity = 100–800 mPa.s. (at 20 °C with 1 °C Chit in 1 °C acetic acid; Mw = 1526.5 g/mol) and κ-Car (viscosity = 5–25 mPa.s.) with food grade quality were produced by Departemen Teknologi Hasil Perairan, Institut Pertanian Bogor (IPB), and CV. Ocean Fresh, Bogor, respectively. The distilled water used in this study was produced by CV. Progo Mulyo, Sleman, Yogyakarta.

Synthesis of Chit/κ-Car-Based PEC Films Modified with gly and TA

This study modified a method from previous study to synthesize Chit/κ-Car-based PEC films (Shahbazi et al., 2016). The comparison of Chit, κ-Car, gly, and TA was determined by the composition ratio shown in **Table 1**. Briefly, the κ-Car powder was dissolved in hot distilled water, followed by additions of a 1% acetic acid-prepared Chit solution until it formed PEC. TA and gly were added to the PEC solution after being agitated with a magnetic stirrer until homogenous. The mixture had a total volume of 10 mL. After being agitated until homogenous for 24 hours, the mixture was cast in a teflon petri dish, and dried at 50 °C. The film was peeled off from the teflon petri dish after it had dried. The whole sample was prepared to move on to the characterization and performance testing phases of the process. The entire performance testing, including TS, E, and WAC of the synthesized

Table 1. The composition of Chit, κ-Car, gly, and TA

PEC films	Chit (g)	κ-Car (g)	gly (g)	TA (g)
PEC 1	0.27	**0.03**	0.02	0.01
PEC 2	0.21	**0.09**	0.02	0.01
PEC 3	0.15	**0.15**	0.02	0.01
PEC 4	0.21	0.09	**0.01**	0.01
PEC 5	0.21	0.09	**0.02**	0.01
PEC 6	0.21	0.09	**0.03**	0.01
PEC 7	0.21	0.09	0.03	**0.05**
PEC 8	0.21	0.09	0.03	**0.10**
PEC 9	0.21	0.09	0.03	**0.20**

composites, was conducted three times to obtain the mean data and its standard deviation. The data processing methods for the TS, E, and WAC test results, as well as the antioxidant release, were processed using OriginPro 2024 software version 10.1.0.170. The proposed interaction estimation was illustrated using ChemDraw 20.0 software.

Characterization of PEC Films

The FTIR (Fourier Transform Infra-Red) Spectrometer Shimadzu Prestige 21 model was used to identify some functional groups of PEC films between 400 and 4000 cm^{-1}. The TS and elongation (E) tests were analyzed using the Universal Testing Machine Zwick 0.5. The water absorption capacity (WAC) of the film was determined by immersing a dry (water-free) PEC film of known mass into 10 mL of distilled water for an hour. The percentage of water absorption was measured by Equation 1 (Ishartono et al., 2021).

$$WAC\ (\%) = \frac{M_1 - M_0}{M_0} \times 100\% \quad (1)$$

where M_0 (g) and M_1 (g), respectively, are weights of dry and swollen samples, while WAC (%) is dried sample's capacity to absorb water.

Release Profile of TA

TA's release profile from Chit/κ-Car-based PEC films was assessed in vitro using ethanol 96% medium based on a modified previous method (Leal et al., 2018). An analysis of the TA concentration was performed using a spectrophotometer UV-Vis Perkin Elmer at a wavelength of 278 nm after the TA-embedded Chit/κ-Car-based PEC films had been submerged in 25 mL of 96% ethanol for each of the time intervals (1, 3, 6, 24, 48, 72, 96, and 120 h).

Generally, to investigate the kinetic profile of TA release, zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models were used to learn the dissolution of active agents such as drugs (Dash et al., 2010; Leal et al., 2018). The zero-order kinetics of release assumes that the release of the active substance in the form of antioxidants occurs slowly and at a constant rate, regardless of the active substance concentration. Equation 2 can be used to express the zero-order release kinetics model mathematically.

$$[Q]_t = [Q]_0 + k_0 t \quad (2)$$

where $[Q]_t$ is the concentration of TA dissolved in time t, $[Q]_0$ is the initial concentration of urea in the release medium ($Q_0 = 0$), and K_0 is a zero-order release constant.

The first-order kinetic model, which can be expressed by the following Equation 3, presupposes that the dissolution rate is dependent on the active agent concentration.

$$\log C = \log C_0 - \frac{K.t}{2.303} \quad (3)$$

where C_0 is the initial concentration of TA, C is the remaining concentration of TA in the PEC films, and K is the first-order release constant expressed in units of $time^{-1}$.

The Higuchi model, which can be expressed by the following Equation 4, presumes that the initial active agent (TA) in the matrix is significantly higher than its solubility.

$$Q = K_H.t^{1/2} \quad (4)$$

where Q is the dissolution percentage of TA in time t and K_H is the Higuchi dissolution rate constant.

The Korsmeyer-Peppas kinetic model describes active agents such as TA dissolution from a polymeric matrix and can be expressed as the following Equation 5.

$$M_t/M_\infty = K.t^n \quad (5)$$

where M_t/M_∞ is a fraction of TA released at time *t*, K is a constant release rate, and n is an exponent of release in the function of time *t* which supplies the transport mechanism.

TA Bioactivity Assays

The antioxidant property test was carried out in two ways: using the fixed reaction time and the steady state method (Mishra et al., 2012). The stable radical DPPH was used in the fixed reaction time method to assess the antioxidant activity of the Chit/κ-Car-based PEC films. This method was carried out by immersing the Chit/κ-Car-based PEC films, which had carried TA, into 25 mL of 96% ethanol. Then, 0.5 mL of the test solution containing released TA was pipetted for each time unit (1, 3, 6, 24, 48, 72, 96, and 120 h) and reacted with 3.5 mL of 75 μM DPPH radical solution in 96% ethanol. The mixture was stirred and left in a dark, room-temperature environment for 30 minutes. The absorbance of DPPH radicals was measured using a UV-Vis spectrophotometer at 517 nm with 0.5 mL of

96% ethanol as a control. Equation 6 was used to determine the radical scavenging activity (RSA) value:

$$\text{RSA (\%)} = \left(1-\frac{A_{sample}}{A_{control}}\right) \times 100\% \quad (6)$$

where $A_{control}$ and A_{sample} represent the radical (DPPH) absorbances at 517 nm in the absence and presence of antioxidants, respectively.

In the steady state method, the same steps were taken to determine the percentage of DPPH that remained after interacting with TA. The estimation of the residual DPPH using the steady state method is represented by Equation 7 below:

$$\text{DPPH}^{\cdot} \text{ remaining (\%)} = \frac{A_{sample}}{A_{control}} \times 100\% \quad (7)$$

where $A_{control}$ and A_{sample} represent the radical (DPPH) absorbances at 517 nm in the initial and steady states of antioxidants, respectively.

RESULTS AND DISCUSSION

Synthesis of Chit/κ-Car-Based PEC Films

Polycationic and polyanionic molecule interactions lead to the formation of PEC films. The formation of Chit/κ-Car-based PEC films occurred optimally when the polymer's ionic group was ionized (Araujo et al., 2014; Li et al., 2013). At pH values between Chit's pKa of 6.3 and κ-Car's pKa of 2.8, PEC films of the two molecules were formed. In this preliminary study, Chit/κ-Car-based PEC films were formed at pH 3.5. At low pH (pH 2.8) or at high pH (pH > 6.3), the ionic interactions formed were not optimal. The formation of PEC solution at low pH (pH 2.8) caused κ-Car not to ionize to $-OSO_3^-$ even though Chit was protonated to $-NH_3^+$. However, the development of PEC films at high pH (pH > 6.3) resulted in the ionization of κ-Car to $-OSO_3^-$ and the unprotonation of Chit. As a result, to form optimal Chit/κ-Car-based PEC films, an appropriate pH mixture was required.

Characterization of Chit/κ-Car-Based PEC Films

The FTIR spectra were used to predict the intermolecular interactions and are presented in **Figure 1**. In **Figure 1a**, the absorption at 1659 cm^{-1} referred to the C=O stretching of the amide I group of Chit, which contained an acetyl group. In addition, the absorption at 1597 cm^{-1} was attributed to the overlapping absorption of amide II from the amide group with the bending vibrations of the H-N-H group of the amine group (Araujo et al., 2014). The spectra of κ-Car in **Figure 1b** showed a characteristic absorption at 1258 cm^{-1}, which could be described to an asymmetrical S=O stretching in the sulfate esters. The absorption at 926 cm^{-1} was ascribed to the C–O–C stretching of 3,6-anhydrous-D-galactose, while at 849 cm^{-1}, it was ascribed to C–O–S stretching of galactose-4-sulfate (Kanmani & Rhim, 2014). The characteristic of the formation of Chit/κ-Car-based PEC films in **Figure 1c** was also observed by a new absorption at 1588 cm^{-1} with fairly sharp intensity. This could be assigned to the interaction between the $-NH_3^+$ group of Chit and the $-OSO_3^-$ a group of κ-Car (C. Li et al., 2013). In **Figure 1e** (Chit/κ-Car-based PEC films/gly), the absorption appeared at 3387 cm^{-1} as O-H stretching, which experienced a shift in wavenumber from 3364 cm^{-1} in **Figure 1d** (gly). The widening of the absorption band at this wavenumber indicates hydrogen bonds were formed between Chit/κ-Car-based PEC films and gly (Kammoun et al., 2013).

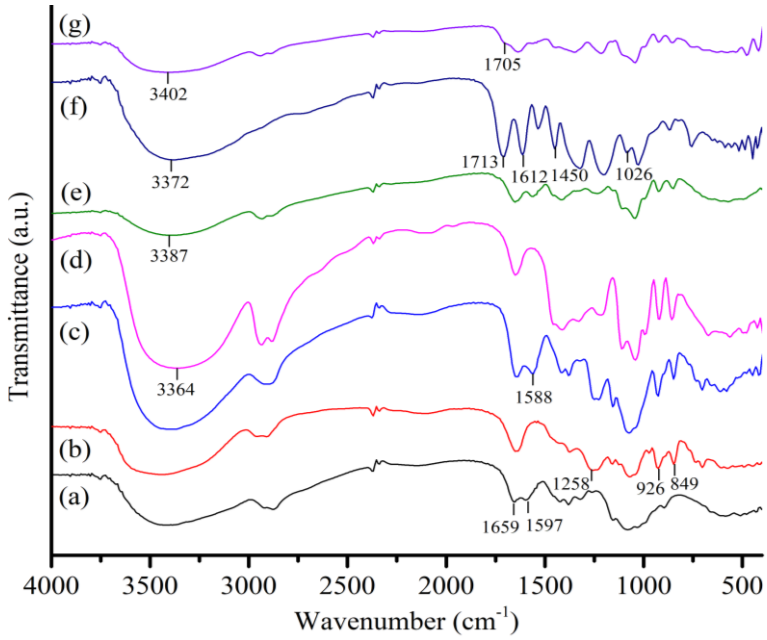

Figure 1. FTIR spectra of (**a**) Chit, (**b**) κ-Car, (**c**) Chit/κ-Car, (**d**) gly, (**e**) Chit/κ-Car/gly, (**f**) TA, and (**g**) Chit/κ-Car/gly/TA

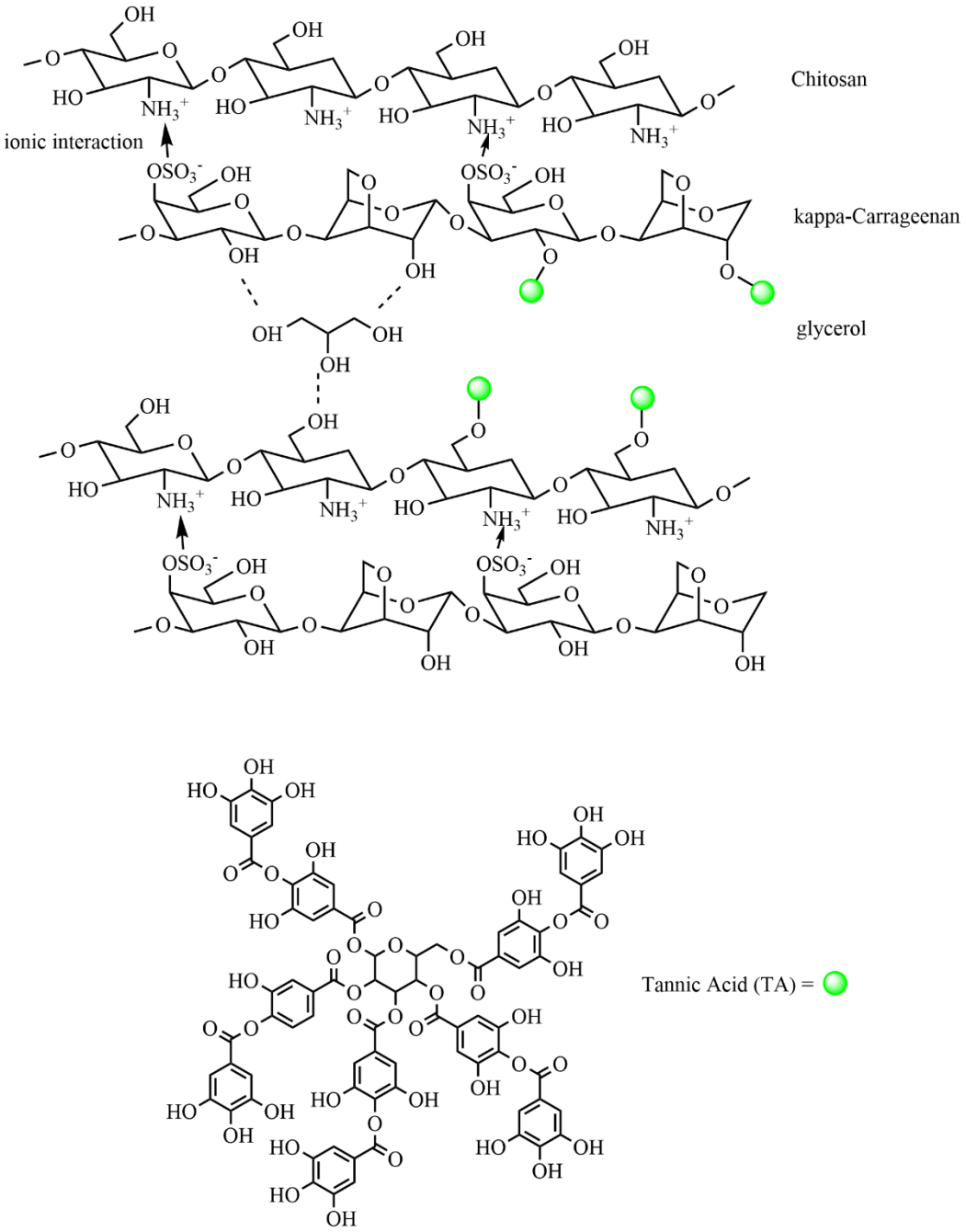

Figure 2. The proposed illustration mechanisms between Chit, κ-Car, gly, and TA

The characteristic absorption that appeared at 1713 cm^{-1} was associated with the C=O carbonyl stretching of TA in **Figure 1f**. Furthermore, the absorption at 3372 cm^{-1} in **Figure 1f** was ascribed to the O-H stretching, 1026 cm^{-1} was assigned to the ether group, and 1612 and 1450 cm^{-1} could be assigned to the C=C aromatic stretching. After the addition of TA to the PEC films, the absorption of the O-H group in **Figure 1g** shifted from 3387 to 3402 cm^{-1}. A shift also occurred from 1713 to 1705 cm^{-1}. These shifts in the absorption wave number indicated hydrogen bonds were formed between TA and Chit/κ-Car-based PEC films/gly (Aelenei et al., 2009; Zhou et al., 2016). Furthermore, based on these analyses, the proposed illustration mechanisms including ionic interaction and hydrogen bonding between Chit, κ-Car, gly, and TA, respectively, were provided in **Figure 2** with some modifications (Jing et al., 2019; Li et al., 2020; Rohaeti et al., 2016).

Effect of Chit/κ-Car, gly, and TA Composition on TS, E, and WAC Properties of PEC Films

Figures 3, **4,** and **5** displayed the results of the mechanical properties on the prepared films, which took the form of a TS, E, and WAC test, respectively. The TS value represents the maximum tension strength that the film can retain when stretched, whereas the E value represents the maximum length experienced by the film when stretching begins until the film breaks. According to **Figure 3**, the addition of κ-Car to the PEC films increased TS significantly. The addition of TA to

the Chit/κ-Car PEC films also resulted in an increase in TS. This is due to the formation of hydrogen bonds in the mixture, which creates a strong structure (Shahbazi et al., 2016). TA promotes the development of additional hydrogen bonds between $-NH_3^+$ in Chit and $-OH^-$ in TA, causing a stronger bond (Rubentheren et al., 2015). However, adding gly to the Chit/κ-Car-based PEC films decreased their TS. The addition of gly, a plasticizing agent for the films, results in a decrease in TS. Incorporating plasticizers into the PEC films enhanced the polymer chains' mobility and expanded the space between them, resulting in a more fragile film structure (Cerqueira et al., 2012). Furthermore, the results of film mechanical testing using the Universal Testing Machine Zwick 0.5 instrument also provided data on elongation (E).

Elongation (E) shows the flexibility and elasticity of the film. **Figure 4** revealed that the addition of κ-Car and TA in the Chit/κ-Car-based PEC films decreased their flexibility and elasticity. Weak intermolecular interactions between $-NH_3^+$ in Chit and $-OSO_3^-$ in κ-Car were the cause of the decrease in E values (Shahbazi et l., 2016). Moreover, the addition of TA in the Chit/κ-Car-based PEC films resulted in a strong interaction between the filler and the matrix, which decreased the E values. This restricted the matrix's movement, which had an effect on lowering the E values (C. Li et al., 2013). Various effects were demonstrated by the addition of gly to the Chit/κ-Car PEC films, which resulted in an increase in E value. Acting as a plasticizer, gly improved the elasticity and flexibility of the composite films by increasing chain mobility and lowering intermolecular interactions along the polymer chain, so producing more percent elongation and flexibility (Kozlowska et al., 2018).

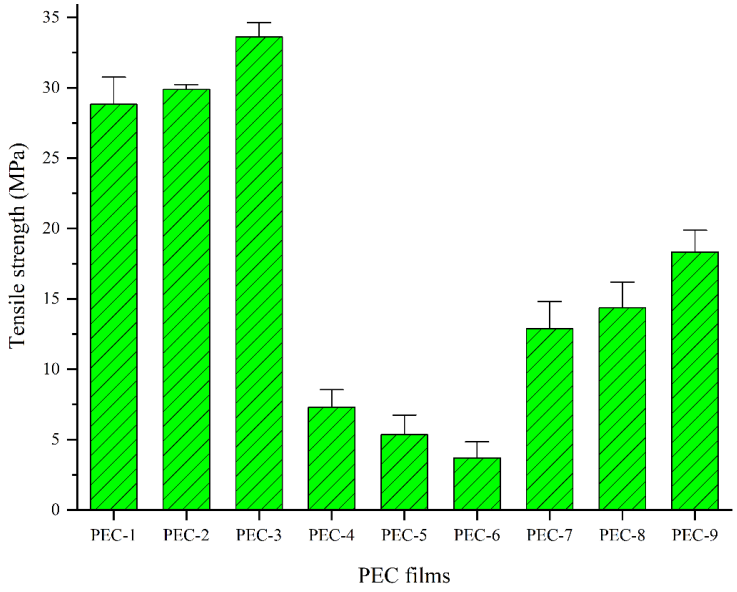

Figure 3. The TS value of various PEC films composition

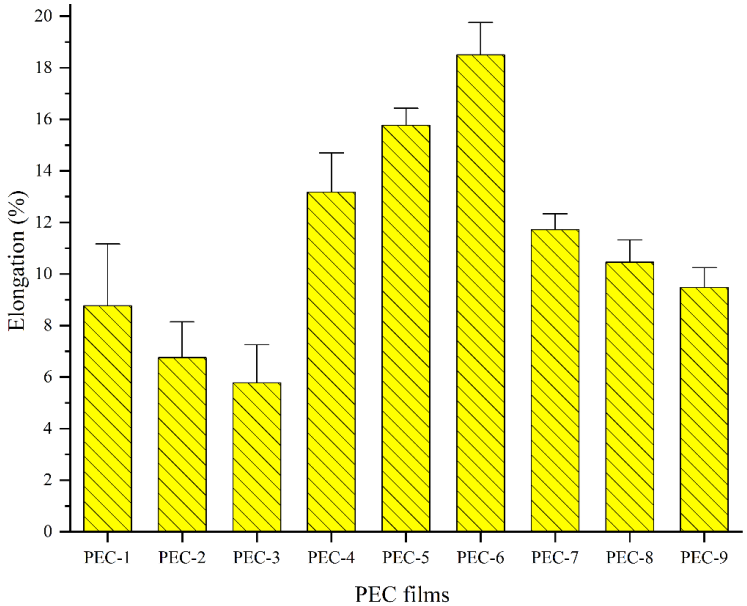

Figure 4. The E value of various PEC films composition

The idea of microstructural behaviours helps to explain why TS and E have an inverse connection in science (Cerqueira et al., 2012; Kozlowska et al., 2018). The atoms in PEC films migrate and change positions in response to tensile stress in order to resist the pulling force. Materials possessing a high tensile strength generally have a greater density and a more organised crystalline structure, characterised by closely bound atoms. Consequently, the application of stress results in minimal movement of atoms, resulting in reduced elongation values. On the other hand, materials that have a low TS value typically have a less compact structure, which gives atoms greater flexibility to migrate when subjected to stress. As a result, these materials exhibit higher E values. Hence, this phenomenon elucidates the observed negative correlation between tensile strength and elongation in materials (Kozlowska et al., 2018).

Figure 5 displays the water absorption capacity (WAC) of the Chit/κ-Car-based PEC films. The WAC test on the film can be used to determine its hydrophilicity. In **Figure 5**, the addition of κ-Car, gly, and TA significantly increased the value of WAC. This can be attributed to the presence of hydroxy groups (-OH) in each of its constituent components (Kozlowska et al., 2018; Shahbazi et al., 2016). The amounts of hydroxyl groups in the Chit/κ-Car-based PEC films increased with the concentration of κ-Car, gly, and TA. As a result, this had an impact on increasing the value of the WAC of Chit/κ-Car-based PEC films. According to the study of tests done with TS, E, and WAC, each testing method serves a specific purpose. According to physical studies, TS and E testing can help support the PEC film composite. WAC testing, on the other hand, involves chemistry studies that look at how the chemicals interact with water molecules. In order to build and improve active packaging for food products, these three testing parameters work together.

According to the mechanical test results in the form of TS, E, and WAC, the addition of TA into Chit/κ-Car-based PEC films will also affect the chemical interactions that occur in the films. The higher concentration of TA added can lead to a weakening of the interaction between TA and Chit/κ-Car (Aelenei et al., 2009). The higher concentration of TA will increase the percentage of its release due to weak interactions, thereby accelerating the release of TA from the Chit/κ-Car-based PEC films into the release medium.

Release Profile of TA

The fabrication of active packaging films in the form of antioxidant-based packaging is based on the ability to release antioxidants onto the food surface at a controlled rate. The antioxidant's solubility in the release medium (food simulant) affects the release rate. TA is an antioxidant compound that has hydrophobic properties, so its release is more suitable when carried out in a release medium that represents fatty foods. The release of TA from the Chit/κ-Car-based PEC films was conducted in ethanol medium as a fatty food simulant due to its hydrophobic properties similar to those of fatty foods. The release profile of TA from Chit/κ-Car-based PEC films is shown in **Figure 6**. In general, the overall PEC films exhibited the same release profile, beginning with a significant initial release (burst effect), followed by a slower and more sustained release. Based on **Figure 6**, PEC-3, PEC-6, and PEC-9 films showed the highest percentage of TA release, which were 54.17%, 60.77%, and 81.12% over a period of 120 h. It can also be concluded that the increase in the composition of κ-Car, gly, and TA themselves leads to a greater dissolution of TA.

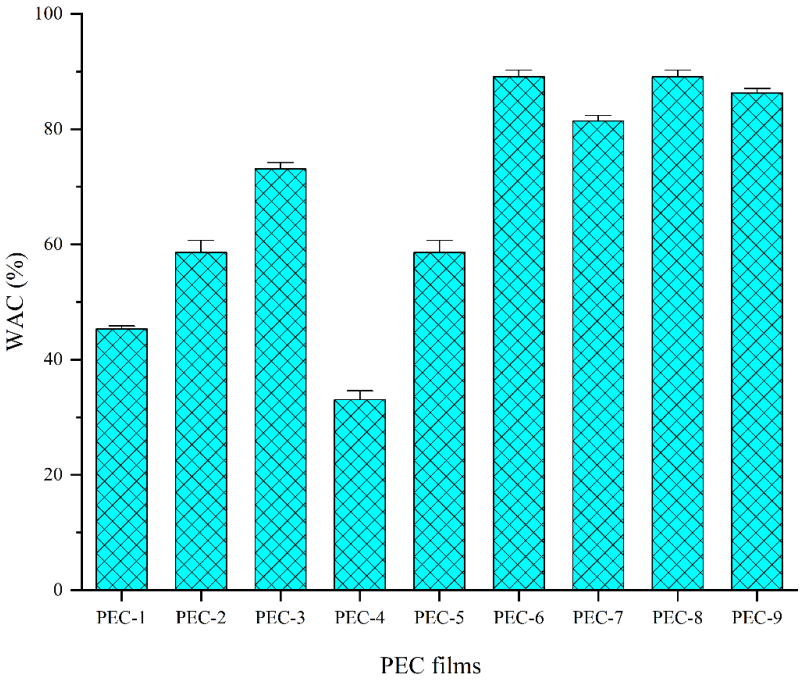

Figure 5. The WAC value of various PEC films composition

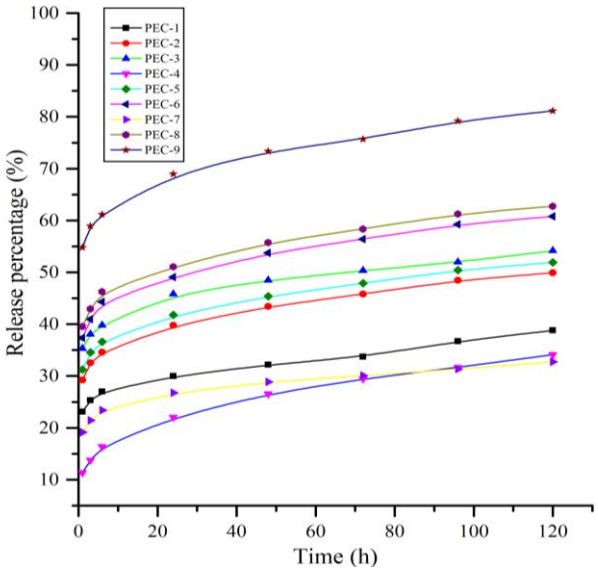

Figure 6. The release profile of TA from Chit/κ-Car-based PEC films

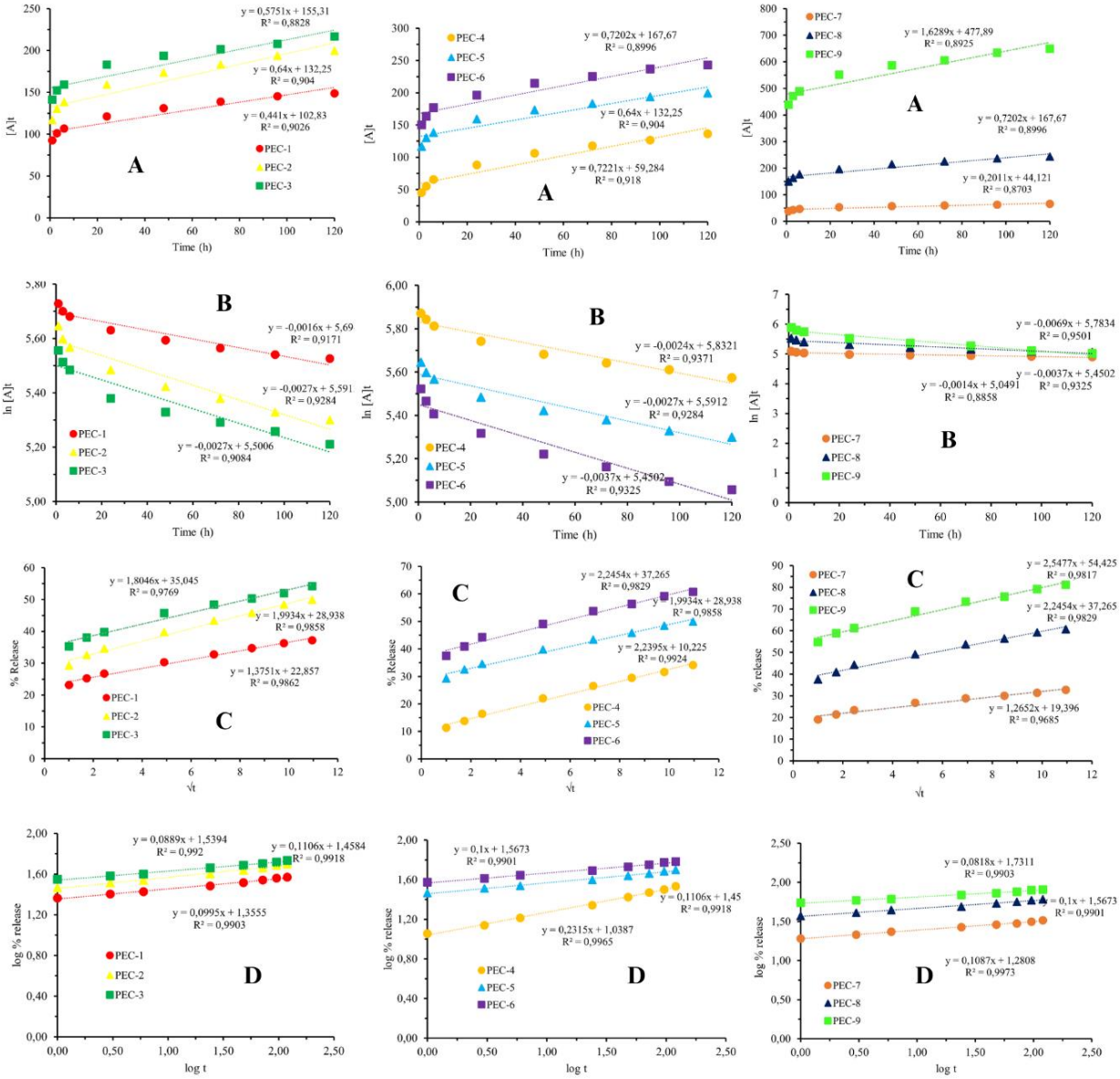

Figure 7. Release profil of TA using various kinetic models: (**A**) zero-order; (**B**) first-order; (**C**) Higuchi; and (**D**) Korsmeyer-Peppas kinetics model

The release mechanism of TA from Chit/κ-Car-based PEC films into the ethanol release medium can be studied using various kinetic models, the results of which can be seen in **Figure 7** and **Table 2**. **Figure 7** revealed data on the TA release profile from 9 PEC films according to the zero-order kinetic model (**A**), first-order kinetic model (**B**), Higuchi model (**C**), and Korsmeyer-Peppas model (**D**). Based on the obtained linear equation data, the Korsmeyer-Peppas model could explain the release mechanism of TA from Chit/κ-Car-based PEC films. This was due to the coefficient of determination (R^2) values obtained for all PEC films, which were close to one and higher compared to other kinetic models. These values indicate the highest linearity relationship between the x-axis and y-axis variables.

Table 2 showed that all variations of TA release from Chit/κ-Car-based PEC films followed the Korsmeyer-Peppas kinetic model, as they have R^2 values approaching 1 in each film. The value of *n* in the Korsmeyer-Peppas kinetic model represents the release exponent related to the TA release mechanism from Chit/κ-Car-based PEC films. The obtained values of *n* from the kinetic profile of the Korsmeyer-Peppas model were less than 0.5, ranging from 0.08 to 0.23. These release exponent values (*n*) less than 0.5 indicated that the TA release mechanism followed Fickian diffusion (Leal et al., 2018). This mechanism explained that the release of TA from Chit/κ-Car-based PEC films occurred through diffusion without any film erosion. The proposed mechanism for TA release was as follows: it started with immersing Chit/κ-Car-based PEC films in an ethanol solution as a fatty food simulant. An ethanol solution will interact with the active groups on the surface of the Chit/κ-Car-based PEC films and cause several interactions such as molecular interactions, which imply changes in the mechanical properties and opacity of the film, as well as not very significant swelling (Zhou et al., 2016). The ethanol solution penetrated into the pores of Chit/κ-Car-based PEC films and subsequently dissolving TA. The dissolved TA in the ethanol solution diffused out of the pores of Chit/κ-Car-based PEC films without matrix erosion. Furthermore, the dissolved TA will later be used as an active antioxidant substance that interacts with DPPH.

TA Bioactivity Assays

The antioxidant activity test was conducted on films with various concentrations of TA. This preliminary study used the DPPH radical compound followed by two different methods of antioxidant activity testing, namely the fixed reaction time method and the steady-state method. The fixed reaction time method involved reacting the released TA in the test solution with DPPH and observing its absorbance at a wavelength of 517 nm. On the other hand, for the steady-state method, the film was directly exposed to the DPPH radical solution for 120 h and its absorbance was observed at the same wavelength. The antioxidant activity was determined by the value of RSA in the fixed reaction time method and the remaining DPPH value in the steady-state method in **Figure 8**.

Table 2. Data on TA release using various kinetic models

PEC	Zero-order		First-order		Higuchi		Korsmeyer-Peppas		
	k	r	k	r	k	r	k	r	*n*
PEC-1	0.44	0.9026	0.0016	0.9171	1.38	0.9862	22.67	0.9903	0.09
PEC-2	0.64	0.9040	0.0027	0.9284	1.99	0.9858	28.73	0.9918	0.11
PEC-3	0.58	0.8828	0.0027	0.9084	1.80	0.9769	34.63	0.9920	0.08
PEC-4	0.72	0.9180	0.0024	0.9371	2.24	0.9924	10.93	0.9965	0.23
PEC-5	0.64	0.9040	0.0027	0.9284	1.99	0.9858	28.73	0.9918	0.11
PEC-6	0.72	0.8996	0.0037	0.9325	2.25	0.9829	36.92	0.9901	0.10
PEC-7	0.20	0.8703	0.0014	0.8858	1.27	0.9685	19.09	0.9973	0.10
PEC-8	0.72	0.8996	0.0037	0.9325	2.25	0.9829	36.92	0.9901	0.10
PEC-9	1.63	0.8925	0.0069	0.9501	2.55	0.9817	53.84	0.9903	0.08

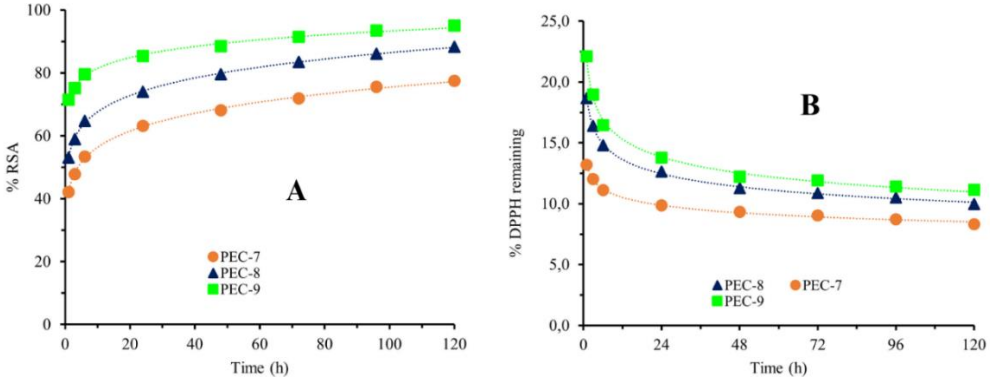

Figure 8. The percentage of RSA (**A**) and remaining DPPH radicals (**B**) value of TA release from Chit/κ-Car-based PEC films

Figure 8 shows that the antioxidant activity increased with the longer release time of TA in the fixed reaction time method (**A**), and the percentage of remaining DPPH radicals decreased with the longer contact time in the steady-state method (**B**). The percentage of RSA values for each film were 77.51% for PEC-7, 88.34% for PEC-8, and 95.04% for PEC-9, respectively. This was due to the longer release time of TA, which leads to a higher concentration of released TA from the Chit/κ-Car-based PEC films. Similarly, in the steady-state method, with a longer contact time of the film, more TA was released, resulting in a lower percentage of remaining DPPH radicals. The percentage decrease of remaining DPPH radicals in each film is 22.10% from the first h to 11.1% at 120 h (PEC-7), 18.76% to 9.96% (PEC-8), and 13.2% to 8.4% (PEC-9). Both results clearly indicated the presence of antioxidant activity from TA against DPPH radicals (Mishra et al., 2012). According to the percentage of RSA and remaining DPPH radical values obtained from the three films, it can be concluded that Chit/κ-Car-based PEC films containing TA have the potential to be antioxidant packaging due to their good antioxidant activity.

CONCLUSIONS

The synthesis of PEC composite film based on Chit and κ-Car modified with gly and incorporated with the antioxidant compound TA has been successfully carried out and the success of the synthesis process has been identified spectroscopically, supported by TS, E, and WAC tests. The incorporation of higher amounts of κ-Car and TA into the Chit/κ-Car-based PEC films led to an increase in tensile strength (TS) and water absorption capacity (WAC), while decreasing the elongation (E). The release behavior of TA from the Chit/κ-Car-based PEC films followed the Korsmeyer-Peppas model, indicating Fickian diffusion with a value of $n < 0.5$. The antioxidant activity test using the DPPH method demonstrated the high antioxidant activity of the PEC films, with RSA values reaching up to 95.04%. Consequently, the Chit/κ-Car-based PEC films showed promise for further development as active packaging materials.

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REFERENCES

Aelenei, N., Popa, M. I., Novac, O., Lisa, G., & Balaita, L. (2009). Tannic acid incorporation in chitosan-based microparticles and in vitro controlled release. *Journal of Materials Science: Materials in Medicine*, *20*(5), 1095–1102. https://doi.org/10.1007/s10856-008-3675-z

Ahmed, M. W., Haque, M. A., Mohibbullah, M., Khan, M. S. I., Islam, M. A., Mondal, M. H. T., & Ahmmed, R. (2022). A review on active packaging for quality and safety of foods: Current trends, applications, prospects and challenges. In *Food Packaging and Shelf Life*. *33*. Elsevier Ltd. https://doi.org/10.1016/j.fpsl.2022.100913

Araujo, J. V., Davidenko, N., Danner, M., Cameron, R. E., & Best, S. M. (2014). Novel porous scaffolds of pH responsive chitosan/carrageenan-based polyelectrolyte complexes for tissue engineering. *Journal of Biomedical Materials Research - Part A*, *102*(12), 4415–4426. https://doi.org/10. 1002/jbm.a.35128

Bhat, V. G., Narasagoudr, S. S., Masti, S. P., Chougale, R. B., & Shanbhag, Y. (2021). Hydroxy citric acid cross-linked chitosan/guar gum/poly(vinyl alcohol) active films for food packaging applications. *International Journal of Biological Macromolecules*, *177*, 166–175. https://doi.org/10.1016/j.ijbiomac.2021.02.109

Bhuimbar, M. v., Bhagwat, P. K., & Dandge, P. B. (2019). Extraction and characterization of acid soluble collagen from fish waste: Development of collagen-chitosan blend as food packaging film. *Journal of Environmental Chemical Engineering*, *7*(2). https://doi.org/10.1016/j.jece.2019.102983

Cerqueira, M. A., Souza, B. W. S., Teixeira, J. A., & Vicente, A. A. (2012). Effects of Interactions between the constituents of chitosan-edible films on their physical properties. *Food and Bioprocess Technology*, *5*(8), 3181–3192. https://doi.org/10.1007/s11947-011-0663-y

Chen, P. H., Kuo, T. Y., Kuo, J. Y., Tseng, Y. P., Wang, D. M., Lai, J. Y., & Hsieh, H. J. (2010). Novel chitosan-pectin composite membranes with enhanced strength, hydrophilicity and controllable disintegration. *Carbohydrate Polymers*, *82*(4), 1236–1242. https://doi.org/10.1016/j.carbpol.2010.06.057

Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica - Drug Research*, *67*(3), 217–223.

de Carvalho, I. O. A. M., de Oliveira, V. S., Chávez, D. W. H., Gamallo, O. D., Castro, R. N., Sawaya, A. C. H. F., Sampaio, G. R., Torres, E. A. F. da S., & Saldanha, T. (2021). The use of lemon juice and its role on polyunsaturated fatty acids and cholesterol oxides formation in thermally prepared sardines. *Journal of Food Composition and Analysis*, *104*(104087). https://doi.org/10.1016/j.jfca.2021.104087

López-de-Dicastillo, C., Castro-López, M. del M., López-Vilariño, J. M., & González-Rodríguez,

M. V. (2013). Immobilization of green tea extract on polypropylene films to control the antioxidant activity in food packaging. *Food Research International*, *53*(1), 522–528. https://doi.org/10.1016/ j.foodres.2013. 05.022

Domínguez, R., Barba, F. J., Gómez, B., Putnik, P., Bursać Kovačević, D., Pateiro, M., Santos, E. M., & Lorenzo, J. M. (2018). Active packaging films with natural antioxidants to be used in meat industry: A review. *Food Research International*, *113*, 93–101. https://doi.org/10.1016/ j.foodres.2018.06.073

El-Meliegy, E., Mabrouk, M., El-Sayed, S. A. M., Abd El- Hady, B. M., Shehata, M. R., & Hosny, W. M. (2018). Novel Fe_2O_3-doped glass/chitosan scaffolds for bone tissue replacement. *Ceramics International*, *44*(8), 9140–9151. https://doi.org/10.1016/j.ceramint.2018.02.122

Erceg, T., Šovljanski, O., Stupar, A., Ugarković, J., Aćimović, M., Pezo, L., Tomić, A., & Todosijević, M. (2023). A comprehensive approach to chitosan-gelatine edible coating with β-cyclodextrin/lemongrass essential oil inclusion complex — Characterization and food application. *International Journal of Biological Macromolecules*, *228*, 400–410. https://doi.org/10.1016/j.ijbiomac.2022.12.132

Gómez-Estaca, J., Calvo, M. M., Sánchez-Faure, A., Montero, P., & Gómez-Guillén, M. C. (2015). Development, properties, and stability of antioxidant shrimp muscle protein films incorporating carotenoid-containing extracts from food by-products. *LWT*, *64*(1), 189–196. https://doi.org/10.1016/j.lwt.2015.05.052

Haghighi, H., de Leo, R., Bedin, E., Pfeifer, F., Siesler, H. W., & Pulvirenti, A. (2019). Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched with LAE (lauroyl arginate ethyl) with antimicrobial activity for food packaging applications. *Food Packaging and Shelf Life*, *19*, 31–39. https://doi.org/10.1016/j.fpsl.2018.11.015

Huang, J. Y., Limqueco, J., Chieng, Y. Y., Li, X., & Zhou, W. (2017). Performance evaluation of a novel food packaging material based on clay/polyvinyl alcohol nanocomposite. *Innovative Food Science and Emerging Technologies*, *43*, 216–222. https://doi.org/ 10.1016/j.ifset.2017.08.012

Ishartono, B., Suyanta, S., & Kartini, I. (2021). Effect of zeolite to clay ratios on the formation of zeolite-clay-white cement composite cylinder as an encapsulant of urea fertilizer. *Key Engineering Materials*, *884*, 196–203. https://doi.org/10.4028/www.scientific.net/kem.884.196

Jayakumar, A., K.V., H., T.S., S., Joseph, M., Mathew, S., G., P., Nair, I. C., & E.K., R. (2019). Starch-PVA composite films with zinc-oxide nanoparticles and phytochemicals as intelligent pH sensing wraps for food packaging application. *International Journal of Biological Macromolecules*, *136*, 395–403. https://doi.org/10.1016/j.ijbiomac.2019.06.018

Jing, Y., Diao, Y., & Yu, X. (2019). Free radical-mediated conjugation of chitosan with tannic acid: Characterization and antioxidant capacity. *Reactive and Functional Polymers*, *135*, 16–22. https://doi.org/10.1016/ j.reactfunctpolym.2018.12.005

Kammoun, M., Haddar, M., Kallel, T. K., Dammak, M., & Sayari, A. (2013). Biological properties and biodegradation studies of chitosan biofilms plasticized with PEG and glycerol. *International Journal of Biological Macromolecules*, *62*, 433–438. https://doi.org/10.1016/j.ijbiomac. 2013.09.025

Kanmani, P., & Rhim, J. W. (2014). Development and characterization of carrageenan/grapefruit seed extract composite films for active packaging. *International Journal of Biological Macromolecules*, *68*, 258–266. https://doi.org/10.1016/j.ijbiomac.2014.05.011

Kozlowska, J., Pauter, K., & Sionkowska, A. (2018). Carrageenan-based hydrogels: Effect of sorbitol and glycerin on the stability, swelling and mechanical properties. *Polymer Testing*, *67*, 7–11. https://doi.org/10.1016/ j.polymertesting.2018.02.016

Lai, W. F., Zhao, S., & Chiou, J. (2021). Antibacterial and clusteroluminogenic hypromellose-graft-chitosan-based polyelectrolyte complex films with high functional flexibility for food packaging. *Carbohydrate Polymers*, *271*. https://doi.org/10.1016/j.carbpol.2021.118447

Leal, A. de S., de Araújo, R., Rocha Souza, G., Nunes Lopes, G. L., Telles Pereira, S., Muálem de Moraes Alves, M., Medeiros Barreto, H., Menezes Carvalho, A. L., Pinheiro Ferreira, P. M., Silva, D., de Amorim Carvalho, F. A., Dantas Lopes, J. A., & Cunha Nunes, L. C. (2018). In vitro bioactivity and cytotoxicity of films based on mesocarp of *Orbignya sp.* and carboxymethylcellulose as a tannic acid release matrix. *Carbohydrate Polymers*, *201*, 113–121. https://doi.org/10.1016/j.carbpol.2018.08.026

Li, C., Hein, S., & Wang, K. (2013). Chitosan-carrageenan polyelectrolyte complex for the delivery of protein drugs. *ISRN Biomaterials*, *2013*, 1–6. https://doi.org/10.5402/2013/ 629807

Li, Y., Li, J., Shi, Z., Wang, Y., Song, X., Wang, L., Han, M., Du, H., He, C., Zhao, W., Su, B., & Zhao, C. (2020). Anticoagulant chitosan-kappa-carrageenan composite hydrogel sorbent for

simultaneous endotoxin and bacteria cleansing in septic blood. *Carbohydrate Polymers*, *243*. https://doi.org/10.1016/j.carbpol.2020.116470

Liu, F., Antoniou, J., Li, Y., Yi, J., Yokoyama, W., Ma, J., & Zhong, F. (2015). Preparation of gelatin films incorporated with tea polyphenol nanoparticles for enhancing controlled-release antioxidant properties. *Journal of Agricultural and Food Chemistry*, *63*(15), 3987–3995. https://doi.org/10.1021/acs.jafc.5b00003

López-de-Dicastillo, C., Catalá, R., Gavara, R., & Hernández-Muñoz, P. (2011). Food applications of active packaging EVOH films containing cyclodextrins for the preferential scavenging of undesirable compounds. *Journal of Food Engineering*, *104*(3), 380–386. https://doi.org/10.1016/j.jfoodeng.2010.12.033

Loukelis, K., Papadogianni, D., & Chatzinikolaidou, M. (2022). Kappa-carrageenan/chitosan/ gelatin scaffolds enriched with potassium chloride for bone tissue engineering. *International Journal of Biological Macromolecules*, *209*, 1720–1730. https://doi.org/10.1016/j.ijbiomac.2022.04.129

Maqsood, S., & Benjakul, S. (2010). Preventive effect of tannic acid in combination with modified atmospheric packaging on the quality losses of the refrigerated ground beef. *Food Control*, *21*(9), 1282–1290. https://doi.org/10.1016/ j.foodcont.2010.02.018

Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH- assay: A critical review and results. *Food Chemistry*, *130*(4), 1036–1043. https://doi.org/10.1016/ j.foodchem.2011.07.127

Mukherjee, D., Azamthulla, M., Santhosh, S., Dath, G., Ghosh, A., Natholia, R., Anbu, J., Teja, B. V., & Muzammil, K. M. (2018). Development and characterization of chitosan-based hydrogels as wound dressing materials. *Journal of Drug Delivery Science and Technology*, *46*, 498–510. https://doi.org/10.1016/j.jddst. 2018.06.008

Nikbakht-Jam, I., Khademi, M., Nosrati, M., Eslami, S., Foroutan-Tanha, M., Sahebkar, A., Tavalaie, S., Ghayour-Mobarhan, M., Ferns, G. A. A., Hadizadeh, F., Tabassi, S. A. S., Mohajeri, S. A., & Emamian, M. (2015). Effect of crocin extracted from saffron on pro-oxidant–anti-oxidant balance in subjects with metabolic syndrome: A randomized, placebo-controlled clinical trial. *European Journal of Integrative Medicine*, *8*(3), 307–312. https://doi.org/10. 1016/j.eujim.2015.12.008

Rohaeti, E., Laksono, E. W., & Rakhmawati, A. (2016). Bacterial cellulose from rice waste and its composite which are deposited nanoparticles as an antimicrobial material. *ALCHEMY Jurnal Penelitian Kimia*, *12*(1), 70. https://doi.org/10. 20961/alchemy.v12i1.946

Rubentheren, V., Ward, T. A., Chee, C. Y., & Tang, C. K. (2015). Processing and analysis of chitosan nanocomposites reinforced with chitin whiskers and tannic acid as a crosslinker. *Carbohydrate Polymers*, *115*, 379–387. https://doi.org/ 10.1016/j.carbpol.2014.09.007

Shahbazi, M., Rajabzadeh, G., Ettelaie, R., & Rafe, A. (2016). Kinetic study of κ-carrageenan degradation and its impact on mechanical and structural properties of chitosan/κ-carrageenan film. *Carbohydrate Polymers*, *142*, 167–176. https://doi.org/10.1016/j.carbpol.2016.01.037

Torres-Arreola, W., Soto-Valdez, H., Peralta, E., Cárdenas-López, J. L., & Ezquerra-Brauer, J. M. (2007). Effect of a low-density polyethylene film containing butylated hydroxytoluene on lipid oxidation and protein quality of sierra fish (*Scomberomorus sierra*) muscle during frozen storage. *Journal of Agricultural and Food Chemistry*, *55*(15), 6140–6146. https://doi.org/10.1021/jf070418h

Tripathi, S., Mehrotra, G. K., Dutta, P. K. (2009). Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications. *International Journal of Biological Macromolecules*, *45*(4), 372–376. https://doi.org/10.1016/j.ijbiomac.2009.07.006

Woranuch, S., Yoksana, R., & Akashi, M. (2015). Ferulic acid-coupled chitosan: Thermal stability and utilization as an antioxidant for biodegradable active packaging film. *Carbohydrate Polymers*, *115*, 744–751. https://doi.org/10.1016/j.carbpol.2014.06.074

Zhang, X., Xiao, G., Wang, Y., Zhao, Y., Su, H., & Tan, T. (2017). Preparation of chitosan-TiO_2 composite film with efficient antimicrobial activities under visible light for food packaging applications. *Carbohydrate Polymers*, *169*, 101–107. https://doi.org/10.1016/j.carbpol. 2017.03.073

Zhou, B., Hu, X., Zhu, J., Wang, Z., Wang, X., & Wang, M. (2016). Release properties of tannic acid from hydrogen bond driven antioxidative cellulose nanofibrous films. *International Journal of Biological Macromolecules*, *91*, 68–74. https://doi.org/10.1016/j.ijbiomac.2016. 05.0 84