SYNTHESIS OF POLYELECTROLYTE COMPLEX FILMS OF CHITOSAN-ALGINATE BY ADDITION OF KELOR LEAVES EXTRACT (*Moringa oleifera*) FOR FOOD PACKAGING

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Abstract
This research aims to synthesize the polyelectrolyte complex (PEC) using chitosan and alginate as matrix film with the addition of kelor leaves extract (*Moringa oleifera*). The preparation of PEC films of chitosan-alginate by addition of kelor leaves extract (*Moringa oleifera*) had been implemented. The kelor leaves was prepared by drying process under the sun for removing water content and the dried kelor leaves was extracted using maceration technique. The final concentrations of film solution used in this study were 0.0% (Film E0), 0.50% (Film E1), 0.75% (Film E2), and 1.00% (Film E3). The PEC chitosan-alginate films with addition of kelor extract were prepared in room temperature (± 25 °C) and pH of film solution was ± 4.0. The result of film's characterization using FTIR spectrophotometer showed that PEC films of chitosan-alginate were formed through the molecular interaction between chitosan which protonated into amine groups ($–NH_3^+$) and alginate which dissociated into carboxylate groups ($–COO^–$). Based on FTIR spectrum, it also showed that there were some wavenumber shifts after addition of kelor leaves extract into PEC films of chitosan-alginate. It was confirmed that there were the changing of molecular interaction between PEC chitosan-alginate films due to presence of kelor extract, indicating that PEC chitosan-alginate films with kelor extract had been formed. Morphological analysis using a scanning electron microscope (SEM) displayed both porous and heterogeneous distribution on the surface after addition of kelor extract.

***Keywords**: alginate, chitosan, films, food packaging, kelor leaves extract*

Introduction
Active packaging is one of the packaging techniques which has either antioxidant or antibacterial agents. In recent times, active packaging is one of the promising systems in food packaging areas that has been developed widely. This packaging technique has a good ability both to increase shelf life of food and to maintain the quality of food from lipid oxidation. One of the active packaging techniques that have been developed lately is antioxidant packaging. Antioxidant packaging was conducted by addition of an antioxidant agent to the packaging films or matrix films. The development of this technique has been used widely, especially in food areas. In some previous research, active packaging fabrication used synthetic antioxidant agents such as BHA (*butylated hydroxyanisole*) and BHT (*butylated hydroxytoluene*). Nowadays, the utilization of synthetic antioxidants is replaced by natural antioxidants, such as extract leaves of some plants which have great antioxidant activity. These kinds of antioxidant agents are considered as both an effective agent to use and an alternative solution to keep both healthy dan safe for human. (Fang et al, 2017).

Some previous studies in active packaging used some natural extracts as antioxidant agent. Dicastillo et al, (2013) used green tea extract on polypropylene film. Addition of green tea extract gave good antioxidant activity. The effect of addition in composite films of chitosan was studied by Peng et al, (2013). Peng et al, (2013) used green tea extract and black tea extract as antioxidant agents. From this study, it indicated that incorporating of tea extracts into chitosan films increased DPPH radical scavenging ability significantly. Perumalla & Hettiarachchy (2011) also reported that green tea and grape seed extract were potential antioxidant agents that can be applicated in food packaging due to its antibacterial activity against some pathogens. Other studies from Li et al, (2014) showed great antioxidant activity in gelatin films with addition of grape seed extract, gingko leaf extract, ginger extract, and green tea extract. These results show that incorporating of those extracts into gelatin film is one of an alternative choice to be implemented into food packaging.

Development of packaging films that uses natural polymers which is biodegradable, renewable, and nontoxic, have been conducted lately. These natural polymers such as chitosan, alginate, carrageenan and others, were used as the matrix component of films. Chitosan has been known as polysaccharide that consists of monomer N-acetylglucosamine and D-glucosamine. It is obtained from deacetylation of chitin, the major component of invertebrate exoskeleton. The use of chitosan as matrix films for food packaging has been used. However, these films sometimes showed low mechanical properties. Previous studies had been proved that the formation of polyelectrolyte complex (PEC) films from chitosan and alginate could increase tensile strength of films compared with films that formed from one polymer only. Polyelectrolyte complex film is formed between two polymers including polycation and polyanion. Chitosan is polycationic polymers that consists of amine groups ($–NH_3^+$) while alginate is known as polyanion polymers which has carboxylate groups ($–COO^–$). Based on previous studies from Kulig & Zimoch-korzycka (2017), Vogt et al, (2008), and Yan et al, (2000), PEC films of chitosan-alginate had good mechanical properties and very stable over a temperature range of 4–37 °C. These PEC films were also flexible and transparent. That is why, polyelectrolyte complex (PEC) films of chitosan-alginate are potentially to be applied in food packaging.

Kelor (Moringa oleifera) is one of popular plants that usually consumed by Indonesian people. In Indonesia especially in Lombok Island, people believe that this plant is very potential to treat some infectious illness. It also has been known as an antitumor and antiinflammation plant due to its components. Kelor (Figure 1) consists of amino acid, some minerals, glucosinolates and isothiocyanate compounds. The stem of kelor has alkaloid compounds such as moringinine and moringine. Kelor also consists of kaempferol, rhamnetin, isoquersitrin dan kaempferritin (Sreelatha & Padma, 2009; Berawi et al, 2019). Moreover, kelor contains saponins, tannins, steroids, phenolic acids, carotenoids, polyphenols, phytates, flavonoids and terpenes (Milla et al, 2021). Based on Sreelatha & Padma (2009), kelor exhibited very high antioxidant activity. Assessment of IC_{50} value using DPPH radical of water extract of kelor leaves resulted in 18.15 μg/ml on matured leaves while 19.12 μg/ml on tender leaves. So, kelor leaves extract can be potentially used as antioxidant agent in food packaging.

Figure 1. Kelor leaves

In this research, PEC films will be prepared from chitosan and alginate. Kelor extract leaves as antioxidant agent will be added into these PEC films. Characterization of PEC films-extract will be conducted using FTIR spectrophotometer to identify if PEC films of chitosan-alginate incorporated by kelor leaves extract have been formed or not. Morphology on surface of PEC films will be observed using a scanning microscope electron.

Research Methods

Materials

Chitosan (deacetylated degree: 94%) was purchased from Phy Edumedia Malang-Jawa Timur, food grade alginate was purchased from Buana Chem Bandung-Jawa Barat, distilled water, acetic acid 2% as the solvent, kelor leaves were obtained from Praya-Lombok Tengah, Nusa Tenggara Barat.

Instrumentations

Analytical balance, universal indicator pH paper Merck, aluminum foil, filter paper, measuring flask, beaker glass, erlenmeyer, dropper pipette, mortar, stir bar, spatula, magnetic stirrer, petri dish, FTIR spectrophotometer instrument, and a scanning electron microscope (SEM) instrument type JEOL JCM-7000.

Preparation of kelor leaves

The leaves were chopped to small pieces. They were washed and dried under the sun for 4 hours (7 am until 12 pm). The dried leaves were mashed using mortar. The leaves sample were kept on dry container at 25 ºC.

Extraction of kelor leaves

Extraction process was conducted according to method described by Susanty, Yudistirani, and S.A., Islam, (2019) with some modification. Dried powder of kelor leaves (10 g) was macerated with distilled water (ratio 1:5) for 48 h. The liquid extracts were filtered with filter paper. The filtrates were evaporated using a rotary evaporator to remove the solvent.

Synthesis of films

Films was prepared according to Hapsari et al, (2020) and Riyandari, (2020) with some modification. Alginate solution (7.5 mg) was prepared and dissolved in 6 ml of distilled water (solution A). Chitosan solution also was prepared by dissolved 30 mg chitosan into 24 mL of acetic acid 2% (solution B). pH measurement of film solution was measured using universal indicator pH paper. PEC chitosan-alginate films were prepared by mixing both solution A and solution B, then stirred them for 1 hour using magnetic stirrer. After stirring the film solution, kelor leaves extract was added into film solution until it reached final concentrations of 0.50%, 0.75%, dan 1.00% (%v/v). Film solution was stirred until it was homogenous on hotplate (27 ºC) for 24 hours using magnetic stirrer. Then, film solution was casted into petri dish with diameter 60 mm. Films were dried for 96 h at room temperature (± 25 °C). After drying, the films were kept in a box containing silica to prevent film from contaminants.

Characterization of films using FTIR spectrophotometer

Functional groups and molecular interaction of the PEC film-kelor extract was characterized by using FTIR spectrophotometer. The films were kept in dry container at 25 ºC for 2 weeks. The dried films were scanned from 4000 to 400 cm^{-1}.

Morphological analysis

The surface of PEC films was observed using a scanning electron microscope. Each sample of film was coated by gold. Then, it was observed using an accelerating voltage of 5 kV.

Results and Discussion

Preparation and extraction of kelor leaves extract

Bioactive components of kelor leaves were determined by extracting sample. In this research, sample was extracted with maceration technique for 48 h. Maceration of kelor leaves used distilled water as a solvent. Based on Sreelatha & Padma (2009), kelor exhibited very high antioxidant activity. Assessment of IC_{50} of water extract of kelor leaves resulted in very good antioxidant activity. IC_{50} of water extract was under 20 µg ml^{-1}. Besides, water extract is safer and more effective to use in human so it is an alternative to use as antioxidant agent in active food packaging.

Synthesis of PEC films with addition of kelor extract

The presence polycationic groups of chitosan ($-NH_3^+$) and polyanionic groups in alginate ($-COO^-$) caused electrostatic interaction between amine groups ($-NH_3^+$) and carboxylate groups ($-COO^-$), indicating to formation of polyelectrolyte complex (PEC) films of chitosan-alginate. The formation of PEC films of chitosan-alginate depends on pH value and ionic strength Chitosan-alginate PEC film so PEC film solution was prepared at pH ± 4.0. Since the chitosan's pKa value is 6.3 while alginate has pKa value at range 3.4–3.7 (Lawrie et al, 2007). In this present work, PEC film was prepared at ± 4.0. Based on Yan et al, (2000), the interaction ions of chitosan and alginate must be occurred at optimum pH, so that cation groups of chitosan and anion groups of alginates were dissociated largely. The electrostatic interaction between amine groups ($-NH_3^+$) and carboxylate groups ($-COO^-$) will be formed strongly and PEC films will result in better mechanical properties.

The products of PEC chitosan-alginate films with addition of kelor extract were showed in Figure 2. Based on the results, it showed that PEC films of chitosan-alginate without kelor extract were more transparent than PEC films with addition of kelor leaves extract. The increasing of extract concentration into PEC films did not show significant changes on film E1 (films with final concentration 0.50%) and E2 (films with final concentration 0.75%). Film E3 with highest concentration of kelor leaves extract showed bit yellow color on its surface.

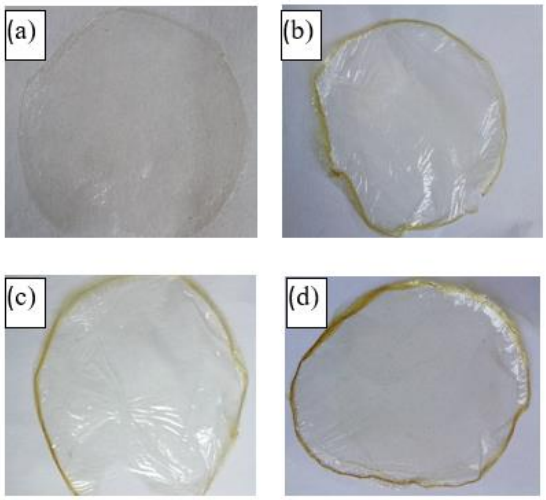

Figure 2. Product of films, (a) PEC film without kelor extract; (b) PEC film with 0.5% extract; (c) PEC film with 0.75% extract; (d) PEC film with 1.0% extract

Characterization of films

Characterization of films using FTIR spectrophotometer is used to observe if PEC films with addition of kelor extract were either formed or not. The spectrum of chitosan and alginate were showed in Figure 3. Analysis of functional groups of chitosan and alginate was described on Table 1.

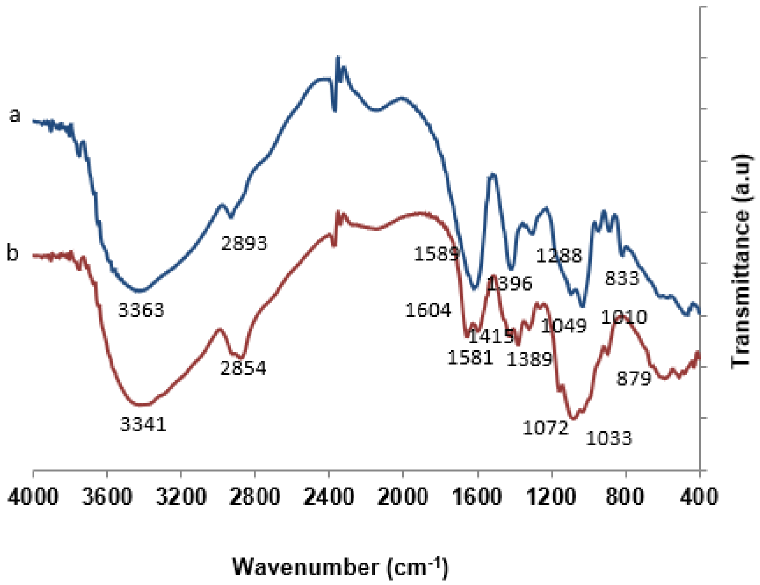

Figure 3. FTIR spectrum of (a) alginate, (b) chitosan

Table 1. Characteristics of functional groups of chitosan and alginate

Materials	Wavenumber (cm^{-1})	Functional groups
Chitosan	3341	Overlap of O–H and N–H bonds
	2854	Aliphatic stretching of C−H from alkyl groups
	1604	Stretching of C=O of amide groups consisted of acetyl (amide I)
	1581	Overlap of amide II (υC-N and δN-H) with bending vibration of H-N-H from amine groups (δN-H)
	1415 & 1389	–CH_2 and –CH_3 bonds
	1072& 1033	Stretching of C−O−C in saccharide structures
	879	Stretching of C−C
Alginate	3363	Stretching of O−H
	2893	Stretching of C−H aliphatic
	1589	Antisymmetric stretching of COO^- groups
	1396	Symmetric stretching of COO^- groups
	1288	Skeletal vibration of alginate
	1049 & 1010	Antisymmetric stretching of C−O−C
	833	Stretching of C−C

Spectrum of PEC films of chitosan-alginate (Figure 4) showed that there were some wavenumber shifts of functional groups on both chitosan and alginate. This indicated that the presence of molecular interaction in formation of PEC films. Spectrum of chitosan (Figure 3) showed that characteristics band of chitosan (amine groups) moved from 1581 cm^{-1} to 1600 cm^{-1}. This shifting showed that there were electronical changes in chitosan solution after addition alginate solution. From alginate spectrum (Figure 3), carboxylate groups also moved to higher wavenumber from 1589 to 1600 cm^{-1}. It indicated that there was interaction between amine groups in chitosan and carboxylate groups in alginate. Another absorption of C−O−C bond in both chitosan and alginate has also moved slightly to lower wavenumber. After formation of PEC films, C−O−C bond moved and detected in 1036 cm^{-1} (spectrum a in Figure 4).

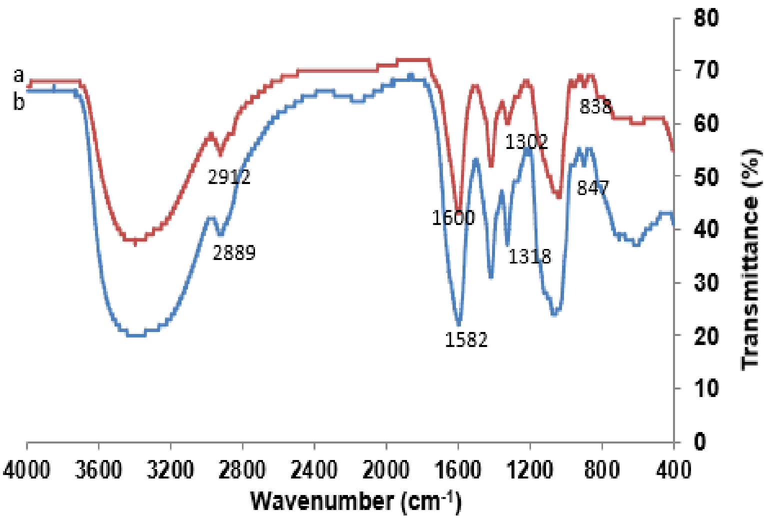

Figure 4. FTIR Spectrum, (a) PEC films of chitosan-alginate; (b) PEC films with kelor extract

Another characteristics absorption of chitosan-alginate PEC films was detected in 1600 cm^{-1}. This absorption indicates that there is a molecular interaction between ionized amine groups of chitosan (–NH_3^+) and ionized carboxylate groups (–COO^-) of alginate, leading to the formation of a polyelectrolyte complex (PEC). From spectrum on Figure 4, this absorption has high intensity, implying strong electrostatic interaction between carboxylate groups of alginate were dissociated into COO^- groups which complexed with protonated amine groups of chitosan (Smitha et al, 2005). Absorption of C=O stretching of amide groups from chitosan does not exist in spectrum of PEC chitosan-alginate. This confirmed that it may be covered due to the high ionic interaction (Hapsari et al, 2020).

The presence kelor extract in PEC chitosan-alginate films was characterized by the changing of some intensity of bands in spectrum b (Figure 4). After addition of kelor extract, it showed that the intensity of some peaks increased. The increasing intensity and widening peaks occurred in some absorption peaks including 3400, 2889, 1582, and 1318 cm^{-1}. Absorption band at 1036 cm^{-1} shift to 1061 cm^{-1} after addition of kelor extract. All these changing confirmed that there were the changing of molecular interaction between PEC chitosan-alginate films due to presence of kelor extract, indicating that PEC chitosan-alginate films with kelor extract had been formed.

Morphological analysis of films

Morphology of surface films were shown in Figure 5. Observation on PEC film without kelor extract showed homogenous morphology on its surface. Based on Castel-Molieres et al, (2018), structure of PEC chitosan-alginate films were mostly influenced by the homogenization technique. However, the presence of some grains on surface indicated that either chitosan or alginate was not completely dissolved. After addition of kelor extract, the morphology of PEC film displayed a porous formation on the surface the film. Hetereogenous distribution on surface of PEC films with kelor extract is may be caused by segregation of some components of kelor extract which were not dissolved in film solution. So, the PEC films containing kelor extract became rougher. Same resulted also occurred in Li et al, (2014) which use natural antioxidant such as ginger extract, green tea extract, grape seed extract, and gingko leaf extract. Addition of extract in at higher level concentration caused gelatin films became rougher.

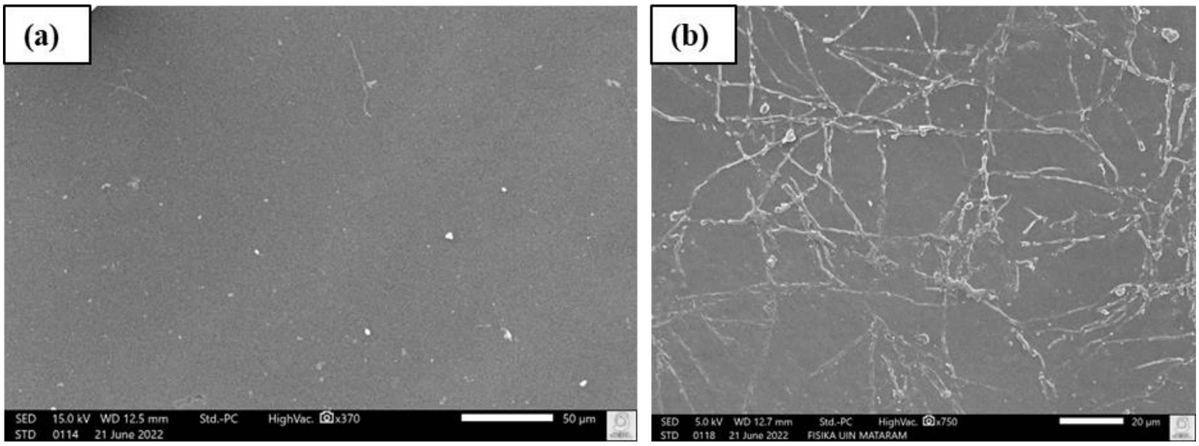

Figure 5. Morphology on both surface, (a) PEC films without kelor extract; (b) PEC films-kelor extract with final concentration of 1%.

Conclusions

In present work, synthesis of PEC film from chitosan and alginate with addition of kelor leaves extract has been implemented. Based on film characterization using FTIR spectrophotometer, PEC chitosan-alginate films containing kelor extract has been formed. There was no significant difference on appearance the films after addition kelor extract. However, addition of kelor leaves extract at highest level concentration caused film became bit yellow compared with other films.

Suggestion

It is necessary to study about other compositions and homogenization technique to increase some properties of PEC chitosan-alginate films with kelor extract. Besides, the properties of these films such as mechanical properties, water absorption, water permeability, antioxidant activity, and others are important things to do to know if these films can be applied for food preservation.

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