Characterization of Chitosan-Based Active Film with Addition of Young Coconut (*Cocos nucifera*) Leaf Extract

Darmono Erma[1], Supriyadi Supriyadi[2], Umar Santoso[2]*

[1]Agriculture and Plantation Service, Province of Central Java, Jl. Gatot Subroto Tarubudaya Ungaran, Central Java, Indonesia
[2]Departement of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia
*Corresponding author: Umar Santoso, Email: umar_s@ugm.ac.id

Submitted: February 18, 2021; Revised: September 23, 2021; Accepted: September 24, 2021

ABSTRACT

This research was aimed to investigate the potential of young coconut (*Cocos nucifera* L.) leaf extract as an antioxidant and antibacterial active compounds. In this study, the physical, mechanical, antioxidant, and antibacterial activity of chitosan-based film with addition of young coconut leaf extract were examined. The young coconut leaf extract was prepared by maceration using ethanol for 48 hours at room temperature. The antioxidant and antibacterial activities were determined by the DPPH and well diffusion methods, respectively. The total phenolic and flavonoids were analyzed using spectrophotometric method. The film was prepared using 1.0 and 1.5% chitosan solution added with young coconut leaf extract at 0, 0.1, and 0.3%. The characterization of the film included color, tensile strength, elongation, WVP, film morphology, identification of functional groups, and total phenolic and antioxidant activity release. The results showed that the antioxidant activity of young coconut leaf extract was IC_{50} of 58.61 ppm. The antibacterial activity test showed that the inhibitory zone for *S. aureus, Salmonella sp.,* and *E. coli* were 17,66, 24.40, and 12.27 mm, respectively. Total phenolic and flavonoid contents were 129.27 mg GAE/g extracts and 3.92 mg QE/g extract, respectively. The color of the film was dark. The higher the concentration of chitosan and the young coconut leaf extract, the higher the tensile strength, whereas the value of elongation at break and water vapor permeability decreased. The release of total phenolic was higher in lipophilic food simulants than in an aqueous system. The result of the study reveals that chitosan-based film added with young coconut leaf extract had great potential as an active biodegradable film for food packaging systems.

Keywords: Active film; antioxidant activity; chitosan; coconut leaf

INTRODUCTION

Packaging is one of the essential stages in the food processing industry. In the last two decades, a decomposable biodegradable film has been used in this industry in response to environmental concerns regarding the accumulation of plastics that pollutes the environment. The food packaging process involving integrating the components of active compounds into the material is expanding. Several plant extracts, including green tea (Siripatrawan and Harte, 2010), *Lycium barbarum* fruit (Wang et al., 2015), and mango leaf (Rambabu et al*.,* 2019), have been extensively used as an antioxidant and antimicrobial additive in active packagings.

In Indonesia, some active studies on food packaging used active compounds from traditional leaf extracts such as teak (Oka et al., 2016) and klutuk banana (Safinta et al., 2019). The conventional packaging is made from natural materials commonly

DOI: http://doi.org/10.22146/agritech.64181
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

used for traditional foods, such as leaves (banana leaves, corn, coconut/anau (aren), guava, and teak leaves). Due to the development of technology and lifestyle, the community has abandoned the traditional packaging because it is considered cheap, unhygienic, impractical, and identified with slums (Noviadji, 2015). Therefore, innovation is needed to maintain its leaves as a source of active agents in the wrapping material.

The young coconut (*Cocos nucifera* L) leaf (*janur*) is one of the traditional packaging leaves. *Janur* is commonly used to wrap traditional food such as ketupat, clorot, dumbeg, and legondo (Central Java specialty foods). The main phenolic compounds in this leaf are *p*-hydroxybenzoic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzaldehyde, and vanillic acid (Dey et al., 2005). *p*-hydroxybenzoic acid in rice hull was an antimicrobial compound capable of inhibiting gram-positive and gram-negative bacteria (Cho et al., 1998).

The research aims to investigate young coconut leaf extract's antioxidant and antibacterial activities, its physical and mechanical properties and the release of total phenolic from chitosan-based films integrated at various concentrations.

MATERIALS AND METHODS

Chemicals and Reagents

The young coconut leaves (age 1 month) were obtained from a tree grown in Kulonprogo, Yogyakarta, commercial chitosan with 95% degree of deacetylation (produced by CV Chi-Multiguna, Indramayu*), 2,2-diphenyl-1-picrylhydrazyl (DPHH)* from Sigma Aldrich, acetic acid (99%), glycerol, ethyl acetate, Folin Ciocalteu reagent, methanol, ethanol, and other chemical were laboratory grade.

Preparation of Young Coconut Leaves Extract

The young coconut leaf extract was prepared according to the method of Madikizela et al. (2014). The leaves were first washed with water until clean, drained, and cut into small pieces. They were then dried with a cabinet dryer at 50 °C for 24 hours. Afterward, it was milled with a blender and sifted with a sieve of 40 mesh size. The extraction process was conducted by the maceration method with 70% ethanol for 48 hours. Finally, the result was filtered using Whatman 41 filter paper, evaporated by rotary vacuum evaporator at 40 °C, and blown with nitrogen gas to obtain a paste-shaped extract.

Active Film Preparation

The chitosan film was prepared following the slightly modified method of Rambabu et al. (2019). The solution was prepared by adding 1.0 and 1.5% chitosan in 1% acetic acid, stirring thoroughly at 60 °C using a magnetic stirrer. The glycerol of 0.5 mL was added as a plasticizer and mixed. Subsequently, the young coconut leaf extract was included in different concentrations from 0, 0.1, and 0.3% and ultrasonicated for 10 minutes. The solution was poured into a Teflon tray for molding and then dried at 50 °C for 20 hours in a cabinet dryer at RH 50% to set the film.

Characterization of Young Coconut Leaf Extract

Antioxidant activity and IC_{50}

The analysis of the antioxidant activity using the DPPH method refers to the approach developed by Molyneux (2004).

IC_{50} measurement

A sample extract of 10 mg was dissolved in 10 mL of methanol in a measuring flask as a 1000 ppm solution. Reaction tubes were prepared to collect 0.01, 0.02, 0.03, 0.04, and 0.05 mL from the 1000 ppm solution. Afterward, 0.99, 0.98, 0.97, 0.96, and 0.95 mL of methanol were added, followed by 2 mL of 0.1 mM DPPH, vortexed, and kept at room temperature for 30 minutes in a dark room. The solution was measured for absorbance at a max λ of 517 nm. The result was the regression curve equation of young coconut leaf extract at a concentration of 10-50 mg/L. Furthermore, the calculation of the IC_{50} is the number 50 (the number that shows the extract concentration that can inhibit the oxidation process by 50% (Molyneux, 2004)) on the ordinate value (y). This implies that the concentration (x) of young coconut leaf extract, which can inhibit 50% of free radicals, is obtained.

Antioxidant activity

An amount of 0.01 mL of a 1000 mg/L young coconut leaf extract in a 1000 ppm solution was combined with 0.99 mL of methanol and 2 mL of a 0.1 mM DPPH solution. The mixture was then vortexed and incubated at room temperature for 30 minutes in a dark room. This solution was measured for absorbance at λ max 517 nm. The following equation calculates the Radical Scavenging Activity (RSA).

$$\% \text{ RSA} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \text{x } 100\% \quad (1)$$

Antibacterial activity

The antibacterial activity test of young coconut leaf extract was conducted against Staphylococcus

aureus, Salmonella sp., and Escherichia coli bacteria by the well diffusion method described by Marini et al. (2007). The extract was used at a concentration of 1000 ppm. Furthermore, the cultivated bacterial culture collected from 0.1 mL of culture (10^6-10^7 CFU/mL) was planted in Mueller-Hinton for the pour plate technique. A total of 0.1 mL of extract was dropped into each well with a diameter of 6 mm, then incubated at 37 °C for 24 hours. Chloramphenicol was used as a positive control. Finally, the antibacterial activity was calculated from the diameter of the clear zone formed around the well.

Total phenolic content

Total phenolic content was determined according to the method developed by Kaur et al. (2015). In amount, 0.2 mL of the 1000 mg/L young coconut leaf extract solution was added with 0.8 mL methanol, 5 mL of 10% folic ciocalteau, and 4 mL of Na_2CO_3, after which it was vortexed and allowed to stand for 60 minutes. The absorbance was measured at a 765 nm wavelength. Furthermore, the result was the ordinate value (y) in the regression equation of the standard gallic acid curve at a concentration of 10-100 mg/L. The total phenolic in the sample solution (x) was expressed as mg equivalent gallic acid/g (mg GAE/g leaf extract).

The formula for calculating the total phenolic content (Equation 2).

$$\mathrm{TPC} = \frac{\mathrm{TP \ x \ FP \ x \ v}}{\mathrm{m}} \quad (2)$$

Where TPC, TP, FP, v, and m are: total phenolic content (mg GAE/g leaf extract), total phenolic (mg GAE/mL), dilution factor, extract volume (mL), and mass sample (g), respectively.

Total flavonoids content

Total flavonoid content was determined according to the method developed by Stanković (2011)in vitro antioxidant activity, total phenolic content and concentration of flavonoids of five different extracts, from the whole herb of Marrubium peregrinum L. (Lamiaceae. A 100 mg sample extract was dissolved in 10 mL methanol in a measuring flask. The solution of young coconut leaf extract of 0.5 mL was added to 0.5 mL methanol, followed by 1 mL of 2% $AlCl_3$ and 1 mL of 20 mM CH_3COONa. The mixture was incubated for one hour at room temperature. Afterward, the absorbance was determined using the UV-Vis spectrophotometric method at a maximum wavelength of 435 nm. The result was the ordinate value (y), which was entered into the regression equation of the quercetin standard curve at a concentration of 8-24 mg/L. Finally, the total flavonoids in the sample solution acquired (x) are expressed as mg equivalence quercetin/g (mg QE/g leaf extract).

Physical and mechanical properties of active film

Color

The color of the film was measured by chromameter for lightness (L), redness (a), and blueness (b) values. The chitosan film was placed on the plate reader, which was subsequently placed on the chromameter. Furthermore, the reader plate was irradiated with rays from the instrument. The chitosan film color was measured in three repetitions.

Water vapor permeability

The film's water vapor permeability (WVP) was measured using a modification of the ASTM E-96 standard method according to Nur Hanani et al. (2012). The vial was filled with 6 mL of distilled water, and the film sample was tightly closed over it and bound with rubber. RH and temperature of the desiccator were controlled at 50 ± 5% and 23 ± 2 °C, respectively. The vial weight was recorded at 1-hour intervals for 9 hours. WVP was calculated using equation 3.

$$\mathrm{WVP} = \frac{\text{(amount of permeant (g) / time (s) x film thickness (mm)}}{\text{film area}(\mathrm{m}^2) \text{ x water vapor pressure difference (kPa)}} \quad (3)$$

Mechanical properties of the active film

Tensile strength (TS) and elongation at break (EAB) film were measured using the Zwick Universal testing machine BL-GR500N model. The film samples were sized at 2.5 by 10 centimeters. Furthermore, the test rate is 10 mm/min, and the result was averaged after three repetitions.

Electronic scanning microscopy (SEM)

SEM analysis was used to study the morphology of the film. The micrographs of the film were obtained from the MiniSEM SNE-3200M with an accelerating voltage of 20 kV. SEM was observed on the sample's surface and obtained at x1000 magnification.

Fourier transform infrared spectroscopy (FTIR)

Fourier transforms infrared spectroscopy (FTIR) was used to characterize the presence of specific chemical groups of a compound. A 2 mg film sample was turned into pellets and then analyzed by FTIR using transmittance mode. Finally, the spectra were obtained in the 4000 to 400 cm^{-1} (IR PRESTIGE-21 SHIMADZU).

Active compounds release

The release of phenolic content and the antioxidant activity of the film were measured based on the method of López De Dicastillo et al. (2016) by immersing the sample material in food simulations following the European Regulations: Simulant A, 10% ethanol, serves as aqueous food simulant, while simulant D1, 50% ethanol, serves as lipophilic food simulant. Release analysis was conducted twice at 40ºC. Furthermore, the film sample with an area of 3 cm2 was soaked in vials containing 5 mL of simulant A and D1. Food simulants A and D1, which received film, are collected periodically at 0, 2, 4, 6, 8, 12, 24, 48, and 72 hours. Finally, the food simulants' total phenolic content and antioxidant activity were analyzed.

Statistical analysis

The method used was a completely randomized design with chitosan and young coconut leaf extract treatments. The concentration of chitosan is 1.0 and 1.5%. Meanwhile, the young coconut leaf extract concentrations were 0, 0.1, and 0.3%, respectively. Each was conducted with 3 replications. The data were analyzed using ANOVA and DMRT (Duncan's Multiple Range Test) continuation tests with a significance level of 5%.

RESULT AND DISCUSSION

Characteristics of young coconut leaf extract

The young coconut leaves were subjected to several tests, including antioxidant activity, total phenolic, total flavonoid, and antibacterial activity of the extract to be added in the film making. The results of the antioxidant activity test were compared with BHT (butylated hydroxytoluene) as a standard. Table 1 shows the antioxidant activity, total phenolic, and total flavonoids.

Antioxidant activity

The value of the antioxidant activity and IC_{50} ethanol extract of young coconut leaves (8.97 ± 0.34 at 0.01 mg/mL and IC_{50} 58.61 ± 7.12 ppm) is smaller than BHT (26.49 ± 2.79 at 0.01 mg/mL and IC_{50} 10.80 ± 3.63 ppm) (Table 1). Specifically, an antioxidant compound is said to be very strong, strong, moderate, and weak when the IC_{50} value is less than 50 ppm, worth 50-100 ppm, 100-150, and 150-200, respectively (Molyneux, 2004). The IC_{50} values of young coconut leaf extract and BHT are included in the strong and very strong categories, respectively, according to Molyneux (2004).

Sasidharan et al. (2009) reported that palm oil leaf methanol extract has an antioxidant activity of 50.14 ± 1.71 at 1.0 mg/mL with an IC_{50} of 810 ppm. The difference in the antioxidant activity of an extract was influenced by factors used in the extraction, including solvent type, extraction time, solvent concentration, extraction temperature, and particle size of samples (Chew et al., 2011).

Total phenolic content

Table 1 shows that the total phenolic content of the young coconut leaf extract was 129.27 ± 2.06 mg GAE/g extract. Hasanah (2019) reported that the total phenolic content in the ethyl acetate fraction was 171.11 mg GAE/g and 171.33 mg GAE/g for fresh and steamed *janur.* In the butanol fraction, it was 120.97 mg GAE/g (fresh *janur*) and 90.55 mg GAE/g (steamed *janur*). Furthermore, the difference could be attributed to the extraction procedure. Naczk and Shahidi (2004) stated that the efficiency of phenolic compounds extraction was affected by the procedure, sample particle size, conditions of storage, and the presence of interferences. The solvent used influences the yield due to differences in the polarities and solubility of the different samples (Butsat and Siriamornpun, 2016).

Research conducted by Velioglu et al. (1998) shows that extracts derived from plants (fruits, leaves, and vegetables) directly correlate the total phenolic content and its antioxidant activity. The main phenolic compounds bound to the walls of young coconut leaves are identified as *p*-hydroxybenzoic and ferulic acid, while the concentration of dissolved phenolic metabolites in young leaves includes ferulic, *p*-coumaric, vanillic, *p*-hydroxybenzoic, *p*-hydroxy, benzaldehyde, and vanillin acids (Dey et al., 2005; Hasanah, 2019).

Table 1. IC_{50} antioxidant activity, total phenolic, and total flavonoids of young coconut leaf extract

Sample	IC_{50} (ppm)	Antioxidant activity (%RSA)	Total phenolic (mg GAE/g extract)	Total flavonoids (mg QE/g extract)
Young coconut leaf extract	58.61 ± 7.12	8.97 ± 0.34	129.27 ± 2.06	3.92 ± 0.10
BHT	10.80 ± 3.63	26.49 ± 2.79		

The results are represented as the means ± standard deviation (n = 3)

Table 2. Antibacterial activity of young coconut leaf extract

Sample	Concentration	Inhibition zone diameter (mm) for bacteria		
	(ppm)	*S. aureus*	*Salmonella sp.*	*E. coli*
Young coconut leaf extract	1000	17.66 ± 0.02	24.40 ± 0.04	12.27 ± 0.01
Chloramphenicol	50	14.83 ± 0.06	20.57 ± 0.06	15.97 ± 0.12

The results are mean ± standard deviation (n = 3).

Table 3. Color of chitosan film

Sample	L (lightness)	a (redness)	b (blueness)
A (Chit 1%)	50.61 ± 1.12^{r}	-0.04 ± 0.06^{p}	6.14 ± 1.60^{p}
B (Chit 1% + extract 0.1%)	45.28 ± 1.70^{q}	0.22 ± 0.40^{pq}	16.59 ± 1.59^{s}
C (Chit 1% + extract 0.3%)	45.10 ± 1.17^{q}	0.89 ± 0.66^{q}	20.89 ± 1.45^{s}
D (Chit 1.5%)	48.73 ± 0.16^{r}	-0.06 ± 0.08^{p}	10.14 ± 0.47^{q}
E (Chit 1.5% + extract 0.1%)	43.71 ± 0.38^{pq}	0.15 ± 0.29^{pq}	13.04 ± 1.55^{r}
F (Chit 1.5% + extract 0.3%)	42.90 ± 1.35^{p}	0.83 ± 0.76^{q}	18.98 ± 0.62^{s}

The results are mean ± standard deviation (n = 3). Different letters in the same column show significant differences ($p < 0.05$)

Total flavonoids content

The total flavonoid content obtained from young coconut leaf extract was 3.920 ± 0.10 mg QE/g extract, as shown in Tabe 1. Research by Katja & Edi (2008) on methanol extracts of old leaves of coconut varieties in Manado (DMA) and Genjah Yellow Nias Coconut (GKN) obtained total flavonoids of 3.725 mg QE/g and 4.625 mg QE/g, respectively. Flavonoids are plants' most common and widely distributed group of phenolic compounds (Jeevani et al., 2011). They have potential as antioxidants because of the hydroxyl groups attached to aromatic carbon rings. The hydroxyl group can be a hydrogen atom donor that can capture free radicals (Amic et al., 2003).

Antibacterial activity

Antibacterial activity test of young coconut leaf extract was conducted on gram-positive and gram-negative bacteria. The results are shown in Table 2.

The antibacterial activity of young coconut leaf extract against *S. aureus, Salmonella sp,* and *E. coli* was 17,66 ± 0.01, 24.40 ± 0.04, and 12.27 ± 0.01 mm, respectively, as indicated by the inhibitory zone. It can be classified into three levels such as weak (inhibition zone ≤12 mm), moderate (inhibition zone 12-20 mm), and strong (inhibition zone ≥ 20 mm) (Lv et al., 2013). Therefore, the young coconut leaf extract is in the moderate to strong category of antibacterial activity (inhibitory zone 12-24 mm), according to Lv et al. (2013).

Ifesan et al. (2013) reported the antimicrobial activity of ethanol extract of coconut leaves (*Cocos nucifera L*) at a concentration of 0.1 mg/mL could inhibit six out of eight microorganisms such as *Acinetobacter spp.* (8.0 mm), *Bacillus cereus* (7.0 mm), *Escherichia coli* (3.0 mm), *Shigella dysenteriae* (5.0 mm), *Staphylococcus aureus* (- mm), *Salmonella typhi* (3.0 mm), *Aspergillus niger* (3.0 mm), and *Aspergillus flavus* (- mm). It is suspected that phenolic acids such as *p*-hydroxybenzoic acid in young coconut leaf extract are antibacterial compounds, as reported by Cho et al. (1998). Finally, the *p*-hydroxybenzoic acid compounds in rice hulls can inhibit gram-positive and gram-negative bacteria.

Physical and mechanical properties of chitosan film

Color

Color is important to the film's appearance, affecting consumer acceptance of the packaged product.

The difference in chitosan concentration had no significant effect ($p>0.05$) on the L (lightness) and a (redness) values, which were considerably affected by the addition of leaf extracts ($p<0.05$). Chitosan- young coconut leaf extract films exhibited substantially lower

Table 4. Thickness, water vapor permeability, tensile strength (TS), elongation at break (EAB) of chitosan film

Sample	Thickness (mm)	WVP (x10^{-12} g/m s Pa)	TS (MPa)	EAB (%)
A (Chit 1%)	0.040 ± 0.01^{a}	4.38 ± 1.39^{a}	1.25 ± 0.83^{a}	6.02 ± 1.27^{b}
B (Chit 1% + extract 0.1%)	0.052 ± 0.01^{ab}	4.12 ± 0.45^{a}	1.84 ± 0.97^{ab}	4.19 ± 1.01^{a}
C (Chit 1% + extract 0.3%)	0.052 ± 0.01^{ab}	3.70 ± 0.30^{a}	1.49 ± 0.39^{a}	3.22 ± 0.30^{a}
D (Chit 1.5%)	0.055 ± 0.01^{ab}	4.28 ± 0.45^{a}	2.24 ± 0.19^{ab}	7.48 ± 0.37^{b}
E (Chit 1.5% + extract 0.1%)	0.058 ± 0.01^{b}	3.98 ± 0.52^{a}	3.14 ± 0.96^{b}	7.40 ± 1.04^{b}
F (Chit 1.5% + extract 0.3%)	0.060 ± 0.01^{b}	3.97 ± 0.65^{a}	3.10 ± 0.86^{b}	7.06 ± 1.10^{b}

The results are mean ± standard deviation (n = 3). Different letters in the same column show significant differences (p<0.05).

L values than the control. The value of a (redness) increased with higher extract concentration and was statistically different from controls at 0.3% (p0.05). The same phenomenon occurred at value b (blueness), where chitosan and extract concentrations make the film even more yellow, as shown in Table 3. The color change was due to the addition of young coconut leaf extract. This is because the phenolic component of young coconut leaf extract causes scattering and refraction of light, resulting in a darker film (Hopkins et al., 2015)moisture barrier and swelling properties of soy protein isolate (SPI.

Water vapor permeability

Water vapor permeability (WVP) is important in selecting food packaging films. The smaller the value, the higher the shelf life of food.

The results showed that the addition of young coconut leaf extract decreased the value of WVP but was not significantly different (p>0.05) in the chitosan and the leaf extract concentrations, as shown in Table 4. This is due to young coconut leaf extract filling the structural gaps in the chitosan polysaccharide chain, thereby inhibiting channels and pathways for water transportation across the film (Siripatrawan and Harte, 2010).

Another possible reason for reducing WVP film chitosan extracts of young coconut leaves could be an increase in density. This is due to the higher density of film shown by compactly accumulating chitosan chains, which can reduce the interstitial space in the matrix, hence, fewer water molecules can pass through the film (Wang et al., 2015). According to Nur Fatin Nazurah & Nur Hanani (2017), the WVP of the active film is influenced by the uniform interaction and distribution of the extract, temperature, humidity, and concentration of the plasticizer.

Mechanical properties of chitosan film

Elastic, flexible, and high-strength properties are desirable attributes of food packaging. The tensile strength values of chitosan films increased and differed significantly with adding chitosan. TS is considerably increased in the chitosan film containing 0.3% extract than in the control film containing 0.1%. The EAB value decreased with the increasing extract concentration but was not significantly different. Meanwhile, it increased with the addition of chitosan and differed considerably.

The increase in TS, as shown in Table 4, can be attributed to the interaction of the phenolic compound extract with the amine group of the polymer chain, thereby causing crosslinking. This interconnected network produces a rigid structure (Song et al., 2013). The decrease in elongation at break value (Table 4) may be due to the interaction between the extract components at high concentrations with chitosan that will produce a crosslinking effect that reduces the volume and mobility of polymer-free molecules causing a decrease in elongation (Bodini et al., 2013).

According to JIS (Japanese Industrial Standard) Z 1707 - 1975 in Nurindra et al. (2015), plastic film for food packaging has a minimum tensile strength value of 0.392 MPa and elongation at break of at least 70%. The active film treatments have tensile strength and elongation at break values above and below the JIS standard.

Electronic scanning microscopy (SEM)

SEM analysis is conducted to examine the morphology of the chitosan film with the addition of young coconut leaf extract. The surface of the chitosan film at 1.0% (A) and 1.5% (D) is smoother, and increasing the concentration of the extract of young coconut leaves further enhances the surface roughness of the film. The results of a similar SEM study by Nguyen

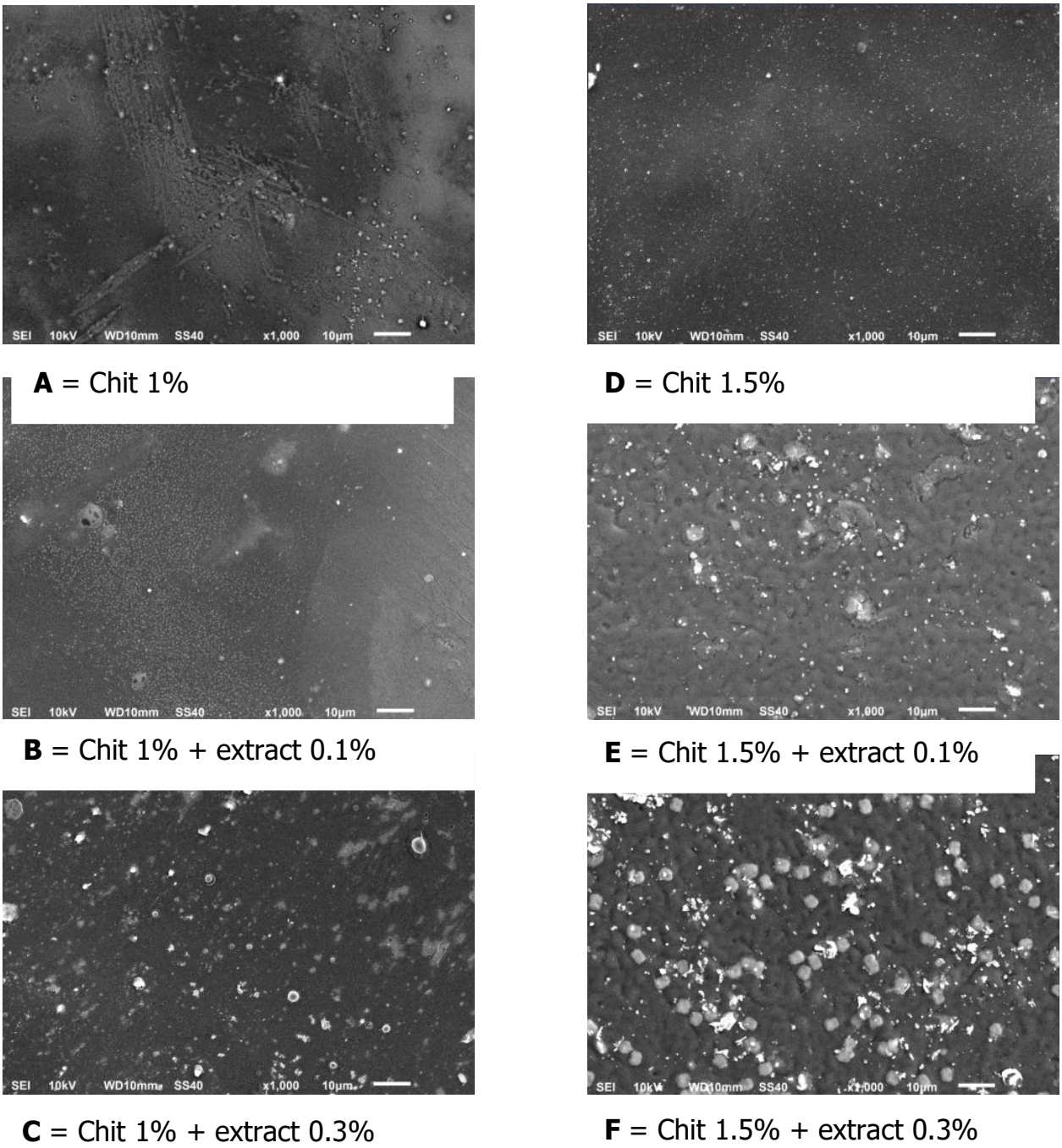


Figure 1. Surface SEM images of the chitosan film with *janur* extract

et al. (2020) stated that the chitosan film control looks smooth, and the surface is homogeneous but with the addition of *Sonneratia caseolaris* (L.) Engl. Leaf extract (SCELE) becomes rough. Furthermore, the surface morphology of chitosan-SCELE-1% shows protrusions and irregularities. Rambabu et al. (2019) reported that adding mango leaf extract to the chitosan polymer increases the density and produces a more compact, thicker film due to the interaction. The active film with a solid cross-section reduces oxygen permeability and increases the shelf life of preserved foodstuffs (Atarés et al., 2011).

Fourier transform infrared spectroscopy (FTIR)

When the two components are mixed, the physical mixture and chemical interaction are reflected by changes in the characteristics of the peak spectrum. The active film samples indicate similar FTIR spectrum

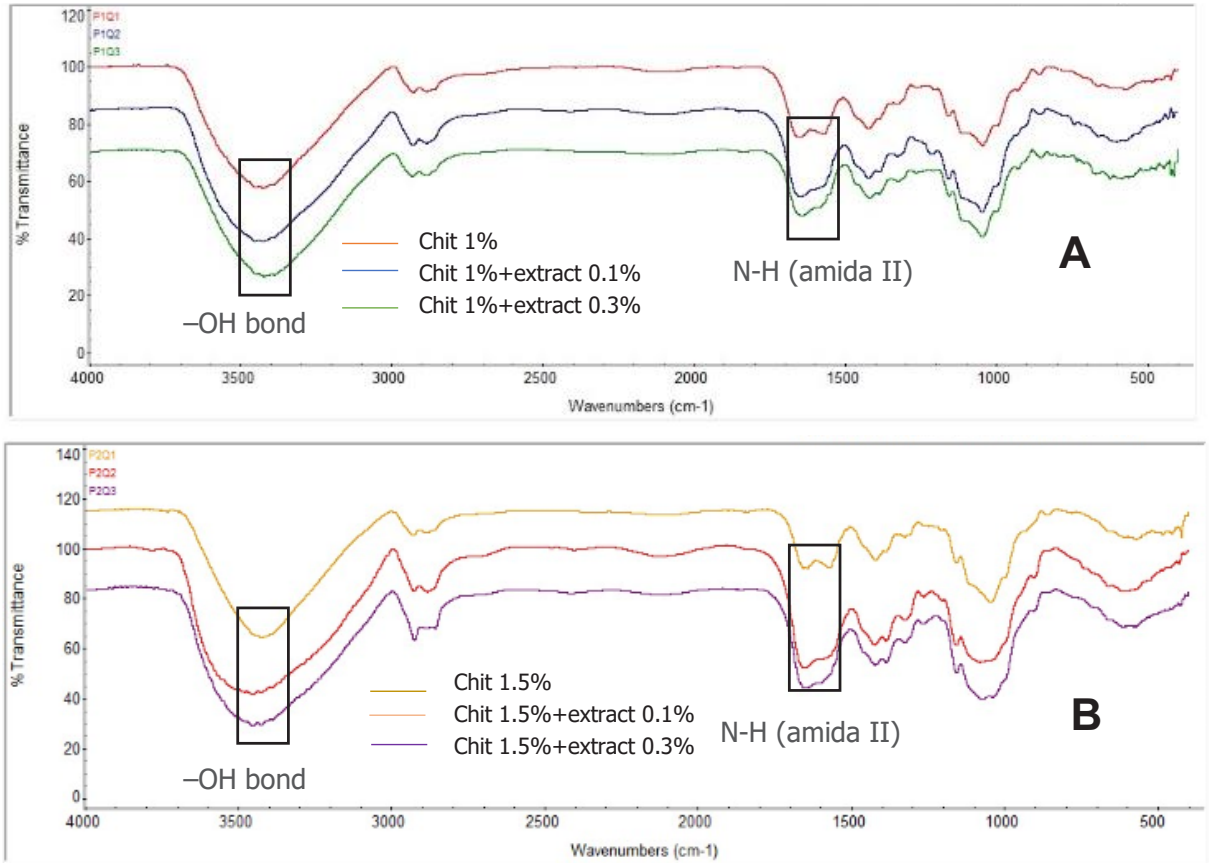


Figure 2. FTIR spectrum of chitosan film with *janur* extract. A = Chit 1%, B = Chit 1.5%

patterns, with most peaks showing the characteristics of chitosan films but with different transmission intensities at certain peaks, as shown in Figure 2.

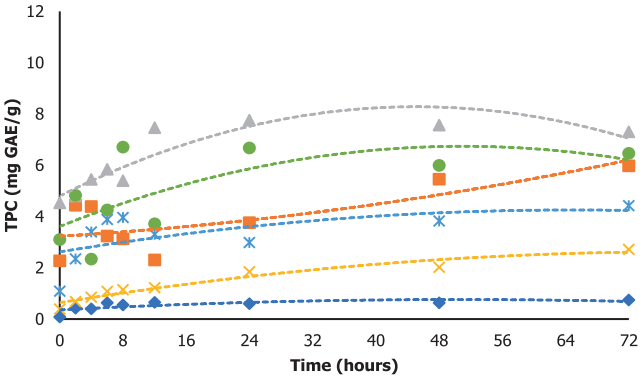
Peak NH stretches in amino groups (3500-3300 cm^{-1}), OH stretches (3400-3200 cm^{-1}), CH stretches (3000-2850 cm^{-1}), C = O (amide I) (1638-1648 cm^{-1}), and CO (1280-1000 cm^{-1}) can be observed on the films that using chitosan as a base (Siripatrawan and Vitchayakitti, 2016). The changes that occur are stretching the peak width at 3500-3300 cm^{-1} and the absence of bending N-H (amide II) (1568 cm^{-1}) on the active film, which is administered young coconut leaf extract. This broad peak corresponds to the stretching vibrations of the -NH2 and the -OH group (Wang et al., 2015).

Intermolecular hydrogen bonds formed between the extract of the *Pistacia terebinthus* stem and the film matrix produce a broader peak of the -OH bond (3253.14 cm^{-1}) than the spectrum of the chitosan control (Kaya et al., 2018). FT-IR analysis confirmed the formation of hydrogen bonds between polyphenol compounds (stretching the width of the peak at 3500-3300 cm^{-1}) in young coconut leaf extract and chitosan, which significantly affected the physical and mechanical properties of the film (Siripatrawan and Vitchayakitti, 2016).

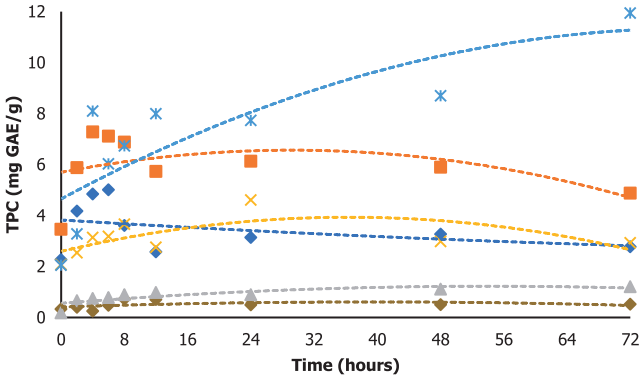
Active compounds release

The main mechanism of the release process is the migration of total phenolic content to protect food products, which in this test are in the form of food simulants. Figure 3 shows the release of total phenolic content in food simulants. Figure 4 shows the release of antioxidant activity. The migration of total phenolic content (Figure 3) shows that the chitosan film with the addition of a high concentration of young coconut leaf extract has a higher content than the control because of the high initial antioxidant concentration in the leaf extract. The release of total phenolic content in lipophilic food simulants is greater than in the aqueous counterpart. Lipophilic food simulants have a higher ethanol content, easily dissolving the phenolic compounds in young coconut leaf extracts.

Some samples grow exponentially (an increase in the form of a fixed percentage of the whole at a certain time) to the maximum rate of migration of phenolic content. However, the release rate is proportional to

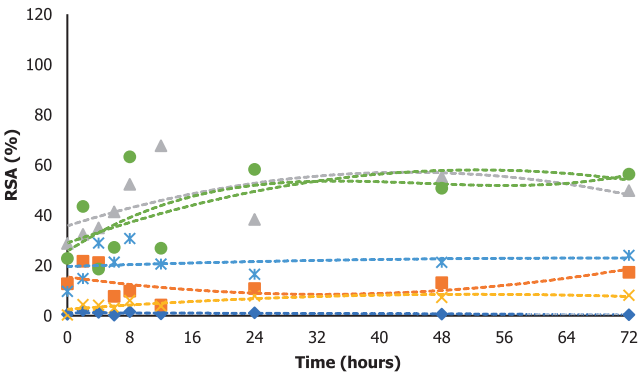


A = release of total phenolic content in aqueous food simulant: (♦) chit 1%, (x) chit 1.5%, (■) chit 1%+extract 0.1%, (ж) chit 1.5%+extract 0.1%, (▲) chit 1%+extract 0.3%, (●) chit 1.5%+extract 0.3%.

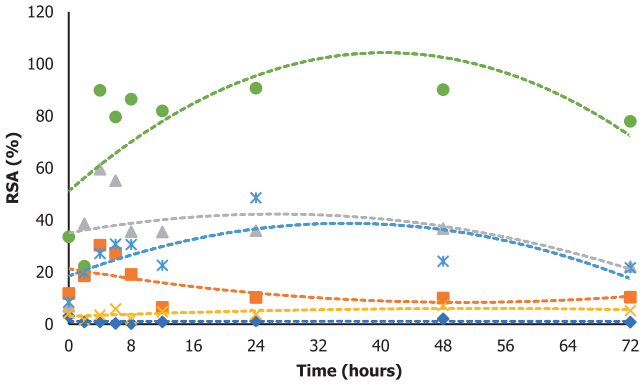


B = release of total phenolic content in a lipophilic food simulant: (♦) chit 1%, (▲) chit 1.5%, (♦) chit 1%+extract 0.1%, (x) chit 1.5%+extract 0.1%, (■) chit 1%+extract 0.3%, (ж) chit 1.5%+extract 0.3%

Figure 3. Release of total phenolic content, A is an aqueous foods simulant; B is a lipophilic food simulant



A = release of antioxidant activity in aqueous food simulant: (♦) chit 1%, (x) chit 1.5%, (■) chit 1%+extract 0.1%, (ж) chit 1.5%+extract 0.1%, (▲) chit 1%+extract 0.3%, (●) chit 1.5%+extract 0.3%.



B = release of antioxidant activity in a lipophilic food simulant: (♦) chit 1%, (x) chit 1.5%, (■) chit 1%+extract 0.1%, (ж) chit 1.5%+extract 0.1%, (▲) chit 1%+extract 0.3%, (●) chit 1.5%+extract 0.3%.

Figure 4. The release of antioxidant activity, A is an aqueous foods simulant; B is a lipophilic food simulant

the concentration of phenolic compounds incorporated in the film (Calatayud et al., 2013). Exponential growth has been demonstrated in other studies with hydrophilic material (Calatayud et al., 2013; López-De-Dicastillo et al., 2010).

The initial concentration of antioxidants in the film was the first important factor for releasing compounds from the polymer matrix into the simulants. The higher the initial antioxidant concentration the greater the number of antioxidants released (chit 1% + extract 0.3% and chit 1.5% + extract 0.3% treatments). Furthermore, the second important factor was the food simulant (López-De-Dicastillo et al., 2010). The release rate at equilibrium depends on the compatibility between migrants with simulants and polyphenols, which are compounds from plant extracts that are very soluble in ethanol compared to water (López De Dicastillo et al., 201and the addition of the extract increased the water and oxygen barrier at low relative humidity but increased the water sensitivity, the glass transition temperature, and the crystallinity of the films and improved their thermal resistance. An analysis by HPLC revealed that the antioxidant components of the extract suffered partial degradation during extrusion, reducing the content of catechin gallates and increasing the concentration of free gallic acid. Exposure of the films to various food simulants showed that the liquid simulants increased their capacity to reduce DPPH• and ABTS •+ radicals. The release of green tea extract components into the simulant monitored by HPLC showed that all compounds present in the green tea extract were partially released, although the extent and kinetics of release were dependent on the type of food. In aqueous food simulants, gallic acid was the main antioxidant

component released with partition coefficient values ca. 200. In 95% ethanol (fatty food simulant).

The release *of* antioxidant activity (Figure 4) shows similar results to total phenolic content. Chitosan films containing high concentrations of young coconut leaf extract have a higher release value of the antioxidant activity. It also has more release of antioxidant activity in lipophilic food simulants. The highest migration of phenolic content in the active film of 1.5% chitosan treated with 0.3% *janur* extract at 72 hours was 11.95 mg GAE/g (Figure 3B) or 9%. In other studies, the amount measured in food simulations was around 12% of the maqui berry extract (Aristotelia chilensis) (López De Dicastillo et al., 2016). Therefore, the phenolic component of the extract of young coconut leaves could partly be a cross-linker agent in the film.

CONCLUSION

The results showed the antioxidant activity of the extract of young coconut leaf could be classified as in the strong category (IC_{50} is 58.61 ppm). The extract also has remarkable antibacterial activity, which can be classified into moderate to strong categories (inhibition zone 12,27-24,40 mm). Furthermore, the antioxidant and antibacterial activity is related to the high content of phenolic and flavonoid compounds. Adding young coconut leaf extract into chitosan film darkens the appearance, increases tensile strength, and decreases the elongation at the break. The WVP film was insignificant due to the addition of young coconut leaf extract. Interactions between hydrophilic groups of chitosan and phenolic compounds of young coconut leaf extract were confirmed by FTIR analysis. Considering the results, chitosan film with the addition of young coconut leaf extract has great potential to be used as a film for packaging lipophilic foods, such as wingko and gethuk.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Research Technology and Higher Education of Republic Indonesia for financially supporting this research through the Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) program 2019.

REFERENCES

Amic D, Dusanka D, A., Beslo D, and Trinasjtia. 2003. Structure-radical scavenging activity relationships of flavonoids. Croatia Chemica Acta 76:55-61.

Atarés, L., Pérez-Masiá, R., and Chiralt, A. 2011. The role of some antioxidants in the HPMC film properties and lipid protection in coated toasted almonds. Journal of Food Engineering, 104(4), 649–656.

Bodini, R. B., Sobral, P. J. A., Favaro-Trindade, C. S., and Carvalho, R. A. 2013. Properties of gelatin-based films with added ethanol-propolis extract. LWT - Food Science and Technology, 51(1), 104–110.

Butsat, S., and Siriamornpun, S. 2016. Effect of solvent types and extraction times on phenolic and flavonoid contents and antioxidant activity in leaf extracts of *Amomum chinense* C. International Food Research Journal 23(1): 180–187.

Calatayud, M., López-De-Dicastillo, C., López-Carballo, G., Vélez, D., Hernández Muñoz, P., and Gavara, R. 2013. Active films based on cocoa extract with antioxidant, antimicrobial, and biological applications. Food Chemistry, 139(1–4), 51–58.

Chew, K.K., Ng, S.Y., Thoo, Y.Y., Khoo, M.Z., Wan Aida, W.M.and Ho, C.W. 2011. Effect of ethanol concentration, extraction time, and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella Asiatica* extracts. International Food Research Journal 18: 571-578.

Cho, J.-Y., Moon, J.-H., Seong, K.-Y., & Park, K.-H. et al. 1998. Antimicrobial activity of 4-hydroxybenzoic acid and trans 4-hydroxycinnamic acid isolated and identified from rice hull. In Bioscience, Biotechnology, and Biochemistry (Vol. 62, Issue 11, pp. 2273–2276).

Dey, G., *Chakraborty*, M., and Mitra, A. 2005. Profiling C6-C3 and C6-C1 phenolic metabolites in Cocos nucifera. Journal of Plant Physiology, 162(4), 375–381.

Hasanah, U. 2019. Perubahan Komponen Volatil, Aktivitas Antioksidan dan Antimikrobia Daun Kelapa (*Cocos Nucifera L.*) Muda Akibat Pengukusan. MSc. thesis. Department of Food and Agricultural Product Technology. Universitas Gadjah Mada, Indonesia.

Hopkins, E. J., Chang, C., Lam, R. S. H., and Nickerson, M. T. 2015. Effects of flaxseed oil concentration on the performance of a soy protein isolate-based emulsion-type film. Food Research International, 67, 418–425.

Ifesan, B., Fashakin, J. F., Ebosele F., and Oyerinde. A. S. 2013. Antioxidant and Antimicrobial Properties of Selected Plant Leaves. European Journal of Medicinal Plants, 3(3), 465–473.

Jeevani O, W., M. M., Karim, A. A. and Bhat, R. 2011. Evaluation of nutritional quality of torch ginger (*Etlingera elatior* Jack.) inflorescence. International Food Research Journal 18(4): 1415-1420.

Katja, D. G, and Edi, S. 2008. Analyses of phytochemistry content and quenching oxygen singlet activities from

coconut leaves. Chemistry Progress. Vol. 1, No. 2: 78-84.

Kaur, A., Kaur, M., Kaur, P., Kaur, H., Kaur, S., and Kaur, K. 2015. Estimation and comparison of total phenolic and total antioxidants in green tea and black tea. Global Journal of Bio-Science and Biotechnology, 4(1), 116–120.

Kaya, M., Khadem, S., Cakmak, Y. S., Mujtaba, M., Ilk, S., Akyuz, L., Salaberria, A. M., Labidi, J., Abdulqadir, A. H., and Deligöz, E. 2018. Antioxidative and antimicrobial edible chitosan films blended with stem, leaf, and seed extracts of *Pistacia terebinthus* for active food packaging. RSC Advances, 8(8), 3941–3950.

López-De-Dicastillo, C., Alonso, J. M., Catalá, R., Gavara, R., and Hernández-Munoz, P. 2010. Improving the antioxidant protection of packaged food by incorporating natural flavonoids into ethylene-vinyl alcohol copolymer (EVOH) films. Journal of Agricultural and Food Chemistry, 58(20), 10958–10964.

López De Dicastillo, C., Nerín, C., Alfaro, P., Catalá, R., Gavara, R., and Hernández-Muñoz, P. 2011. Development of new antioxidant active packaging films based on ethylene vinyl alcohol copolymer (EVOH) and green tea extract. Journal of Agricultural and Food Chemistry, 59(14), 7832–7840.

López De Dicastillo, C., Rodríguez, F., Guarda, A., and Galotto, M. J. 2016. Antioxidant films based on crosslinked methyl cellulose and native Chilean berry for food packaging applications. Carbohydrate Polymers, 136, 1052–1060.

Lv, F., Liang, H., Yuan, Q., and Li, C. 2011. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Research International, 44(9), 3057–3064.

Madikizela, B., Aderogba, M. A., Finnie, J. F., and Van Staden, J. 2014. Isolation and characterization of antimicrobial compounds from *Terminalia phanerophlebia* Engl. & Diels leaf extracts. Journal of Ethnopharmacology, 156, 228–234.

Marini, M., Bondi, M., Iseppi, R., Toselli, M., and Pilati, F. 2007. Preparation and antibacterial activity of hybrid materials containing quaternary ammonium salts via the sol-gel process. European Polymer Journal, 43(8), 3621–3628.

Molyneux P. 2004. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology, 26(May), 211–219.

Naczk, M., and Shahidi, F. 2004. Extraction and analysis of phenolics in food. Journal of Chromatography A 1054: 95–111.

Nguyen, T. T., Thi Dao, U. T., Thi Bui, Q. P., Bach, G. L., Ha Thuc, C. N., and Ha Thuc, H. 2020. Enhanced antimicrobial activities and physiochemical properties of edible film based on chitosan incorporated with *Sonneratia caseolaris* (L.) Engl. leaf extract. Progress in Organic Coatings, 140, 105487.

Noviadji, B.R. 2014. Desain Kemasan Tradisional Dalam Konteks Kekinian. Jurnal Fakultas Desain. Vol 1(1).

Nurindra, A. P., M. A. Alamsjah, and Sudarno. 2015. Characterization of Edible Film from Propagules Mangrove Lindur (*Bruguiera Gymnorrhiza*) Starch with Addition of Carboxymethyl Cellulose (CMC) as Plasticizer. Jurnal Ilmiah Perikanan dan Kelautan Vol. 7 No. 2.

Nur Fatin Nazurah, R., and Nur Hanani, Z. A. 2017. Physicochemical characterization of kappa-carrageenan (Euchema cottoni) based films incorporated with various plant oils. Carbohydrate Polymers, 157, 1479–1487.

Nur Hanani, Z. A., Roos, Y. H., and Kerry, J. P. 2012. Use of beef, pork, and fish gelatin sources in the manufacture of films and assessment of their composition and mechanical properties. Food Hydrocolloids, 29(1), 144–151.

Oka A. A., K. A. Wiyana, I. M. Sugitha, and I.N.S. Miwada, 2016. Identifikasi Sifat Fungsional Dari Daun Jati, Kelor dan Kayu Manis dan Potensinya sebagai Sumber Antioksidan pada Edible Film. Jurnal Sain Peternakan Indonesia Vol. 11 No 1. ISSN 1978-3000

Rambabu, K., Bharath, G., Banat, F., Show, P. L., and Cocoletzi, H. H. 2019. Mango leaf extract incorporated chitosan antioxidant film for active food packaging. International Journal of Biological Macromolecules, 126, 1234–1243.

Safinta, N. R., Santoso, U., and Supriyadi. 2019. Ekstrak Daun Pisang Klutuk (*Musa balbisiana Colla*) sebagai Bahan Tambahan pada Pembuatan Kemasan Aktif berbasis Methyl Cellulose. CHEMICA: Jurnal Teknik Kimia. Vol. 6, No. 1, June 2019, pp. 07-14. ISSN 2355-8776

Sasidharan, S., Sharmini, R., Vijayarathna, S., Yoga Latha, L., Vijenthi, R., Amala, R., and Amutha, S. 2009. Antioxidant and hepatoprotective activity of methanolic extracts of *Elaeis guineensis Jacq* leaf. Pharmacologyonline, 3, 84–90.

Siripatrawan, U., and Harte, B. R. 2010. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. Food Hydrocolloids, 24(8), 770–775.

Siripatrawan, U., and Vitchayakitti, W. 2016. Improving functional properties of chitosan films as active food packaging by incorporating with propolis. Food Hydrocolloids, 61, 695–702.

Song, X., Zhou, C., Fu, F., Chen, Z., and Wu, Q. 2013. Effect of high-pressure homogenization on particle size and film properties of soy protein isolate. Industrial Crops and Products, 43(1), 538–544.

Stanković, M. 2011. Total phenolic content, flavonoid concentration, and antioxidant activity of *Marrubium peregrinum* L. extracts. Kragujevac Journal of Science, 33(33), 63–72.

Velioglu, Y. S., Mazza, G., Gao, L., and Oomah, B. D. 1998. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. Journal of Agricultural and Food Chemistry, 46(10), 4113–4117.

Wang, Q., Tian, F., Feng, Z., Fan, X., Pan, Z., and Zhou, J. 2015. Antioxidant activity and physicochemical properties of chitosan films incorporated with *Lycium barbarum* fruit extract for active food packaging. International Journal of Food Science and Technology, 50(2), 458–464.