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ORIGINAL RESEARCH PAPER

EFFECTS OF KESUM LEAF EXTRACT SUPPLEMENTATION ON CHARACTERISTICS OF DURIAN SEEDS STARCH (*DURIO ZIBETHINUS*) – CHITOSAN EDIBLE FILM

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Abstract: Edible films are thin layers formed on food surface to maintain quality and extend shelf life of food. One of the edible packagings used as a wrapper on food is an edible film from durian seed starch-chitosan. Additional supplementation from active ingredients can be used as antimicrobials, such as kesum leaf extract (KLE). The purpose of this study was to evaluate the effect of KLE on the physical properties of edible film of durian seed starch - chitosan. This research used completely randomized design (CRD) with one factor. In this research, treatments of the edible film with different concentrations of KLE (K_0: 0 %, K_1: 0.2 %, K_2: 0.4 %, K_3: 0.6 %, K_4: 0.8 %, K_5:1.0 %, and K_6: 1.2 %) were performed. The variables observed were microstructure of edible film evaluated using scanning electron microscopy (SEM) and physical properties of edible film, including WVTR (water vapor transmission rate), tensile strength and elongation. The results showed that the surface of the SEM edible film in K_0 was flat, very tight and non-porous, while that in K_1 to K_6 was very hollow and uneven, with non-uniform size. This indicated that KLE supplementation decreased homogeneity, resulting in changes in the surface morphology of the films. KLE supplementation had no significant effect on WVTR and elongation, but it had a significant effect on tensile strength.

Keywords: *chitosan, edible film, kesum leaf extract, tensile strength, WVT*

INTRODUCTION

Edible packaging is a wrapper or coating used in food products to maintain quality and extend shelf life. Edible packaging used in food products consists of two types, namely edible coating and edible film. Edible coating is commonly applied directly to the product surface as additional protection, while edible film is applied after it is printed in sheet form. Starch is a potential material for edible film manufacture as it has characteristics, such as ability to form a strong thin-layer or film. Among many materials used in edible film manufacture, durian seed starch seems to be a good material since it has ability to protect the product against oxygen from the environment [1]. However, because of its low mechanical strength and hydrophilic, it is unconsidered as a good barrier against water vapor. The low resistance against water and poor ability as a water vapor barrier can affect its stability and mechanical properties [2]. Meanwhile, low stability of film will shorten the shelf life. Therefore, it is recommended to use other polymers in order to overcome this shortage, such as by the use of chitosan, a hydrophobic biopolymer.

Chitosan has become a promising alternative in food product preservation. In addition, the quality of edible film can be enriched with other natural ingredients, which have antioxidant and antimicrobial activity. Edible films incorporated with garlic, rosemary, and oregano oils have already been reported to possess antimicrobial activity [3, 4]. Edible film of Sago starch in combination with lemon grass leaf essential oil at a concentration of 0.4 % is effective to inhibit the growth of *Escherichia coli* and *Salmonella enteritidis* [5]. Other natural ingredients potentially used in sausages edible packaging are green tea [6], chitosan-oregano [7], and oleoresin of lime leaves (*Citrus hystrix* DC.) [8].

Kesum (*Polygonum minus* Huds) is an endemic plant in West Borneo and has a unique flavor and aroma. It is known as an ingredient for Bubur Padas, a special food from the Sambas Malays ethnic group. Kesum leaf has active compounds that can be used as antimicrobial agents. Phytochemical analysis of methanol fraction shows that kesum leaf extract (KLE) contains phenolic group compounds, terpenoids-steroids, flavonoids and alkaloids [9]. Major compounds of kesum leaves are dodecanal (43.47 %), decanal (16.263) and 1-decanol (12.68 %) [10]. Decanal and dodecanal are aldehyde derivative compounds that are effective to increase the flavor and aroma of kesum. Wibowo [9] studied the antimicrobial bioactivity of kesum essential oils and found that kesum is potentially effective to inhibit the growth of *Escherichia coli* and *Basilus subtilis.*

Kesum has been proven to be effective to repair the damage of lung and lung cancer [9]. Mackeen *et al.* [11] reported that the ethanol extract of kesum leaf is cytotoxic against HeLa cells, the lethal concentration 50 (LC_{50}) is 30 $\mu g \cdot mL^{-1}$. Wibowo *et al.* [9] reported that the *n*-hexane fraction in kesum leaf extract can inhibit cell proliferation of lung in animal exposed with benzopirena.

Edible packaging from durian seeds starch - chitosan can be used in food products to maintain quality [12]. Edible packaging can only maintain the quality of food products by preventing oxidation, but pathogen bacteria can damage edible packaging. So that the supplementation of antimicrobial active ingredients is needed. Therefore, supplementation with kesum leaf extract is expected to enhance the effectiveness of durian seed starch - chitosan as matrix-forming film, antimicrobial and antioxidant for food products. The purpose of this study was to evaluate the effect of kesum leaf extract on the physical properties of edible film of durian seed starch - chitosan.

MATERIALS AND METHODS

Materials

The reagents and solvents used in this study (glycerol, ethanol, acetic acid, sodium hydroxide, hydrochloric acid) were purchased from Merck (Germany), and were used without prior purification. The aquadest distilled water was obtained from Waterone (Indonesia). The materials used in this study were shrimp shells, durian seed, oven (Memmert UN55 Oven, Germany), evaporator (Rotary Evaporator IKA RV 10 Digital V, Germany), analytical scales (Analytical Balance ME104E, USA) plastic film printing plates, WVTR plates, universal testing machine (Zwick Z.05 Texture Analyzer, Zwick, Toni Technik, Indentec Ltd., Germany), SEM JSM-6510LA Series (Jeol Ltd., Japan), beaker glass (Pyrex, USA), measuring cup, hot plate stirer (IKA C Mag HS7, Germany), rod mixers and glass cup.

Chitosan preparation

Chitosan was been made using the modified method by Rahayu and Purnavita [13]. The shrimp shells waste was dried, mashed, then sifted with a size of 100 mesh. The shrimp shells were deproteination using 2.0 N NaOH solution with a ratio of 1: 6 (w/v) while stirring and heated at 80 °C for 1 hour. After separating from the solution, the shells were washed with water until they are neutral. They were then dried at a temperature of 70-80 °C for 24 hours in an oven (Memmert UN 55 Universal Oven, Germany). The homogenate from deproteination was demineralized using a 1.5 N HCl solution (ratio 1:12 w/v) and stirred at room temperature for 1 hour. After being filtered, the homogenate was washed with water until neutral, then dried at 70-80 °C for 24 hours in the oven to get dry chitin. The deacetylation process was carried out by boiling chitin in a 50 % NaOH solution with a ratio of 1:20 (w/v) at 90 °C in 120 minutes. The homogenate was then separating with liquid, then washed with aquadest until neutral. After that, the homogenate was dried at a temperature of 70-80 °C in the Memmert oven for 24 hours.

Durian seeds starch preparation

Durian seeds starch was produced according to Maherawati *et al.* method [1]. Durian seeds were cut and weighed 400 g and after addition of 1 L water were blended with a food processor (Panasonic MK-F800, Indonesia) for 5 minutes. After storage for 24 hours and decantation, the durian seed homogenate was filtered using Whatman® filter papers, and then the durian seed homogenate was deposited in 1 L of water for 3 days, replacing the settling water every 24 hours. On the fourth day, the water was removed by filtering using Whatman® and the starch slurry was separated. The starch slurry was dried in oven (Memmert UN 55 Universal Oven, Germany) at 50 °C for 24 hours until moisture content was 12 %. The dried starch deposits were crushed using a Panasonic MK-F800 food processor for 1 minutes and mesh sieved with size 100.

Kesum leaf extracts preparation

Kesum leaf extracts were obtained by using a maceration method with ethanol. Fresh chopped kesum leaves (± 0.5 cm) were weighed. They were soaked with 96 % ethanol at room temperature for 24 hours. The ratio between materials and solvent was 1:50. The filtrate was obtained from the pulp, then it was macerated with ethanol for 24 hours during 4 x 24 hours. Separation of the solvent from filtrate was conducted using rotary vacuum evaporator (Rotary Evaporator IKA RV 10 Digital V, Germany) in 100 rpm at temperature 80°C and the process was terminated after the ethanol was entirely evaporated.

Edible films preparation

Edible films were manufactured from ingredients containing 3 % of durian seeds starch, 0.8 % of chitosan, 0.6 % of glycerol and 120 mL of distilled water. Edible films were produced by using Ban *et al.* [14] method with modifications as follows:

- chitosan was dissolved into 10 % acetic acid;
- durian seed starch and chitosan were weighed and dissolved into 120 mL of distilled water;
- the solution was heated at temperature 70 °C and stirred using hotplate magnetic stirrer until it reached the gelatinization;
- glycerol was added and then mixed;
- after the solution reached cold condition, the kesum leaf extracts in different concentration was added (0 % (K_0); 0.2 % (K_1); 0.4 % (K_2); 0.6 % (K_3); 0.8 % (K_4); 1.0 % (K_5) and 1.2% (K_6)); the solutions were then mixed;
- the solution of edible films was then poured into printing plate and dried in oven at temperature 50 °C for about 20 hours;
- if it reached dry condition, the plastic from the mold was removed.

Tensile strength and elongation

Tensile strength and elongation were measured using the modified method by Kim *et al.* [15] and Xu *et al.* [16] using the Instron Universal Testing Machine (Zwick Z.05 Texture Analyzer, Zwick, Toni Technik, Indentec Ltd., Germany). Cut sample film in the form of dimension I, with a width of 5 mm, length of 50 mm, and thickness is determined based on the mean of measurement. The testing speed is 10 mm/minute, with the distance between the tongs is 50 mm.

$$Elongation\ (\%) = \frac{Maximum\ Length\ (mm) - Initial\ Length\ (mm)}{Initial\ Length\ (mm)} \times 100$$

$$Tensile\ strength\ (MPa) = \frac{Force\ maximum\ (N)}{Surface\ area\ on\ film\ (m^2)}$$

Water vapor transmission rate (WVTR)

The water vapor transmission line (WVTR) is determined gravimetrically modified the method by Xu *et al.* [16]. The film sample to be tested is covered in a cup therein containing 10 g of silica gel, and then, it is placed in a container containing a salt solution NaCl 40 % (w/v) (RH = 75 %) at 25 °C. The diameter of the inner cup is 5 cm, and 2 cm high. The water vapor diffused through the film is absorbed by silica gel, thereby increasing its weight. Cup weight is recorded every hour for 7 hours. Linear regression was used to estimate the slope of this line in $g \cdot h^{-1}$.

$$WVTR = \frac{Slope\ cup\ gain\ weight\ \left(\frac{g}{h}\right)}{Surface\ area\ on\ film\ (m^2)}$$

SEM (Scanning Electron Microscopy)

Microstructure of edible film observed using Scanning Electron Microscopy to Lin *et al*. method [17] with a modification. SEM was performed using JSM-6510LA Series (Jeol Ltd. Japan) to evaluate the microstructures of edible coating. Edible films were firstly dried using freeze drying for 19 hours until their water concentration reached 2 % or less. Edible films were then cut into size of 0.5 x 0.5 cm. After preparation was completed, they were coated using gold (Au) at 2.5 kV and 20 mA for 1.5 min or metal in Magnetron Sputtering Device with a vacuum. SEM JSM-6510LA Series (Jeol Ltd. Japan) has 2 vacuum systems. The samples which have been coated with gold were placed on a sample place and transferred into electron microscopy. The samples were then shot. Lastly, the samples were recorded in the monitor and the results were captured.

Data analysis

Research design using completely randomized design (CRD) with 1 factor treatment of concentration of kesum leaf extract. Data obtained were then analyzed by using one way analysis of variance (ANOVA) and significant differences between the mean then tested by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Edible film microstructure

Scanning Electron Microscopy (SEM) analysis was conducted to determine the surface characteristics of biomaterials, including structure visualization, morphology and dimensions. The morphological analysis of antimicrobial film surface supplemented with different kesum leaf extracts is presented in Figure 1. The results showed that the surface of molecular structure was very tight and not hollow.

Figure 1a revealed the results of SEM analysis for control films in K_0 showing a flat, very dense and non-porous on surface morphology. It indicated that the film matrix had a good texture. As shown in Figures 1b-g, differences in the surface structure were observed in K_1 to K_6 indicating that there was an effect of kesum leaf extracts on films. The surface was not smooth, there were white patches resulted from kesum leaf extract. White patches in K_1 to K_6 seemed uneven. This indicated that supplementation of kesum leaf extracts might affect the active ingredients. Kesum leaf extract contains polar organic compounds, which are only soluble in polar solvents. The solvent of durian seeds starch and chitosan as a matrix constituent films are water and acetic acid. The extraction effectiveness of a particular compound is dependent on the solubility of the compound in a solvent.

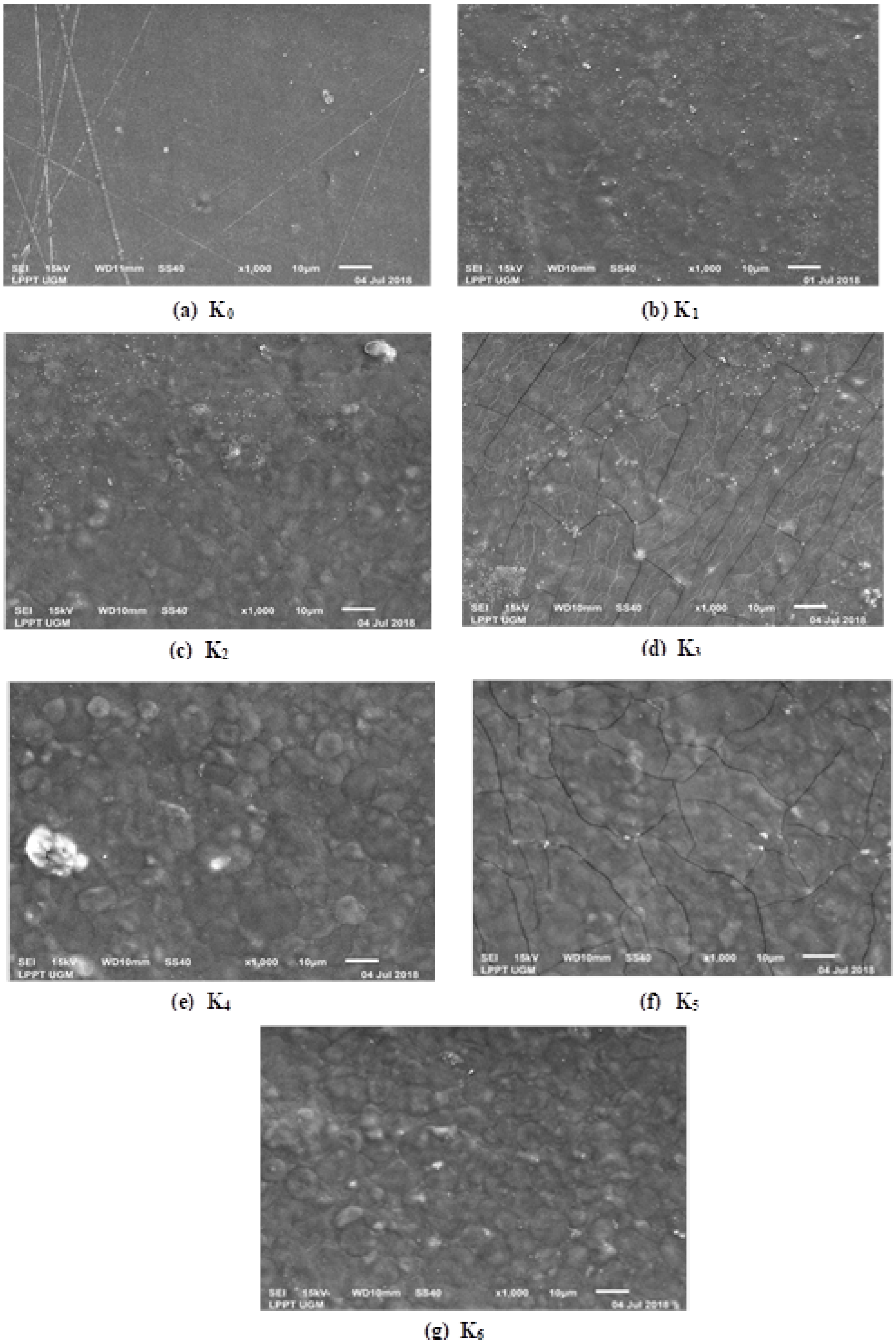

Figure 1. *SEM analysis for edible film surfaces characteristics:*
(a) K_0; (b) K_1; (c) K_2; (d) K_3; (e) K_4; (f) K_5; (g) K_6

The surface characteristics of films were very hollow and uneven, with non-uniform size of granules, as shown in K_4 to K_6. This indicated that the supplementation of kesum leaf extract decreased homogeneity. The supplementation of active ingredients from kesum leaf extracts changed the surface morphology of films. This change will further affect the mechanical properties of films. As reported by Sari *et al.* [18], the supplementation of onion extract decreased the bond strength between amylose

molecules that will further reduce the film matrix density and affect the film porosity. The plasticizer used in film manufacture affects the desired properties. The biopolymes used in film manufacture is durian seed starch-chitosan that are hydrophilic and easy to interact with plasticizers like glycerol.
Meanwhile, the supplementation of active compounds, which has the ability to inhibit the growth of microorganisms is needed to be further investigated its effect on the film characteristics obtained. The polarity effect of each antimicrobial film ingredient must be investigated to gain the maximum film properties and functions, supported by data from mechanical properties such as tensile strength, elongation percentage and Young's modulus.

Water Vapor Transmission Rate (WVTR)

WVTR is an indicator used to evaluate the film permeability towards water vapor or the ability of films to inhibit water vapor transmission. As shown in Figure 2, the various concentrations of kesum leaf extracts did not affect WVTR values.

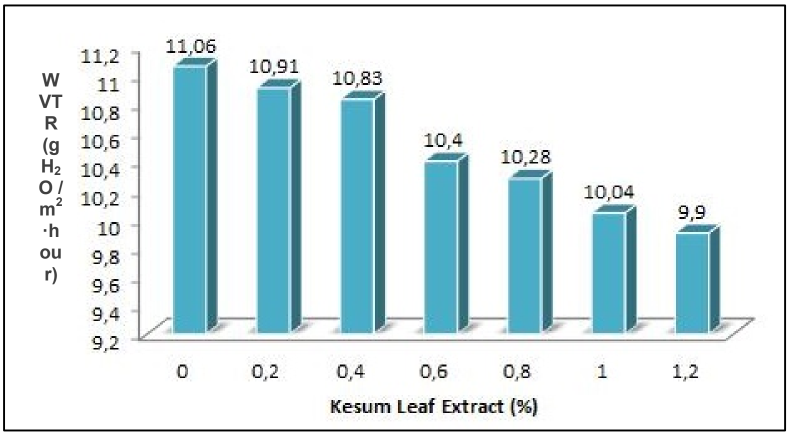

Figure 2. *Effect of kesum leaf extract on edible film water transmission rate*

WVTR analysis of edible film produced from durian seed starch - chitosan with kesum leaf extracts supplementation ranged from 9.90 to 11.06 g $H_2O/m^2\cdot$hour. Generally, WVTR values obtained in this study were quite low. In fact, chitosan is more hydrophobic than starch and this can increase the film's ability as a barrier to water vapor. In this study, the increase of kesum leaf extracts concentration decreased WVTR films. This is because the films is hydrophobic as a result of higher concentration of kesum leaf extract supplemented.
Water vapor migration generally occurs in the hydrophilic film. The ratio between hydrophilic and hydrophobic components in films affects the WVTR value. A greater hydrophobicity of film results in a smaller WVTR value. In addition, a smaller water vapor migration occurring in the product packaged by edible films results in a better properties of edible film in preserving the shelf life of the product packed [2].

Tensile strength

Tensile Strength is measured as the maximum force required to break edible film. The results showed that kesum leaf extract supplementation significantly affected the tensile strength of films. As shown in Figure 3, the values of tensile strength ranged between 26.05 MPa and 29.61 MPa and the highest value was found in kesum leaf extracts at 1.2 % level with value of 29.61 MPa.

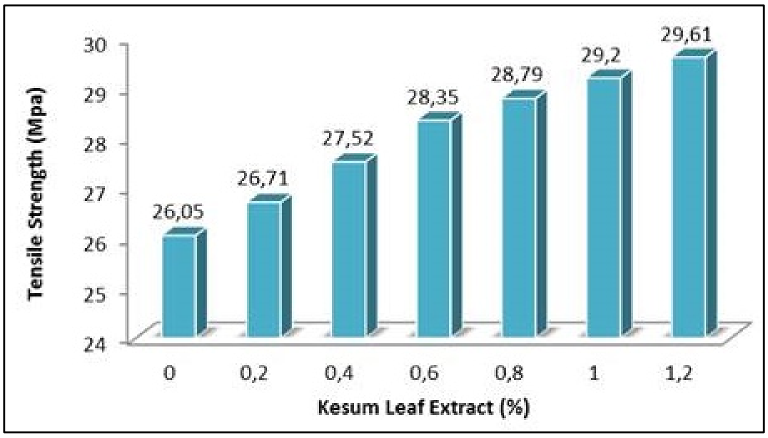

Figure 3. *Effect of kesum leaf extract on edible film tensile strength*

Tensile strength of films is influenced by strength of ingredients used in film manufacture. Manuhara *et al*. [19] reported that mechanical properties of film depend on the strength of materials used in film manufacture. A high chitosan supplementation with a fixed volume increases the number of chitosan molecules resulting in higher strength of films. BNJ analysis showed that tensile strength of films made from starch - chitosan was generally increased, but the elongation was decreased, indicating that the films is more powerful because of chitosan supplementation.

Elongation

Elongation is the maximum extension percentage that an edible film can achieve before it is finally broken/torn. The results showed that kesum leaf extract had no significant effect on film elongation properties. As shown in Figure 4, the values of elongation ranged from 1.803 to 2.227 %.

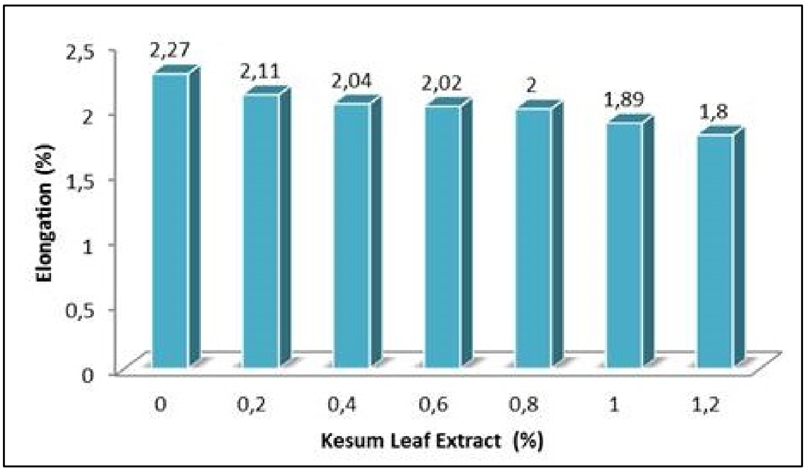

Figure 4. *Effect of kesum leaf extract on edible film elongation*

The highest value for elongation was found in K_0 (0 % KLE) group, while the lowest value was obtained in K_6 (1.2 % KLE) group. This result was in contrast with the values of tensile strength, in which the highest tensile strength was found in f6, while the lowest was found in f_0.
As reported by Setiahadi [20] the increase in tensile strength is usually followed by decrease in elongation values depicted by less elastic films produced. Setiahadi [20] reported that glycerol supplementation will reduce intermolecular forces between molecules in polysaccharide chain, resulting in higher film elongation.
Mean values of film elongation in this study were in agreement with reports by Poeloengasih [21] with elongation values ranging between 1.68 % and 3.48 %. However, the values are higher than reported by Murdianto *et al.* [22] in edible film supplemented with janggelan leaf extract, with range value from 0.14 % to 0.27 %. In this study, we highlight that edible film of durian seed starch - chitosan in combination with kesum leaf extract had relatively high elongation value.

CONCLUSION

According to the results of this study, the surface of SEM edible film in K_0 was flat, very tight and non-porous, while that in K_1 to K_6 was very hollow and uneven, with non-uniform size. Furthermore, KLE supplementation had no significant effect on WVTR and elongation, but it affected tensile strength. The WVTR, tensile strength and elongation of edible films from durian seeds starch-chitosan supplemented with KLE ranged between 9.90 and 11.06 g $H_2O/m^2 \cdot$hour, between 26.05 MPa and 29.61 MPa and between 1.803 and 2.27 %, respectively.

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REFERENCES

1. Maherawati, Lestari, R.B., Haryadi: Karateristik Pati dari Batang Sagu Kalimantan Barat pada Tahap Pertumbuhan yang Berbeda [Characteristics of Starch from Western Kalimantan Sago Trunks at Different Growth Stages] (in Indonesian), *Agritech*, **2011**, **31** (1), 9-13, https://doi.org/10.22146/agritech.9720;
2. García, N.L., Ribba, L., Dufresne, A., Aranguren, M., Goyanes, S.: Effect of Glycerol on the Morphology of Nanocomposites Made from Thermoplastic Starch and Starch Nanocrystals, *Carbohydrate Polymers*, **2011**, **84** (1), 203-210;
3. Pranoto, Y., Salokhe, V.M., Rakshit, S.K.: Physical and Antibacterial Properties of Alginate-Based Edible Film Incorporated with Garlic Oil, *Food Research International*, **2005**, **38** (3), 267-272;
4. Seydim, A.C., Sarikus, G.: Antimicrobial Activity of Whey Protein-Based Edible Films Incorporated with Oregano, Rosemary and Garlic Essential Oil, *Food Research International*, **2006**, **39** (5), 639-644;

5. Maizura, M., Fazilah, A., Norziah, M.H., Karim, A.A.: Antibacterial Activity and Mechanical Properties of Partially Hydrolyzed Sago Starch–Alginate Edible Film Containing Lemongrass Oil, *Journal of Food Science*, **2008**, **72** (6), C324-C330;
6. Siripatrawan, U., Noipha, S.: Active Film from Chitosan Incorporating Green Tea Extract for Shelf Life Extension of Pork Sausages, *Food Hydrocolloids*, **2012**, **27** (1), 102-108;
7. Krkić, N., Šojić, B., Lazič, V., Petrović, L., Mandič, A., Sedej, I., Tomović, V.: Lipid Oxidative Changes in Chitosan-Oregano Coated Traditional Dry Fermented Sausage Petrovská klobása, *Meat Science*, **2013**, **93** (3), 767-770;
8. Utami, R., Kawiji, Khasanah, L.U., Narinda, A.H.: Pengaruh Oleoresin Daun Jeruk Purut (Citrus hystrix DC) pada Edible Coating terhadap Kualitas Sosis Sapi Beku [The Effect of Kaffir Lime (*Citrus hystrix DC.*) Refrigated Leave Oleoresin on Edible Coating to Beef Sausage Quality] (in Indonesian), *Jurnal Teknologi Industri Pertanian (Journal of Agroindustrial Technology)*, **2015**, **25** (2), 116-124;
9. Wibowo, M.A.: Uji Antimikroba Fraksi Metanol dan Dietil Eter Daun Tanaman Kesum [The Antimicrobe Test of Fraction Methanol and Diethyl Ether of Kesum Leaves] (in Indonesian), *Agripura*, **2009**, **3** (2), 410-414;
10. Baharum, S.N., Bunawan, H., Ghani, M.A., Mustapha, W.A.W., Noor, N.M.: Analysis of the Chemical Composition of the Essential Oil of *Polygonum Minus* Huds. Using Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry (GC-TOF MS), *Molecules*, **2010**, **15** (10), 7006-7015;
11. Mackeen, M.M., Ali, A.M., El-Sharkawy, S.H., Manap, M.Y., Salleh, K.M., Lajis, N.H., Kawazu, K.: Antimicrobial and Cytotoxic Properties of Some Malaysian Traditional Vegetables (Ulam), *International Journal of Pharmacognosy*, **1997**, **35** (3), 174-178;
12. Pratomo, P., Lestari, R.B., Raharjo, D.: Aplikasi Edible Film Pati Biji Durian dengan Penambahan Kitosan Sebagai Bahan Pengemas Primer Dodol Lidah Buaya Selama Penyimpanan (in Indonesian), *Jurnal Sains Mahasiswa Peternakan*, **2015**, **4** (2), 5 pages;
13. Rahayu, L.H., Purnavita, S.: Optimasi Pembuatan Kitosan Dari Kitin Limbah Cangkang Rajungan (*Portunus pelagicus*) Untuk Adsorben Ion Logam Merkuri (in Indonesian), *Reaktor,* **2007**, **11** (1), 45-49;
14. Ban, W., Song, J., Argyropoulos, D.S., Lucia, L.A.: Improving the Physical and Chemical Functionally of Starch-derived Films with Biopolymers, *Journal of Applied Polymer Science*, **2006**, **100** (3), 2542-2548;
15. Kim, K.W., Ko, C.J., Park, H.J.: Mechanical Properties, Water Vapor Permeabilities and Solubilities of Highly Carboxymethylated Starch-Based Edible Film, *Journal of Food Science*, **2002**, **67** (1), 218-222;
16. Xu, Y.X., Kim, K.M., Hanna, M.A., Nag, D.: Chitosan-Starch Composite Film: Preparation and Characterization. *Industrial Crops and Products,* **2005**, **21** (2), 185-192;
17. Lin, S., Huff, H.E., Hsieh, F.: Extrusion Process Parameters Sensory Characteristics, and Structural Properties of A High Moisture Soy Protein Meat Analog, *Journal of Food Science*, **2002**, **67** (3), 1066-1072;
18. Sari, Y.W., Syafitri, U., Sanders, J.P.M., Bruins, M.E.: How Biomass Composition Determines Protein Extractability, *Industrial Crops and Products*, **2015**, **70**, 125-133;
19. Manuhara, G.J., Kawiji, Retri, H.: Aplikasi Edible Film Maizena dengan Penambahan Ekstrak Jahe sebagai Antioksidan Alami pada Coating Sosis Sapi [Application of Edible Film from Maizena with Ginger Extract as Natural Antioxidant on Beef Sausage Coating] (in Indonesian), *Jurnal Teknologi Hasil Pertanian*, **2009**, **2** (2), 50-58, http://dx.doi.org/10.20961/jthp.v0i0.12868;
20. Setiahadi, E.: *Preparation and Characterization of Composite Edible / Biodegradable Film Sodium Carboxymethylcellulose Cavendish Banana Stem Semu (Moses cavendishii Lambert ex Paxton)* (in Indonesian), Masters Thesis, Graduate School of UGM Yogyakarta, **2005**;
21. Poeloengasih, C.D.: *Characterization of Composite Edible Films Winged Seed Protein (Psophocarpus tetragonolobus (l., dc) and Tapioca* (in Indonesian), Master Thesis, Graduate School of UGM Yogyakarta, **2002**;
22. Murdianto, W., Marseno, D.W., Haryadi: Sifat Fisik dan Mekanik Edible Film dari Ekstrak Daun Janggelan (*Mesona palustris* BI) [Physical and Mechanical Properties of Edible Film from Janggelan Leaf Extract (*Mesona palustris* Bl)] (in Indonesian), *Agrosains*, **2005**, **18** (3), 353-362.