

THE PHYSICOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF GALACTOMANNAN EDIBLE FILM OF *ARENGA PINNATA* INCORPORATED WITH *ZINGIBER OFFICINALE* ESSENTIAL OIL

JULIATI BR TARIGAN*, IRWANA NAINGGOLAN, JAMARAN KABAN

Department of Chemistry, University of Sumatera Utara, Medan, Indonesia. Email: juliati@usu.ac.id

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ABSTRACT

Objective: This study aimed to demonstrate the incorporation of *Zingiber officinale* essential (ZOE) oil onto galactomannan from *Arenga pinnata* (GAP) matrix and determined the antibacterial activity of the edible films (EF).

Methods: EF was obtained from the incorporation of GAP (0.5, 0.9, or 1.3 g) with ZOE (0.5 or 1 g) using glycerol (0.6 g) and monoglycerol oleic (0.2 g) as a plasticizer.

Results: The thickness of the films increased with the increase of GAP and ZOE oil. However, the tensile strength and water permeability decreased in the fourth EF when the ZOE oil was increased. The maximum value of tensile strength and modulus elongation was obtained with the ratio of GAP to ZOE oil at 0.9, 0.5, 4.502 MPa, and 0.0633 MPa, respectively. The Fourier transform infrared spectrum showed that the interaction occurred between GAP and ZOE oil. The EFs showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Saccharomyces cerevisiae* with the highest activity in the third EF. The total bacteria amount in a colony decreased until the 5th day compared with the control. Furthermore, the third EF could inhibit oxygen migration with a respiratory quotient of 7.71.

Conclusions: This study revealed that EFs from GAP and ZOE could be prepared and have antibacterial activity against several pathogenic bacteria.

Keywords: Edible film, Galactomannan, *Arenga pinnata*, *Zingiber officinale*, Essential oil, Antibacterial.

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INTRODUCTION

Recently, there is increasing interest in the identification of the properties of antibacterial products from natural sources, which are usually used in the preservation of food or health food. The edible film (EF) could give extra protection to food and also has some advantages such as biodegradability and environmental friendliness. The EF can be used to enhance food quality and keep the food fresh in storage. Some compounds, for example, antioxidants, antimicrobials, and flavor can be added to the EF, which can increase its stability, quality, functionality, and safety of the food [1-5]. Based on our research, an EF from galactomannan of *Arenga pinnata* (GAP) incorporated with the essential oil of *Ocimum basilicum* has antioxidant and antibacterial properties [6].

Based on our knowledge, only limited research exists about the characterization and application of galactomannan as EF and coating compared with other polysaccharides. However, current research on galactomannan has shown a new perspective about the properties and usage of galactomannan for EF and coating, based on the specific property of galactomannan solution which forms a viscous liquid with water even in low concentrations [7-9].

One of the galactomannan sources is *A. pinnata* or "kolang-kaling" in Indonesia. Galactomannan contained in *A. pinnata* is 4.58% (w/w) with antioxidant activity (IC_{50}) of 20.45 ppm based on the DPPH method [10]. However, utilization of GAP for EF and coating remains limited. Extract of ZOE oil extracts, water extract, and residue extract has been tested for their antioxidant properties using the DPPH method [10]. ZOE extract can be produced using the hydrodistillation method [11,12] and has been identified as having positive antimicrobial activity against bacteria such as *B. subtilis*, *Staphylococcus aureus*, and *K. pneumonia*, and fungi such as *Aspergillus niger*, *Penicillium notatum*, *Mucor hiemalis*, and *Fusarium oxysporum* [13]. However, to the best of

our knowledge, ZOE has never been incorporated into GAP to form an EF. The physicochemical properties of EF from GAP incorporated with ZOE have to be tested before the EF is applied to food.

To address this issue, we studied the physicochemical properties of EF from GAP incorporated with ZOE. The thickness of the films, water permeability, thermal decomposition, tensile strength, surface morphology, and antibacterial activity was also studied against seven bacteria. Antibacterial activity against seven bacteria was conducted using *Oreochromis niloticus* fish and determined using the standard plate count method.

MATERIALS AND METHODS

Materials

The materials used in this study were galactomannan extracted from *A. pinnata* and *Zingiber officinale*, which were obtained from a local market in Medan, North Sumatera, Indonesia. Glycerol (99.5%) was obtained from the oleochemicals industry in Medan, and calcium chloride was obtained from Merck.

Methods

Preparation of GAP and ZOE

Extraction of GAP in neutral conditions was based on the method of a previous researcher with slight modification [7]. The result of this process has been published [6]. The ZOE used in this study was obtained according to the following method which has been published [13]. The chemical composition of ZOE also has been published.

Preparation of EF from GAP incorporated with ZOE

The EF preparation was performed as described in Cerqueira *et al.* with some changes [7]. The film was prepared using various quantities of galactomannan (0.5, 0.9, and 1.3 g) and ZOE (0.5 and 1.0 g) while the

amounts of glycerol at 0.6 g and monoglycerol oleic (MGO) at 0.2 g were the same for each film. The formulation for each film is described in Table 1. Galactomannan solution was prepared by dissolving galactomannan with water in a 100 mL volumetric flask at room temperature, followed by the addition of glycerol and MGO. After stirring for 2 h, the appropriate weight of ZOE was added and stirred for 5 min. Then, 75 mL of solution was poured onto a glass plate (13 cm × 13 cm) and dried in an oven at 35°C for 20 h and then stored in a desiccator before testing. The physicochemical properties of the EF were determined according to the thickness, water permeability, tensile strength, thermal decomposition, and Fourier transform infrared (FT-IR).

Thickness studies

The thicknesses of the EF were tested using a micrometer (Tricle brand) by measurement at five different points randomly. The mean of the thickness was used to determine water vapor permeability or water permeability transfer rate.

Water vapor permeability studies

Water vapor permeability was determined based on the ASTM E96-00 (ASTM 2000b) method with slight modification and based on a gravimetric test [7]. Cell permeation was tested using a plastic material with a diameter of 4.4 cm and height of 3 cm, containing 25 g of silica gel (0% of humidity and 0 Pa of pressure). Each cell was perforated with a 1.3 cm diameter hole onto which EF was pasted. Next, the cell was stored in a desiccator containing water distillate to form 100% humidity and water vapor pressure of 2337 Pa at 20°C. Each cell was weighed every 2 h for 12 h. Then, the slope was measured based on the increase of silica to time with regression. Each experiment was performed in two replicates. The water vapor transmission rate (WVTR) was calculated based on equation below [14].

$$WVTR = \left(\frac{\Delta W}{\Delta t \cdot A} \right) kg \cdot s^{-1} \cdot m^{-2}$$

Where, DW/Dt = the amount of water absorbed per time

Antimicrobial activity

The antimicrobial activity test of the EF from GAP incorporated with ZOE was conducted following the method described in previous research [6]. All the films were cut aseptically with a diameter of films were 6 mm [6].

Table 1: Formulation of EF from GAP incorporated with ZOE

EF	Formulation			
	GAP (g)	ZOE (g)	Glycerol (g)	MGO (g)
EF ₁	0.5	0.5	0.6	0.2
EF ₂	0.5	1.0	0.6	0.2
EF ₃	0.9	0.5	0.6	0.2
EF ₄	0.9	1.0	0.6	0.2
EF ₅	1.3	0.5	0.6	0.2
EF ₆	1.3	1.0	0.6	0.2

EF: Edible film, GAP: Galactomannan from *Arenga pinnata*, ZOE: *Zingiber officinale* essential

Table 2: Thickness, water vapor permeability, tensile strength, and thermal decomposition of GAP EF

Parameter	Sample					
	EF ₁	EF ₂	EF ₃	EF ₄	EF ₅	EF ₆
Thickness of film (×10 ⁻² mm)	3.2	3.8	6.0	6.4	7.8	8.2
WVP (×10 ⁻⁹ kg/s/m/Pa)	3.483	4.665	6.606	6.398	0.721	0.796
Tensile strength (MPa)	20.400	12.280	4.502	2.600	1.920	3.860
Elongation (%)	1200.28	70.050	71.092	55.350	27.610	48.560
Endothermic (°C)	80	80	80	80	80	80
Exothermic (°C)	315	305	325	315	330	325

EF: Edible film, GAP: Galactomannan from *Arenga pinnata*

Determination of bacteria using standard plate count method

The *O. niloticus* fish bought from the local market was cleaned and cut into small sizes to weigh 1 g each and wrapped with EF₃ and ZOE (0.5% in DMSO). The sliced, wrapped fish was stored in plastic containers at 5–10°C for 10 days, with an unwrapped fish as a control. The standard plate count method was used to determine the amount of bacteria growth on day 0, 1, 2, 3, 5, and 9 using a colony counter. Each sample was put into a tube and 10 mL of distillate water was added. Next, the sample was diluted to 10,000 times with distillate water and 10 mL PCA media was added. 1 mL of sample was then poured into a tube and vortexed for 1 min, then poured into a Petri dish and homogenized by shaking. The density of the bacterial cell was counted after incubation for 24 h at 37°C using the equation below [15].

$$\sum \text{Cell} = \sum \text{colony} \times \frac{1}{\text{dillution factor}}$$

Respiration rate analysis

The respiration rates of fresh *O. niloticus* fish wrapped and unwrapped were conducted using a respiratory chamber equipped with a rubber stopper. Approximately 200 g of wrapped and unwrapped fish was placed in the chamber, and the chamber lid was sealed with vacuum grease to avoid in/out O₂ and CO₂ gases. The chamber was stored at 10°C. For determination of O₂ and CO₂ gases concentration, two holes were made using plastic pipe. The concentration of gases was determined using a cosmotector and calculated using the equation below.

$$R_r = \frac{10^3 \times M_w \times \frac{\Delta C}{100} \times \left(V - \frac{W}{A} \right)}{R \times W \times \Delta T \times (273 + t_0)}$$

Where,

R_r = Production rate of CO₂ or consumption rate of O₂ (ml/kg-jam)

M_w = Molecular weight (CO₂ = 44 and O₂ = 32)

ΔC = Deviation concentration of O₂ or CO₂ (%) between two measurements

V = Container volume (l)

R = Constanta gas (0.0821 dm³.atm/K/mol)

W = Weight of sample (kg)

σ = density (kg/L)

t₀ = Storage temperature (°C)

ΔT = Observation interval (jam)

Stress-strain studies

Stress studies were performed by applying a stress instrument to the EF with specific thickness and size. First, the instrument was set up with a load of 100 kgf with stress rate of 50 mm/min and then the film was clamped on that instrument. The film was pulled until broken and the maximum stress (F_{max}) and strain were observed. The strain data were converted to tensile strength (δ) and elongation (ε).

RESULTS AND DISCUSSION

The physicochemical properties of EF from GAP are shown in Table 2. The thickness of the EF was measured using a micrometer, and the

water vapor permeability was calculated based on regression values of the different weights of the desiccant with time, tensile strength, and thermal decomposition of GAP EF.

Water vapor permeability is one of the most important properties which influence the utilization of EF as water could deteriorate the sample [8]. As shown in Table 2, the values of WVP changed with the increasing concentration of GAP in the films at the same concentration of glycerol and MGO. However, further increase of GAP concentration decreased the WVP values quickly, which was presumably due to the increase of a gel network which made the polysaccharide become dense rendering the formation of a more cohesive film structure [9].

The EF production from GAP incorporated with ZOE resulted in six different films: EF₁-EF₆. The thicknesses of the EF were determined at five different points using a micrometer, and the means were calculated in the range of 0.032-0.082 mm, which shows that increasing the amount of GAP and ZOE increased the thickness of the film. The thickness of the GAP EF occurred in the range of 0.032-0.082 mm. This result is similar to the results of other researchers who reported that the hydrophobic addition could reduce WVTR/WVP value [16].

Thermal properties of all EF showed an endothermal temperature of 80°C, which means that the amount of GAP and ZOE does not affect water release from the film. We assumed that the small amount of compound used affects the interaction between water and galactomannan, which remained stable even with the appearance of hydroxyl, aldehyde, and alkene functional group from ZOE. The samples of EF₁-EF₆ were degraded at a temperature above 300°C, which could be used for food that is produced at high temperature. In addition, exothermal temperature decreased with the increase of ZOE, but the effect was contrary to the increase of galactomannan.

Tensile strength and elongation were also affected by compounds in the films, as both values decreased with the addition of ZOE. This was because ZOE acted as a plasticizer which makes the structure became rigid, increasing the mobility, and decreasing crack resistance [7].

Each EF was identified using FT-IR to find out the changes of functional group in galactomannan when interacting with ZOE. Fig. 1 shows the FT-IR spectrum of EF number 3 and GAP. FT-IR was used in this research to study the interaction between two or more compounds. Absorbance data of specific wavelength could give quantitative data [17].

The spectra of galactomannan showed characteristic peaks which were similar to previous reports [6,18]. The stretching vibration peak at 3310 cm⁻¹ is related to OH groups from polysaccharide, while the peak at 2919 cm⁻¹ belongs to the characteristic absorption of stretching -C-H from -CH₂. The peak at 1640 cm⁻¹ represents the polysaccharide bond. The broadband in the area of 900-1200 cm⁻¹ was due to stretching vibration of -C-C-O, C-OH, and C-O-C from the main polymer chain [19]. The peak ascribed to bending vibration C-O from pyranose ring was recorded at 1146 cm⁻¹ while the peak at 871 cm⁻¹ was characteristic to β-D-mannopyranose, and at 813 cm⁻¹ represented the α-D-galactopyranose bond [19]. The FT-IR spectra of GAP and EF₃ were almost similar, which means there was no change in the functional group after mixing. However, there were appreciable changes in the EF₃ spectra in the appearance of a peak at 1735 cm⁻¹ which is related to the stretching vibration of C=O group from ZOE. Furthermore, some differences were also observed after film formation in which the medium absorbance peak of OH group at 3000-3500 cm⁻¹ and the sharp peak of C-OH at 900-1200 cm⁻¹ became weak absorbance, which was probably due to interaction compound between GAP and ZOE [20].

SEM was used to investigate the morphological image of the EF as shown in Fig. 2. The SEM images of the EF, formed from a mixture of GAP, MGO, and ZOE, revealed some changes in the film surface. The smooth surface, as shown in Fig. 2c, was due to the addition of MGO to

the EF rendering a stable film with the ZOE distributed evenly on the surface of the film.

Antibacterial activity

Gupta and Ravishankar reported that ginger paste has antimicrobial activity against *Escherichia coli* [21]. Based on that, the antibacterial activity of the EF and ZOE was studied using fresh *O. niloticus* fish with the standard plate count method. As shown in Fig. 3, the *O. niloticus* fish was used to determine the ability of the EF and ZOE to inhibit bacterial

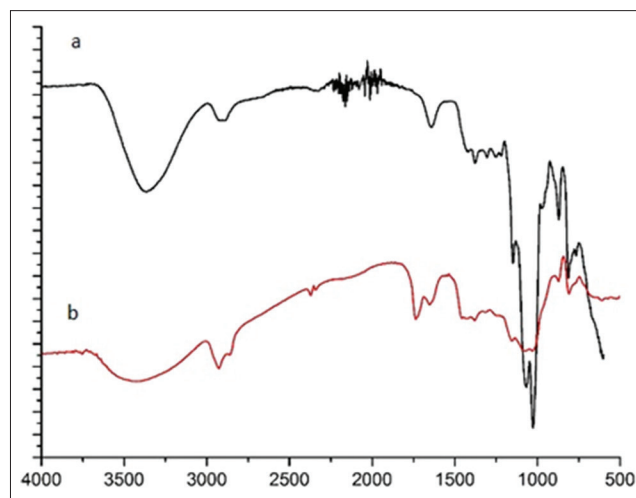


Fig. 1: The Fourier transform infrared spectrum of galactomannan from *Arenga pinnata* (a) and edible film₃ (b)

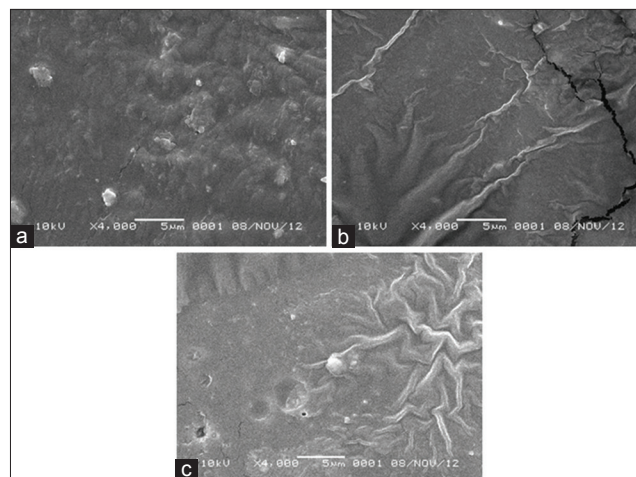


Fig. 2: The image of surface morphology of galactomannan from *Arenga pinnata* (GAP) edible film. (a) SEM EF of GAP + glycerol (0.9 g + 0.6 g), (b) SEM EF of GAP + glycerol + *Zingiber officinale* essential (ZOE) (0.9 g + 0.6 g + 0.5 g), (c) SEM EF galactomannan + glycerol + MGO + ZOE (0.9 g + 0.6 g + 0.2 g + 0.5 g)

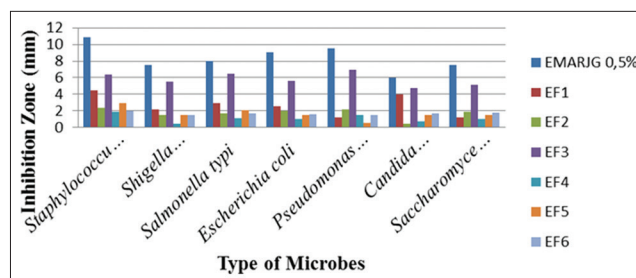


Fig. 3: Antibacterial activities of the edible film (EF₁-EF₆)

growth *in vivo*. The results in Fig. 4 showed that the amount of bacteria until day 5 remains below the standard value compared to the control sample, which means that both EF and ZOE have antimicrobial activities. The lower amount of bacteria compared to the control demonstrated that the active compound contained in the EF and ZOE was released starting from day 1 to day 5. The bacteria amount increased after day 5, which was due to the decrease of the concentration of the active compound in both the EF and ZOE. Therefore, it can be concluded that the EF and ZOE have antimicrobial activity and could inhibit bacterial until day 5.

Respiration rate

Respiration rate is an important factor in the future application of EF [7]. The respiration rate is usually determined by measuring the consumption rate of O₂ or the production rate of CO₂. The respiration

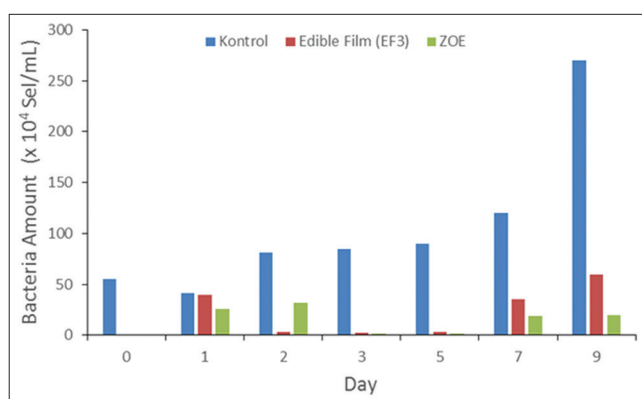


Fig. 4: Estimation of the density of bacteria using standard plate count method

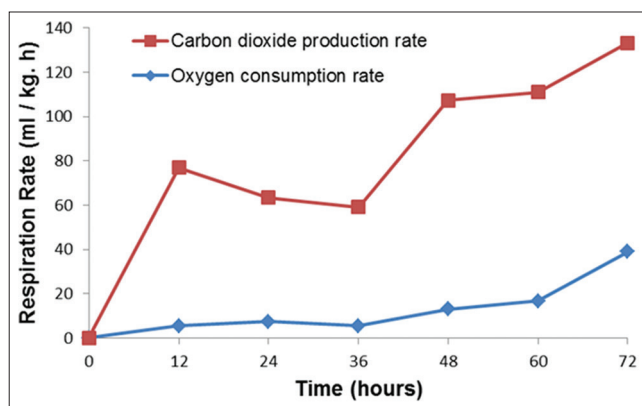


Fig. 5: Respiration rates of unwrapped *Oreochromis niloticus* fish

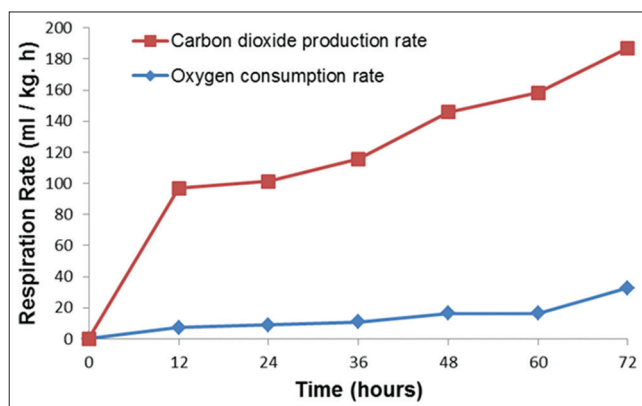


Fig. 6: Respiration rates of wrapped *Oreochromis niloticus* fish

rates of oxygen and carbon dioxide of EF wrapped around fresh *O. niloticus* fish with the determined interval of time at 12 h, at 10°C storage temperature, are presented in Figs. 5 and 6. Significantly different results were found in the oxygen consumption of wrapped and unwrapped fresh *O. niloticus* fish. The wrapped fish sample had a low oxygen consumption rate compared to the unwrapped sample, while the carbon dioxide production rate remained similar for both samples. The lowest oxygen consumption rate was presumably due to the raw material used for the production of the EF, hydrocolloid, which can inhibit oxygen migration. The respiratory quotient (RQ) of the EF was 7.71, which came from the carbon dioxide production rate divided by the oxygen consumption rate, at 101.812 mL/kg hour and 13.204 mL/kg hour, respectively. The RQ value of more than 1 means that substrate is containing oxygen, such as organic acid, which was used in respiration [22].

It can be concluded that the addition of MGO in the preparation of GAP EF makes the surface of the film smooth. ZOE can decrease WVP and the tensile strength of GAP EF. Especially in EF₄, ZOE was able to affect the formation of FT-IR wavelength at 3100–3500 cm⁻¹, 1635 cm⁻¹, and 800–1200 cm⁻¹. The results of the antibacterial activities against seven bacteria showed that the films (EF₂, EF₃, and EF₄) had growth inhibition of *S. aureus*.

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AUTHORS' CONTRIBUTIONS

Juliati Br. Tarigan (JBT), Irwana Nainggolan (IN), and Jamaran Kaban (JK) conducted the experiments. JBT wrote and edited the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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