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Development and characterization of active edible films based on *Pangasius* sp. skin gelatin and carrageenan enriched with avocado peel essential oil

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ABSTRACT: Growing environmental concerns have led to the development of biodegradable alternatives to synthetic polymers. Edible films for food packaging can be formulated from various biopolymers, including gelatin, which is known for its excellent film-forming properties but exhibits poor water vapor resistance. Incorporating κ-carrageenan may enhance the mechanical properties of gelatin films, while avocado peel essential oil could provide antioxidant and antimicrobial functionalities, improving the overall quality of the packaging material. This study aimed to develop and characterize gelatin-based edible films derived from catfish (*Pangasius* sp.) skin, incorporating κ-carrageenan and avocado peel essential oil. The research involved gelatin extraction, film formulation, and evaluation of physicochemical properties. The extracted gelatin had a moisture content of 9.74%, an ash content of 0.55%, a pH of 5.81, and a viscosity of 19 mPas, meeting the SNI 8622:2018 standards. GC-MS analysis identified 22 compounds in the avocado peel essential oil, predominantly terpenoids. The essential oil exhibited antibacterial activity against *Staphylococcus aureus* (12.70 ± 0.38 mm) and *Escherichia coli* (5.94 ± 0.13 mm) and demonstrated potent antioxidant activity with an IC_{50} value of 7.58 mg/l. Incorporating avocado peel essential oil affected the physical properties of the gelatin carrageenan films, decreasing tensile strength, water vapor transmission rate, and brightness index, while increasing elongation at break, film thickness, yellow index, and clarity index. These findings suggest that gelatin-based films enriched with κ-carrageenan and avocado peel essential oil have potential as biodegradable active packaging materials.

KEYWORDS: edible film, gelatin, carrageenan, avocado peel essential oil

INTRODUCTION

Pangas catfish (*Pangasius* sp.) production has increased by 21.08% from 340.44 tons in 2022 to 431.38 tons in 2023 [1]. This growth was accompanied by a proportional increase in processing by-products, including skin, head, bones, and stomach contents [2]. These by-products generally have low economic value, and if not properly managed, may contribute to environmental pollution. Among them, pangasius skin, which constitutes approximately 5.12–6.14% of the raw material weight, has the potential to serve as a valuable source of gelatin, holding a high economic potential [3, 4].

Gelatin is a by-product derived from the hydrolysis of collagen, which is primarily found in animal bones and skin. Both gelatin and collagen are considered halal-sensitive ingredients, as they are commonly sourced from either bovine or porcine origins [5]. Fish skin has been identified as a valuable alternative source of halal collagen and gelatin, offering comparable quality to bovine-derived counterparts, without the associated risk of transmitting bovine spongiform encephalopathy (BSE). Due to its high digestibility, gelatin is widely used in the production of edible films that can be consumed alongside packaged food products [6].

Edible packaging refers to materials intended for consumption produced from food-grade biopolymers, such as lipids, proteins, and polysaccharides. These biopolymers are obtained from plant, animal, or marine sources or as by-products of food processing [7]. Edible films function as carriers of active compounds, including antioxidants, antimicrobials, colorants, and flavorings [8]. The inclusion of antioxidant compounds in edible films and coatings primarily inhibits lipid oxidation, delays the development of off-flavors, and enhances color stability. On the other hand, antimicrobial agents enhance food safety and prolong shelf life by inhibiting or preventing the growth of spoilage and pathogenic microorganisms on food surfaces [9].

The volume of biowaste is increasing annually due to high consumption in local markets, agriculture, and manufacturing sectors. Improper management of biowaste can pose significant risks to the environment and human health. To mitigate these impacts, biowaste has the potential for valorization as a biomaterial in various industrial applications [10]. Avocado (*Persea americana* Mill.) peel essential oil has emerged

as a promising active compound for incorporation into edible films aimed at producing active packaging. Avocado peels and seeds are rich in bioactive phytochemicals, including phenolic acids, condensed tannins, and various flavonoids such as procyanidins, flavonols, hydroxybenzoic acids, and hydroxycinnamic acids [11]. These bioactive compounds exhibit both antioxidant and antimicrobial properties, making avocado peel a suitable natural additive for packaging applications.

Protein-based edible films provide effective barriers to gases, organic vapors, and oils. However, their application in food packaging is limited because of their relatively poor mechanical strength and high-water permeability [12]. Carrageenan has been identified as a promising biomaterial for the development of food packaging films owing to its favorable mechanical properties, water vapor barrier capacity, surface hydrophobicity, light-blocking characteristics, and thermal stability [13]. In addition, carrageenan contributes to antimicrobial activity in active packaging, particularly against *Salmonella* spp. and *E. coli* [14]. The present study aimed to develop and characterize an active edible film based on catfish skin gelatin supplemented with carrageenan and avocado peel essential oil.

MATERIALS AND METHODS

Materials

The materials used in this study including fresh pangas catfish (*Pangasius* sp.) skin was purchased from a local fish processing in Sidoarjo, Indonesia. Avocado (*P. americana*) peel was collected from several avocado juice sellers in Malang, Indonesia. Glacial acetic acid (CH_3COOH; Merck, Darmstadt, Germany), distilled water, sodium hydroxide (NaOH; Merck), sodium metabisulfite (Merck), carrageenan (Sigma-Aldrich, St. Louis, MO, USA), glycerol, anhydrous sodium sulfate (Na_2SO_4; Merck), and 70% ethanol (Merck) were of analytical grade.

Gelatin preparation

Gelatin was prepared according to a modified method of Nurdiani et al [6]. Fresh pangas catfish fish skin was cut into 2 × 2 cm pieces and soaked in 0.1 M NaOH at a ratio of 1:5 (w/v). The alkaline solution was replaced every hour during the soaking process. The skin was then rinsed thoroughly with running water until it reached neutral pH (pH 7). Subsequently, the skin was soaked in 0.6 M acetic acid (CH_3COOH) at a ratio of 1:5 (w/v) for 2 h and again rinsed with running water until a neutral pH was achieved. Extraction was performed by immersing the pretreated skin in distilled water at a ratio of 1:3 (w/v) and heating at 55 °C for 4 h in a water bath. The extract was filtered using Whatman No. 42 filter paper to obtain a gelatin filtrate, which was then dried in a dehydrator at 60 °C for 12 ht. The resulting gelatin sheets were ground into a powder using a grinder.

Avocado peel essential oil extraction

Avocado peel essential oil was extracted according to the method of Kamara et al [15], with some modifications. Avocado peels were cut into 2 × 2 cm pieces, and 500 g of the sample was placed in a distillation flask along with 375 ml of distilled water. The flask was connected to a distillation apparatus, and the distillation process was initiated. During distillation, the volume of distilled water was maintained by periodic addition of water. The resulting distillate was collected, and NaCl was added to facilitate phase separation between the oil and aqueous layers using a separating funnel. The oil phase was then treated with anhydrous Na_2SO_3 to remove residual moisture and filtered. The refractive index of the oil was measured, and the drying process with Na_2SO_3 was continued until a constant refractive index was achieved. The final avocado peel essential oil was weighed and subjected to further analyses.

Characterization of gelatin and avocado peel essential oil

Gelatin characterization includes the determination of moisture and ash content according to AOAC methods [16], pH measurement using a pH meter, and viscosity analysis using a viscometer. Avocado peel essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS) (GCMS-QP2020 NX, Shimadzu, Kyoto, Japan) to determine its chemical composition [17].

Active packaging formulation

Active packaging was formulated based on a modified method of Fitriyani et al [18]. Gelatin (3 g) was dissolved in 100 ml of distilled water, heated at 60 °C for 15 min, and homogenized using a magnetic stirrer. Carrageenan (0.8 g) was dissolved in 100 ml of distilled water, heated at 70 °C for 15 min, and homogenized in the same manner. The gelatin and carrageenan solutions were combined, heated at 70 °C for 15 min, and stirred continuously. Glycerol (0.5 ml) was added to the mixture as a plasticizer, homogenized, and cooled to room temperature. Avocado peel essential oil was added at concentrations of 0.25, 0.5, 0.75, and 1% (v/v), and the mixture was heated at 45 °C for 15 min with constant stirring. The resulting solution (30 ml) was poured into a baking tray and dried in a dehydrator at 45 °C for 15 h. The resulting edible films were cooled to room temperature (25 °C) for 30 min before further testing.

Physical characterization of active packaging

The physical properties of the active packaging films were evaluated through several tests, including tensile strength and elongation, which were measured using a Universal Testing Machine following ASTM D882^{-1}

(AG-X Plus, Shimadzu, Kyoto, Japan) [19]. In addition, the water vapor transmission rate (WVTR) was measured based on the method of Fahrullah et al [20]. For the WVTR test, the edible film was cut into circular shapes with a diameter of 2.8 cm and used to cover a glass container filled with 3 g of silica gel. The covered glass was then placed in a desiccator, and the weight change was recorded every 24 h over a period of 5 days. The WVTR value was calculated using the following formula: the increase in mass (n, in grams) divided by the product of time (t, in days) and the surface area of the film (A, in mm^2). Folding endurance was assessed by cutting the edible film into samples with a thickness of 2 mm, which were then placed between the thumb and index finger. The film was folded gently, and any visible cracks on the film surface were observed and recorded [20]. Film thickness was measured following the method described by Song et al [19], using a micrometer screw (MDC-25M, Mitutoyo, Kawasaki, Japan) with a precision of 0.001 mm. Measurements were taken at five different points on the film surface, and the average value was calculated. The color characteristics of the edible film were analyzed following the method described by Nurdiani et al [17], using a colorimeter (CS-10, Konica Minolta, Tokyo, Japan) with white as the reference background.

Antibacterial activity of active packaging

Antibacterial activity was evaluated using a modified disk diffusion method, employing edible films as antimicrobial disks, as described by Nurdiani et al [17]. The edible films were cut into discs with a diameter of 0.6 mm and sterilized under UV light for 10 min. Mueller-Hinton Agar (MHA) medium was poured into sterile Petri dishes and allowed to cool and solidify. Bacterial cultures of *S. aureus* and *E. coli* at a concentration of 10^7 CFU/ml were collected using sterile cotton swabs and evenly spread onto the surface of MHA medium. After allowing the plates to rest for a few minutes, the sterilized edible film discs were carefully placed on the agar using sterile tweezers. The plates were incubated in an inverted position at 37 °C for 24 h. Following incubation, the inhibition zones (clear zones) around the edible films were measured using a caliper. The presence of bacterial growth underneath the edible films was also examined and documented.

Antioxidant activity of active packaging

The antioxidant activity of the edible film was assessed based on its ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals [17]. To prepare the sample, the film was dissolved in 95% ethanol. The resulting mixture was homogenized using a vortex mixer and then centrifuged at 4,000 rpm for 10 min to separate the supernatant. Subsequently, 1 ml of 0.2 mM DPPH solution was added to 4 ml of the sample. After being allowed to react for 10 min, the absorbance was measured at 517 nm using a spectrophotometer to determine the antioxidant activity.

Table 1 Physicochemical characteristics of pangas catfish fish skin gelatin compared with the National Indonesian Standard (SNI 8622:2018) and previous studies.

Characteristic	Result	SNI*	Ref.**
a. Chemical			
Moisture content (%)	9.74	< 12	6.43
Ash content (%)	0.55	< 3	0.39
pH	5.81	3.8–7.5	5.56
b. Physical			
Viscosity (mPas/cP)	19	> 15	61.66

* National Indonesian Standard (SNI) 8622:2018 [21].
**Nurilmala et al [22].

Data analysis

The experimental data were analyzed by one-way analysis of variance (ANOVA) using Minitab software version 19. When significant differences were detected ($p < 0.05$), the means were further compared using Tukey's post-hoc test.

RESULTS AND DISCUSSION

The quality of fish skin gelatin is influenced by both physical and chemical parameters, which are at par with the National Indonesia Standard (SNI) 8622:2018 [21] and the results of the study by Nurilmala et al [22] (Table 1). The moisture content of gelatin is associated with its shelf life and can affect the brightness of its appearance [22]. The ash content is one of the indicators used to assess the quality and efficiency of the gelatin extraction process [5]. Additionally, the pH value of gelatin influences its properties, including gel strength and viscosity. Gelatin viscosity plays an important role in determining its functional behavior when applied to food products. Low viscosity may result from reduced amino acid content or be affected by factors such as temperature, extraction time, and acid concentration during processing [23]. Gelatin quality may also be influenced by its molecular weight. Nurilmala et al [22] reported that gelatin derived from *Pangasius* skin pretreated with acid and base solutions prior to extraction exhibited a molecular weight ranging from 128–230 kDa.

GC-MS analysis of avocado peel essential oil

GC-MS analysis identified 22 compound peaks in the avocado peel essential oil (Fig. 1). The most abundant compound accounted for 39.28% of the total peak area and was detected at peak number 14 with a retention time of 38.28 min. This compound was identified as *1,6,10,14,18,22-tetracosahexaen-3-ol*, which belongs to the alcohol group and is a derivative of hydrocarbons. According to Zamakshshar et al [24],

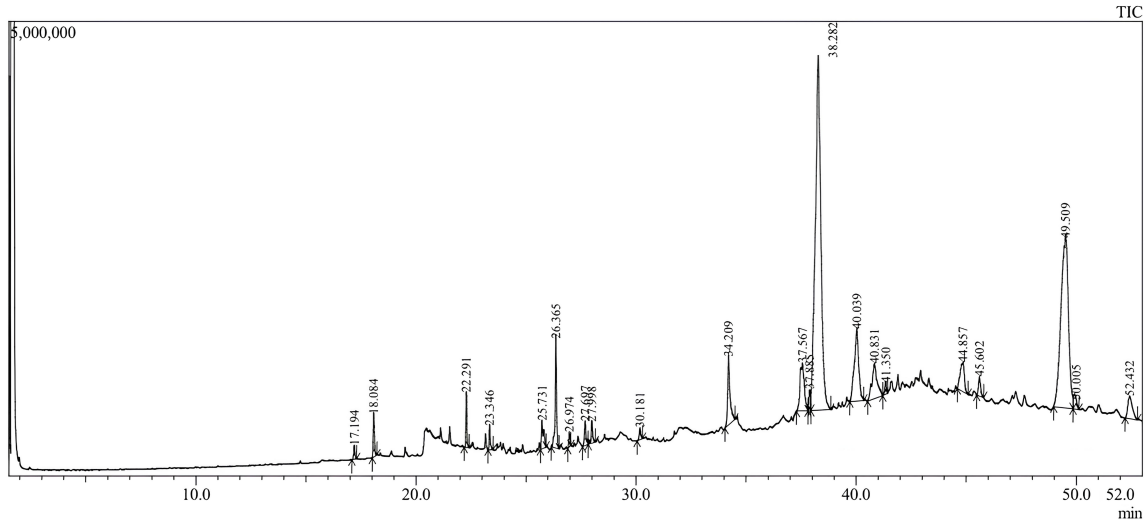


Fig. 1 GC-MS chromatogram of avocado peel essential oil. The chromatogram shows the separation of volatile compounds based on their retention times (min). Each peak corresponds to a compound detected by the mass spectrometer. Major peaks were identified using the NIST library and are presented in Table 2.

1,6,10,14,18,22-tetracosahexaen-3-ol exhibits antibacterial activity against *S. aureus*.

The dominant compounds present in the avocado peel essential oil were terpenoids (Table 2). The most abundant terpenoids in the essential oils were monoterpenes (C_{10}) and sesquiterpenes (C_{10}). These two classes of terpenoids differ in their boiling points, which in turn affects their retention times in GC analysis. In GC systems, compounds with lower boiling points tend to elute first, as they evaporate more readily and are detected earlier. Therefore, the retention time of each compound is directly related to its boiling point [25]. Among the identified terpenoids, caryophyllene has been reported to exhibit both antioxidant and antibacterial properties in avocado peel essential oil [15].

Physical properties of the active packaging films

The physical properties of the active packaging films are shown in Table 3. The incorporation of avocado peel essential oil significantly affected ($p < 0.05$) all parameters except the folding test and film thickness. An increase in the essential oil concentration resulted in decreased tensile strength, water vapor transmission rate, and brightness index. In contrast, higher concentrations of the essential oil led to increases in elongation at break, yellow index, and clarity index.

Tensile strength

The highest tensile strength was observed in the film without avocado peel essential oil (6.78 MPa), whereas the lowest was recorded in the film containing 1% avocado peel essential oil (2.84 MPa). The tensile strength values in this study met the minimum requirement established by the Japanese Industrial Standard (JIS), that is > 0.39 MPa. The addition of essential oils to edible films can lead to a reduction of tensile strength, a phenomenon commonly observed in starch-based and chitosan-based edible films. This reduction occurs because essential oils increase the mobility and flexibility of polymer chains within the film matrix, thereby decreasing rigidity and tensile strength. In addition, the disruption of polymer-polymer interactions and the plasticizing effect of oil droplets incorporated into the edible film matrix further contribute to the decrease in tensile strength [26]. Prasetyaningrum et al [27] reported that the incorporation of clove essential oil (CEO) into an alginate/κ-carrageenan-based edible film matrix resulted in a decrease in tensile strength with increasing CEO concentration. The addition of 3% CEO yielded a tensile strength of 20.96 MPa, which was lower than that obtained with 1.5% CEO, at 28.46 MPa.

Elongation

The highest elongation percentage was observed in the film containing 1% avocado peel essential oil, with a value of 15.73%, whereas the lowest value was recorded in the film without avocado peel essential oil, at 10.36%. These values comply with the JIS, which requires elongation values greater than 10%. An increase in elongation percentage was reported by Prasetyaningrum et al [27], in which the addition of CEO to an alginate/κ-carrageenan-based edible film matrix raised the value from 58% to 70%. This effect is attributed to the strong electrolyte properties of starch-derived film matrices such as alginate or κ-carrageenan, which have low affinity for interacting

Table 2 Identification of compounds in avocado peel essential oil using GC-MS. The peaks are listed according to their retention times (RT). Area (%) represents the relative abundance of each compound, while similarity indicates the percentage of match with the NIST library.

Peak#	R Time	Area	Area%	Height	Height%	Similarity	Base m/z	Compound name
1	17.194	645346	0.37	152209	1.15	97	41.05	Neral
2	18.084	1856053	1.07	480727	3.62	98	69.10	2,6-Octadienal, 3,7-dimethyl, (E)-
3	22.291	2010043	1.16	576127	4.34	96	93.10	Caryophyllene
4	23.346	1004549	0.58	262847	1.98	94	91.05	Alloaromadendrene
5	25.731	2068136	1.20	306571	2.31	94	69.05	Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)
6	26.365	5500736	3.18	1220842	9.20	94	43.05	Caryophyllene oxide
7	26.974	596752	0.35	162603	1.23	90	67.05	(1R,3E,7E,11R)-1,5,5,8,8-Tetramethyl-12-oxabi
8	27.697	1478165	0.85	265114	2.00	95	95.10	tau.-Muurolol
9	27.998	1268535	0.73	242941	1.83	94	95.10	alpha.-Cadinol
10	30.181	668693	0.39	126881	0.96	94	60.05	Tetradecanoid acid
11	34.209	5662401	3.27	762846	5.75	93	73.05	n-Hexadecanoid acid
12	37.567	6999531	4.05	524830	3.95	81	69.05	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (E)-(
13	37.885	1208430	0.70	223523	1.68	84	55.10	Octadecanoid acid
14	38.282	67933712	39.28	3875525	29.20	88	69.05	1,6,10,14,18,22-Tetracosahexan-3-ol,
15	40.039	11082912	6.41	790342	5.95	84	69.05	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentam
16	40.831	6386971	3.69	384117	2.89	88	69.10	1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,
17	41.350	723808	0.42	133316	1.00	77	69.05	2,6,10-Dodecatriene-1,12-diol, 6-(hydroxymet
18	44.857	4252572	2.46	321958	2.43	77	99.10	Rac-glycerol-1,3-dilaurate
19	45.602	1362526	0.79	202395	1.52	86	69.10	2,6,11,15-Tetramethyl-hexadeca-2,6,8,10,14-p
20	49.509	45124765	26.09	1900845	14.32	83	57.10	1-Hydroxy-3-(octanoyloxy)propan-2-yl decan
21	50.005	1552556	0.90	105862	0.80	72	69.10	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-
22	52.432	3548026	2.05	249929	1.88	95	127.10	Glycerol tricaprylate

with hydrophobic compounds like essential oils. The incorporation CEO can hinder the intermolecular interactions between polymer chains.

Thickness

The film containing avocado peel essential oil had a thickness of approximately 0.17–0.20 mm. These values comply with the JIS, which recommends a maximum film thickness of < 0.25 mm. Several factors may influence the thickness of edible films, including the surface area of the mould plate, the volume of the film-forming suspension, and the composition of the film matrix. Furthermore, the drying process and incorporation of glycerol may reduce the intermolecular distance between polymer chains, thus decreasing elasticity and bound water content, ultimately contributing to a thinner film [28]. According to Zhou et al [29], increasing the concentration of cinnamon essential oil incorporated into cassava starch-based edible films significantly increased the film thickness. This increase was attributed to the interaction and physical bonding between the film matrix and the incorporated oil.

Table 3 Physical properties of active edible films with the addition of avocado peel essential oil at different concentrations.

Property	Avocado Peel Essential Oil Concentration (% v/v)				
	0	0.25	0.5	0.75	1
Tensile strength (MPa)	6.78 ± 2.01^{a}	5.94 ± 1.88^{ab}	4.90 ± 1.46^{ab}	3.85 ± 0.69^{ab}	2.84 ± 0.87^{b}
Elongation (%)	10.36 ± 2.05^{b}	13.26 ± 1.16^{ab}	14.09 ± 2.18^{ab}	14.30 ± 3.36^{ab}	15.73 ± 1.41^{a}
Thickness (mm)	0.15 ± 0.01	0.17 ± 0.03	0.18 ± 0.04	0.19 ± 0.03	0.20 ± 0.03
Water vapor transmission rate ($g/m^2/h$)	1.01 ± 0.26^{a}	0.67 ± 0.17^{b}	0.58 ± 0.14^{bc}	0.38 ± 0.14^{bc}	0.27 ± 0.05^{c}
Folding test	5.00	5.00	5.00	5.00	5.00
Color					
L^* (lightness index)	72.73 ± 1.50^{a}	70.79 ± 0.67^{ab}	69.44 ± 2.06^{bc}	67.51 ± 1.05^{cd}	66.11 ± 1.66^{d}
Yi (yellow index)	10.38 ± 2.74^{b}	12.92 ± 2.06^{ab}	13.85 ± 1.21^{ab}	14.41 ± 1.83^{ab}	15.66 ± 2.88^{a}
ΔE (clarity index)	22.08 ± 1.50^{c}	23.12 ± 0.61^{bc}	24.50 ± 2.25^{abc}	26.11 ± 0.57^{ab}	26.48 ± 2.33^{a}

Data are expressed as mean ± standard deviation (SD, $n = 3$). Different superscript letters within the same row indicate statistically significant differences among treatments ($p < 0.05$) according to Tukey's post-hoc tests.

The formation of micro-droplets from the hydrophobic essential oil during the homogenization of the film-forming solution also contributed to the increase in film thickness.

Water vapor transmission rate

The highest WVTR was observed in the film without the addition of avocado peel essential oil, with a value of 1.01 $g/m^2/h$, while the lowest was found in the film containing 1% avocado peel essential oil, with a value of 0.27 $g/m^2/h$. These values are in accordance with the JIS, which sets the maximum permissible WVTR to < 10 $g/m^2/h$. Increasing concentration of lemon essential oil incorporated into edible films resulted in a decrease in WVTR. This phenomenon can be attributed to the reduced water-binding capacity of the film, as well as an increase in the tortuosity factor of the vapor diffusion path caused by the presence of lipid globules from the essential oil [19]. Conversely, several authors [29, 30] reported that the WVTR of films containing essential oils increased, which was associated with a reduction in film cohesion. A lower WVTR indicates better film quality, as it enhances the ability of the film to protect the product, slow down oxidation processes, and maintain the moisture content of the product. The WVTR through hydrophilic composite films is influenced by the diffusion and solubility of water molecules in the film matrix [31].

Folding test

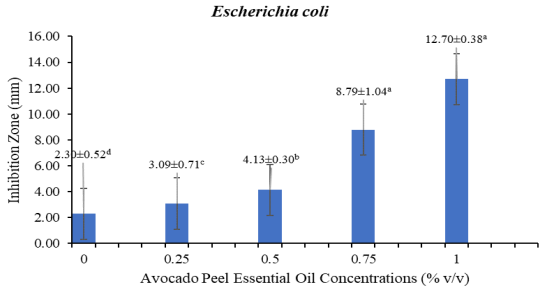
The addition of avocado peel essential oil did not affect the folding test results. All treated samples showed the same value of 5.00, corresponding to the quality level of AA. This quality level indicated that the film remained intact after being folded twice. In comparison, Sutra et al [32] reported that the folding test of ginger starch and tuna skin gelatin films yielded an average value of 3.15, which suggests a slight cracking when folded once. This was attributed to the high concentration of tuna skin gelatin that reduced the elasticity of the film. According to SNI 2372.6:2009, a grade A result in the 4-fold folding test indicated no cracking when folded once. The results of the folding test were closely related to the texture and strength of the gel. Better folding test outcomes are indicative of higher gel quality.

Film color

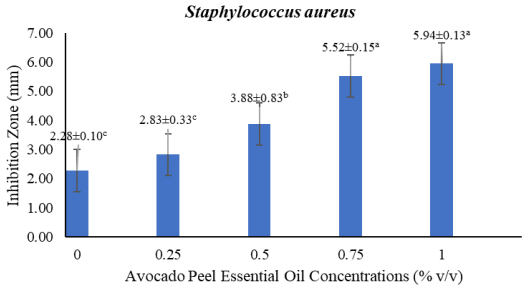
The addition of higher concentrations of avocado peel essential oil resulted in a film color that became increasingly yellow and clear. In the study by Yulianti and Ginting [33], the brightness level (L^*) of the film ranged from 80.3 to 81.7, which is higher than the values observed in this study. Moreover, a characterization study of films without glycerol by Akili et al [34] yielded a clarity index value of 8.67, which was lower than the findings in this study. This difference can be attributed to the addition of glycerol, a clear liquid that enhances the transparency of the composite films without significantly altering the color of the resulting edible packaging.

Antibacterial activity

The edible films enriched with avocado peel essential oil exhibited antibacterial activity against *S. aureus* and *E. coli* (Fig. 2). Increasing the concentration of avocado peel essential oil resulted in a corresponding increase in the diameter of the inhibition zones for both bacterial species. At the highest concentration (1%), the inhibition zones measured 12.70 mm for *S. aureus* and 5.94 mm for *E. coli*. The avocado peel essential oil in this study contained 1,6,10,14,18,22-tetracosahexaen-3-ol, which has been reported to exhibit antibacterial activity against *S. aureus* [24]. In addition, avocado peel essential oil is rich in terpenoids, one of which is β-caryophyllene. This compound has



(a)



(b)

Fig. 2 Antibacterial activity of edible film enriched with avocado peel essential oil at different concentrations (0, 0.25, 0.5, 0.75, and 1% v/v) against: (a) *E. coli* and (b) *S. aureus*. Data are expressed as mean ± standard deviation (SD, $n = 3$). Different superscript letters above the bars indicate statistically significant differences among treatments ($p < 0.05$) according to Tukey's post-hoc test. Bars sharing the same letter are not significantly different.

been shown to possess antibacterial activity against *E. coli*, although its effect is generally less pronounced compared to that against gram-positive bacteria such as *S. aureus* [35]. Based on the inhibition zone diameters, antibacterial activity against *S. aureus* can be classified as strong (diameter > 10 mm), whereas activity against *E. coli* is considered weak (diameter < 10 mm) [36]. In comparison, Kamaraj et al [15] reported that aqueous avocado fruit peel extract (1:50 w/v) produced an inhibition zone of 12.00 mm against *S. aureus* and 9.50 mm against *E. coli*. Prasetyaningrum et al [27] also reported that increasing the concentration of CEO incorporated into edible films enhanced the inhibition zone against *E. coli* (3% CEO = 113.14 mm). The mechanism underlying this increase in inhibition zone is associated with the ability of carrageenan or starch-based film matrices to rapidly release essential oils once the bacteria penetrate the film solution.

Antioxidant activity

The active edible films with avocado peel essential oil exhibited an IC_{50} antioxidant activity of 7.58 mg/l (Table 4). Antioxidant activity is influenced by the bioactive compounds present in avocado peel essential oil. Based on the GC-MS results, the oil is predominantly composed of 1,6,10,14,18,22-tetracosahexaen-3-ol, which belongs to the alcohol group and is a derivative of hydrocarbons. According to Waly et al [37], the presence of alcohol group-related constituents of avocado peel essential oil can enhance its antioxidant effect by donating hydrogen atoms or electrons to free radicals. Itam et al [38] reported that antioxidant activity can be categorized based on IC_{50} values as follows: < 50 mg/l, very strong; 50–100 mg/l, strong; 101–250 mg/l, moderate; 250–500 mg/l, weak; and > 500 mg/l, inactive. Therefore, the antioxidant activity observed in this study falls under the *very strong* category. In comparison, a study by Kamaraj et al [15] reported an IC_{50} value of 71.96 µg/ml and an inhibition rate of 60% for avocado fruit peel extracted using aqueous at 80 °C for 20 min. Compared to other extracted essential oils, rosemary essential oil (variety Typicus) incorporated into edible films exhibited an IC_{50} value of 3.96 µg/ml [39]. The incorporation of CEO into active nanocomposite films based on soy protein isolate (SPI)/microfibrillated cellulose (MFC) induced protein plasticization, increased oxygen permeability/oxygen transmission rate, and reduced water vapor permeability. This incorporation also enhanced the antioxidant activity of the films, which was attributed to the bioactive compounds present in the essential oil. Additionally, the protein-based film matrix played a crucial role in facilitating the release of active compounds from the CEO owing to its good dispersion within the nanocomposite structure [40].

Table 4 Percentage of DPPH inhibition of edible film enriched with avocado peel essential oil at different concentrations.

Avocado peel essential oil (% v/v)	Inhibition (%)
0	34.99 ± 2.02^{d}
0.25	39.79 ± 1.39^{c}
0.5	45.95 ± 0.64^{b}
0.75	49.86 ± 1.28^{a}
1	52.93 ± 1.41^{a}

Data are expressed as mean ± standard deviation (SD, $n = 3$). Different superscript letters (a–d) within the same column indicate statistically significant differences among treatments ($p < 0.05$) according to Tukey's post-hoc test.

CONCLUSION

Gelatin extracted from *Pangasius* fish skin met the quality standards outlined in SNI 8622:2018, with acceptable values for moisture content, ash content, pH, and viscosity. Avocado peel essential oil, which contains 22 identified compounds dominated by terpenoids, exhibited intense antibacterial activity against *S. aureus* and *E. coli*, as well as very strong antioxi-

dant potential with an IC_{50} value of 7.58 mg/l. The incorporation of avocado peel essential oil significantly affected the physical characteristics of the active packaging films based on *Pangasius* fish skin gelatin and κ-carrageenan. Increasing essential oil concentrations resulted in decreased tensile strength, WVTR, and brightness index, while enhancing the elongation at break, film thickness, yellow index, and clarity index. These findings suggest that *Pangasius* fish skin gelatin films enriched with κ-carrageenan and avocado peel essential oil have promising potential as biodegradable active packaging materials with improved functional properties.

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