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Antioxidant and Antibacterial Activities of Pangasian Catfish Skin Gelatin Edible Coating with Carrageenan and Red Ginger Essential Oil

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ABSTRACT Fresh fish are highly perishable due to their high moisture content, requiring proper handling to extend their shelf life. Edible coatings using fish-derived gelatin offer a halal and eco-friendly alternative. This study aimed to enhance the pangasian catfish skin gelatin's antioxidant and antibacterial activities by combining it with carrageenan and red ginger essential oils. This study involved gelatin and edible coating production, followed by testing for antioxidant and antibacterial activities against S. aureus and E. coli. Formula optimization was performed using Response Surface Methodology (RSM) via Design Expert V12, with the results verified through a Paired T-Test in Minitab 19. Adding carrageenan and red ginger essential oils significantly affected the inhibition percentage and antibacterial activity. The optimal formula included 0.5 g carrageenan and 1.5 mL red ginger essential oil. The verification steps showed alignment with predictions for Staphylococcus aureus but not for Escherichia coli.

Keywords: *Edible coating*; carrageenan; pangasian catfish skin gelatin; red ginger essential oil

INTRODUCTION

Edible coating is a type of packaging used to extend the shelf life of foods. It can be applied in liquid form directly to the surface of food and functions as a preservative. The main components of coatings are grouped into three categories: hydrocolloids, lipids, and composites (mixtures) (Rangkuti *et al*., 2019). Hydrocolloids are derived from polysaccharides and proteins. The polysaccharide group includes cellulose, starch, and carrageenan, whereas the protein group comprises collagen, gelatin, and egg whites. The advantage of gelatin-based materials lies in their unique properties, such as the ability to transition into a gel, absorb water, swell reversibly, form films, influence the viscosity of substances, and create colloidal systems (Fauziati *et al*., 2016).

Gelatin is a protein derivative produced through partial collagen degradation from bones, connective tissues, or animal skin. The gelatin currently available on the market is generally made from the bones and skin of cows or pigs, which can pose a problem for Muslims and Hindus (Nurilmala *et al*., 2021). The gelatin added to food products must be halal and healthy, including gelatin derived from fish skin (Nasution *et al*., 2018). Various studies have shown that fish skin gelatin has significant potential for development. One such source is pangasian catfish skin, which has the advantage of being relatively easy to cultivate. The increase in pangasian catfish fish farming has led to various processed pangasian catfish products, increasing waste (industrial by-products) such as skin, heads, and bones, which have not been fully utilized (Oktaviani *et al*., 2017).

The characteristics of edible coating can be enhanced by adding various ingredients to the formulation. Including carrageenan in edible coatings can reduce the risk of pathogenic bacterial growth, extending the product's shelf life. Carrageenan is derived from the galactan polysaccharide class, an intercellular matrix material in seaweeds of the Rhodophyta class. It functions as a viscosity regulator, stabilizer, thickener, etc. There are six classification types of carrageenan, one of which is kappa carrageenan, which is known for its physicochemical properties that enable it to form gels easily. The resulting edible coatings' enhanced properties were modified by adding kappa carrageenan (Prihastuti & Abdassah, 2019).

In addition to carrageenan, incorporating red ginger is believed to improve the quality of edible coatings. Priyono *et al*. (2018) indicated that fresh red ginger is typically used in spices and traditional medicine. In contrast, processed products include pickled ginger, ginger in syrup, dried ginger, powdered ginger, and essential oils. Essential oils are widely used in the perfume industry, pharmaceuticals, medicine, and other applications, particularly spices. Ginger essential oil possesses antimicrobial activity, which can help to maintain food quality, making it a potential natural preservative. Red ginger essential oil also exhibits antioxidant activity (Kawiji *et al*., 2011). This study analyzed the effects of adding carrageenan and red ginger essential oil to edible coatings made from pangasian catfish fish skin gelatine. It is predicted that red ginger essential oil and carrageenan will exhibit antioxidant and antibacterial activities in edible coatings derived from pangasian catfish skin gelatine.

MATERIALS AND METHODS

Materials

Frozen pangasian catfish skin was obtained from a local

supplier. Kappa carrageenan was purchased from the IndoGum (Indonesia) supplier. Red ginger essential oil was purchased from the Essential Oil Institute, Universitas Brawijaya, Malang, Indonesia. Acetic acid, NaOH, 0.5% glycerol, Tween 80, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 96% ethanol, Mueller-Hinton Agar (MHA) medium, nutrient broth, tryptic soy broth, and other chemicals of analytical grade were procured from Merck (Germany) and HiMedia (India). *Escherichia coli* and *Staphylococcus aureus were obtained from the Biomedicine Laboratory, Faculty of Medicine, Universitas Brawijaya Malang, Indonesia.*

Methods

Preparation of fish skin gelatin

Fish skin gelatin was prepared according to a modified version of the method described by Nurdiani *et al*. (2020). The fish skin was cut into small pieces (approximately 1x1 cm) and soaked in a 0.1 M NaOH solution at a ratio of 1:5 (w/v) for 2 hours at room temperature, and the NaOH solution was replaced every hour. After soaking, the samples were washed under running water until the pH returned to neutral (pH = 7). Once the pH was neutral, the samples were soaked in 0.6 M acetic acid solution at a ratio of 1:5 (w/v) for 2 hours at room temperature until the samples swelled. The fish skin was then rewashed with running water until the pH returned neutral (pH = 7). The samples were extracted using distilled water (1:3 w/v) in a water bath at 55–60 °C for 4 hours. The resulting mixture was filtered using a filter paper in a Buchner funnel with the assistance of a vacuum pump. The filtrate was collected in a glass beaker and stored in a dehydrator at 55-60 °C for 24 hours. The concentrated gelatin filtrate was poured into a plastic baking dish and dried in a dehydrator for 8–12 hours at 55-60 °C. The dried gelatin sheets were ground using a grinder to obtain gelatin powder. The gelatin was then analysed for yield, viscosity, and pH.

Preparation of edible coatings

The preparation of edible coatings, with modifications, was based on the method described by Nurdiani *et al*. (2024). The process was initiated by dissolving 3 g of gelatin in 50 mL of distilled water. The gelatin solution was heated using a hotplate and magnetic stirrer at 50 °C for 15 minutes. Commercial kappa carrageenan (1.5 g) was dissolved in 50 mL of distilled water. The kappa carrageenan solution was heated on a hot plate and magnetic stirrer until it reached 100 °C. The gelatin and kappa carrageenan solutions were mixed and homogenized using a hotplate and magnetic stirrer, continuously stirring for 15 minutes. Subsequently, 0.5 mL of glycerol was added to the mixture, stirring for 15 minutes. Finally, red ginger essential oil was added to the edible coating solution.

Experimental design

The edible coating formulation was optimized using response surface methodology (RSM) with Design-Expert version 12 software (Stat-Ease Inc., Minneapolis, MN, USA). The independent variables (factors) were kappa carrageenan (X1) and red ginger essential oil (X2), whereas the dependent variables (responses) were antioxidant activity (% inhibition) and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The range and midpoint values of the two factors were determined based on preliminary research. The experiment was conducted randomly using a central composite design (CCD) with three levels, coded as -1, 0, and +1 (Table 1).

Table 1. Formulation of kappa carrageenan and red ginger essential oil.

Run	Factor	
	Carrageenan (g)	Red ginger essential oil (mL)
1	0.75	0.90
2	0.50	1.50
3	0.75	0.90
4	1.00	0.30
5	0.75	0.05
6	1.00	1.50
7	0.75	0.90
8	0.75	1.74
9	0.75	0.90
10	0.75	0.90
11	0.50	0.30
12	1.10	0.90
13	0.30	0.90

DPPH antioxidant activity test

The antioxidant activity test was performed according to the method described by Afriyah *et al*. (2015). A 5 mL sample was added to 250 mL of 95% ethanol. The sample was then crushed in ethanol and vortexed to ensure complete dissolution. The solution was centrifuged at 4000 rpm for 10 minutes to separate the antioxidant extract from the sediment. A 0.1 mM solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol was prepared, and 2 mL of this solution was added to the antioxidant extract. The degree of colour reduction in the solution indicates the efficiency of free radical scavenging. The mixture was left for 20 minutes, after which the absorbance was measured at λ = 517 nm. The percentage of antioxidant

inhibition was calculated based on the reduction in DPPH color using the following equation:

$$\text{Antioxidant activity} = \left(\frac{\text{blank absorbance - sample absorbance}}{\text{blank absorbance}}\right) \times 100\%$$

Antimicrobial activity test

The agar diffusion method assessed the antimicrobial activity (Kirby-Bauer disc diffusion). Antibacterial activity was assessed using paper discs with a diameter of 6 mm. The inhibitory activity was tested against *Escherichia coli* and *Staphylococcus aureus* as test microorganisms. The bacterial suspension was first inoculated into a liquid medium to prepare bacterial cultures. Mueller-Hinton Agar (MHA) was also prepared, poured into sterile Petri dishes, and allowed to solidify at room temperature. A sterile cotton swab was used to collect the bacterial culture, which was then gently spread evenly across the surface of the solidified MHA medium. Sterile paper discs were soaked in edible coating solutions of different formulations for 5 minutes. The paper discs containing the edible coating samples were placed on the agar surface using sterile tweezers. The plates were then incubated in an inverted position at 37 °C for 24 hours. After incubation, a clear zone of inhibition appeared around the paper discs. The diameters of the zones were measured using calipers to evaluate the antimicrobial activity of the edible coatings (Khairani *et al.*, 2017). Chloramphenicol was used as the positive control.

Statistical analysis

Data was analyzed using multiple regression analysis with Design-Expert version 12 software (Stat-Ease Inc., Minneapolis, MN, USA). Condition optimization was performed using a central composite design (CCD) based on the response surface methodology (RSM). The predicted optimal treatment results were verified through paired t-test analysis using the Minitab 19 software (Minitab Pty Ltd., Sydney, NSW, Australia).

RESULTS AND DISCUSSION

Characteristics of fish skin gelatin

Pangasian catfish skin gelatin was analyzed to determine its characteristics as a raw material for use in edible coatings as the final product. The gelatin was evaluated in yield (%), viscosity (mPa·s), and pH value. The results of gelatin analysis are presented in the table below.

Table 2. Analysis of gelatin yield, viscosity, and pH.

Parameter	Results	GMIA Standard*	Reference**
Yield (%)	12.60	18.11	-
Viscosity (mPa.s)	29.00	15-75 (Tipe A) 20-75 (Tipe B)	61.66
pH	5.90	3.8-5.5 (Tipe A) 5.0-7.5 (Tipe B)	5.56

Notes: *GMIA, 2019; **Nurilmala *et al.*, 2021.

The yield of pangasian catfish fish skin gelatin was 12.6%, which was lower than the standard by the Gelatin Manufacturers Institute of America (GMIA, 2019). The yield is an essential parameter for assessing the efficiency of a processing method. It also plays a role in financial analysis, as it allows the estimation of the quantity of raw material required to produce a specific volume of a product. The yield was calculated as the ratio of the dry weight of gelatin to the weight of the extracted skin (Lombu *et al.*, 2015). Several factors can affect gelatin yield, including the pre-treatment process, extraction temperature, and the type of fish skin used (Nurilmala *et al.*, 2021). The low yield may result from incomplete hydrolysis or collagen loss during washing. Yield values can vary depending on the proximate composition of the fish skin, collagen content, dissolved skin components, fish species, age, and extraction method (Nasution *et al.*, 2018).

The viscosity of pate fish skin gelatin is lower than that reported in the literature, but still meets the GMIA standard for type B gelatin, which ranges from 20 to 75 mPa·s. The variation in viscosity is likely due to natural differences in fish species and the influence of the extraction methods (Nasution *et al.*, 2018). Viscosity is an essential physical property in determining gelatin quality, as it significantly affects gel properties, particularly the gelation and melting points—higher viscosity results in a higher melting point and gel formation rate than gelatin with lower viscosity. The viscosity of gelatin is closely related to its molecular weight and the length of its amino acid chains (Hasdar & Rahmawati, 2016).

The pH of pangasian catfish fish skin gelatin is higher than values reported in the literature but meets the GMIA standard of 5.0-7.5 for type B gelatin. Measuring the pH of gelatin is vital because it affects other properties, such as viscosity and gel strength. The maximum gel strength occurred at pH 5, whereas the viscosity is at its minimum, highlighting the importance of pH for the rheological properties of gelatin. The pH values of 5 and 8 were chosen because gelatin's melting and gelling temperatures are more stable in the pH range of 5-9, which corresponds to a more robust gel structure. The gel should exhibit a stronger structure when the pH is far from the isoelectric point, and the structure becomes weaker as it approaches the isoelectric point. pH also affects viscosity. The higher the acid concentration, the more acid cations are trapped in ossein, resulting in a lower or more acidic pH. The collagen hydrolysis process continues with the decomposition of collagen polymers, where hydrogen bonds with water molecules and free amino acid groups are crucial for the strength of gela-

tin. Changes in pH can also increase or decrease the viscosity of gelatin within a pH range of 6-8 (Febriana *et al.*, 2021).

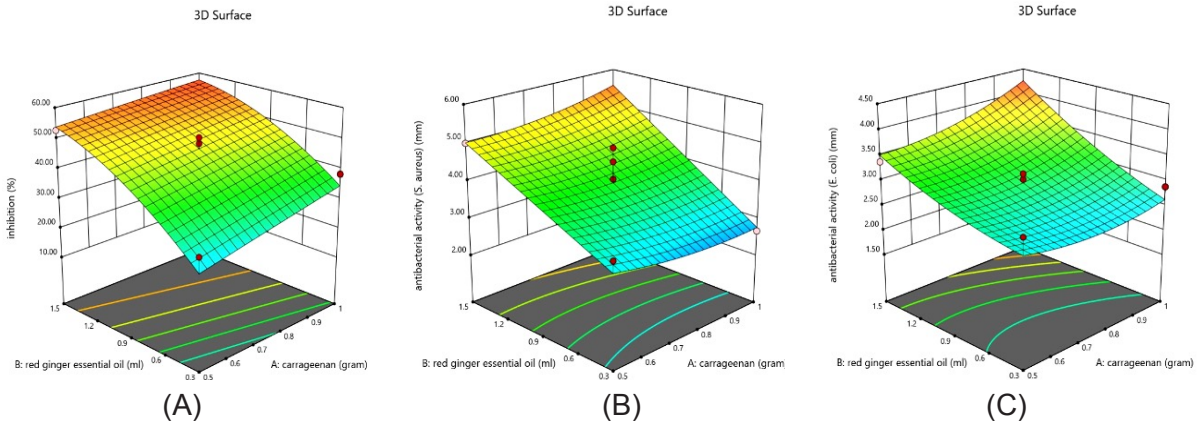
Edible coating analysis using response surface methodology (RSM)

Response Surface Methodology (RSM) was employed to optimize the conditions for edible coatings made from pangasian catfish fish skin, focusing on antioxidant parameters (% inhibition) and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Table 3 presents the effects of kappa carrageenan and red ginger essential oil on the antioxidant activity (% inhibition) and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

Table 3. The effects of kappa carrageenan and red ginger essential oils on antioxidant activity (% Inhibition) and antimicrobial activity.

	Independent (Factor)		Dependent (Response)		
Run	Carrageenan (g)	Red ginger essential oil (mL)	Antioxidant (% inhibition)	Antimicrobial Staphylococcus aureus (mm)	Antimicrobial Escherichia coli (mm)
	(X1)	(X2)	(Y1)	(Y2)	(Y3)
1	0.75	0.90	44.57±0.59	4.52±0.71	2.79±0.78
2	0.50	1.50	52.68±0.06	5.00±0.05	3.88±0.82
3	0.75	0.90	48.38±3.73	4.06±0.34	2.88±0.48
4	1.00	0.30	38.32±1.62	2.66±0.81	2.88±0.49
5	0.75	0.05	14.20±4.17	2.14±0.20	1.95±0.02
6	1.00	1.50	55.34±1.03	5.14±0.58	4.04±1.20
7	0.75	0.90	45.56±0.86	2.83±0.59	3.03±0.78
8	0.75	1.74	59.93±0.89	5.89±0.90	4.35±0.38
9	0.75	0.90	50.37±0.14	4.88±0.47	2.91±0.29
10	0.75	0.90	43.67±0.40	3.41±1.02	3.14±0.28
11	0.50	0.30	29.42±0.17	3.41±0.10	2.98±1.20
12	1.10	0.90	51.04±0.58	4.68±0.04	3.55±0.83
13	0.30	0.90	38.68±0.03	4.19±0.98	3.11±074

The effect of the two independent variables, kappa carrageenan (X1) and red ginger essential oil (X2), on the response values is illustrated in Figure 1. A three-dimensional (3D) response surface was developed to depict the relationship between the two independent factors, kappa carrageenan (g) and red ginger essential oil (mL), and the dependent factors: antioxidant activity (% inhibition) and antimicrobial activity against *S. aureus* and *E. coli*. The results of the response surface plot, highlighting the influence of kappa carrageenan and red ginger essential oils, are displayed in Figure 1.



(A) (B) (C)

Figure 1. Response surface plots of the effect of kappa carrageenan and red ginger essential oil on (A) antioxidant (% inhibition), (B) antimicrobial *Staphylococcus aureus*, and (C) antimicrobial *Escherichia coli*.

Antioxidant activity of the enriched edible coating

The results of the antioxidant response analysis (% inhibition) ranged from 14.20% to 59.93% (Table 3) and were significantly influenced ($p < 0.05$), with a p-value of 0.0006. Therefore, this model can predict the optimal antioxidant conditions (% inhibition) in the edible coating production process. The highest antioxidant activity was achieved by adding 0.75 g of carrageenan and 1.74 mL of essential oil, resulting in 59.93% inhibition. The lowest inhibition value was recorded with the addition of 0.75 g of carrageenan and 0.05 mL of essential oil, which resulted in 14.20% inhibition. Based on the % inhibition results, the average IC50 value for adding carrageenan and essential oils was 101.14 ppm. According to Nasution *et al*. (2015), a compound is classified as a powerful antioxidant if its IC50 value is less than 50 ppm, strong if its IC50 value is between 50 and 100 ppm, medium if the IC50 value is between 100 and 150 ppm, and weak if its IC50 value is between 151 and 200 ppm. The IC50 results for the edible coating made from pangasian catfish fish skin with the addition of carrageenan and essential oils can be categorized as moderate because the IC50 falls within the range of 100-150 ppm.

The antioxidant activities of edible coatings are influenced by the antioxidant compounds present in the material and their ability to reduce free radicals. Essential oils contain phenolic compounds, which are believed to play a significant role in the antioxidant activity of edible coatings due to their free radical scavenging mechanism involving the -OH group. Generally, a higher total phenol content corresponds to an increased antioxidant activity. Carrageenan in edible films exhibits antioxidant activity even without adding essential oils. This is likely because carrageenan has the potential to possess antioxidant properties. However, the antioxidant activity of the active fraction of carrageenan is lower than that of the crude extract. According to research by Triadmojo *et al*. (2021), red ginger essential oil has an IC_{50} value of 25.27 µg/The incorporation of red ginger essential oil into edible films has the potential to enhance the antioxidant activity, surpassing that of previous edible films without this addition.

Red ginger essential oil exhibited an antioxidant activity (% DPPH radical capture) of 16.61%. In comparison, red galangal essential oil showed an activity of 22.22%—the essential oil from the rhizome of *Zingiber officinale* var. *Theilade rubrum* contains monoterpenoid compounds, including camphene (14.5%), geranial (14.3%), and geranyl acetate (13.7%). Atmaka et al. (2021) state that carrageenan can significantly protect antioxidant compounds. The greater the number of double helices formed by carrageenan, the stronger its ability to protect phenolic compounds from the heating process, resulting in less damage to these compounds. Therefore, a higher concentration of kappa carrageenan leads to better protection of antioxidant compounds and an increase in the antioxidant activity of green grass jelly gel by 1.167%. According to Sarpina *et al*. (2018), gingerol in red ginger is a non-volatile phenolic compound that acts as a natural antioxidant. In addition, based on research conducted by Triadmojo *et al*. (2021), an edible film without adding red ginger essential oil has an IC_{50} value of 10.0 µg/mL.

In contrast, an edible film with red ginger essential oil added has an IC_{50} value of 25.27 µg/mL. Additionally, an edible film supplemented with vitamin C exhibits an IC_{50} value of 20.14 µg/mL. Incorporating red ginger essential oil into edible films can enhance antioxidant activity compared to edible films without the addition.

Antimicrobial activity of enriched edible coating

The results of the analysis of the antimicrobial response against *Staphylococcus aureus* ranged from 2.14 to 5.89 mm (Table 3) and were significantly influenced ($p<0.05$). Therefore, this model can predict the optimal conditions for inhibiting *S. aureus* growth in the edible coating production process. The edible coating produced the highest antibacterial activity, adding 0.75 g of carrageenan and 1.74 mL of essential oil, resulting in an inhibition zone of 5.89 mm.

Based on research conducted by Iriana *et al*. (2022), ginger can inhibit microbial activity in pressure-cooked milkfish when red ginger essential oil is added. The inhibitory effects on these bacteria were attributed to terpenoid and flavonoid compounds. Terpenoids are compounds that can bind to lipids and proteins in the cell membranes, causing cell lysis. Damage to bacterial cell membranes disrupts nutrient transport processes, leading to deficiencies in essential nutrients for growth. Flavonoids are compounds that denature bacterial cell proteins and damage bacterial enzyme systems. The values for adding carrageenan and red ginger essential oils were lower than those reported by Sholehal & Romadhon (2016), who reported inhibition zones ranging from 2.2 to 6.7 mm. The largest zone of inhibition was observed in the treatment with 1% essential oil and 1.5-2.5 grams of carrageenan. This larger inhibition zone occurs because *Staphylococcus aureus* is a gram-positive bacterium. The rigidity of the bacterial cell wall, caused by the peptidoglycan layer and its thickness, renders gram-positive bacteria resistant to osmotic lysis.

This finding is supported by Khayum *et al*. (2019), who stated that the essential oil contained in red ginger rhizomes contains alcohol, which can also enhance its antibacterial action. The greater the number of alcohol molecules, the stronger the antimicrobial action of red ginger rhizomes. The difference in the diameter of the inhibition zone can be attributed to two factors influencing the quality of the extract: biological factors, such as the type of plant, the plant's origin, the storage conditions, and the specific part of the plant used; and chemical factors, such as the types of active compounds present in the plants, the methods employed, and the solvents used. According to research by Hutabarat *et al*. (2016), bacteria can combat antibacterial compounds. Optimal inhibitory activity of antibacterial substances on the growth of certain bacteria occurs only when optimal treatment is provided, regardless of the extract concentration.

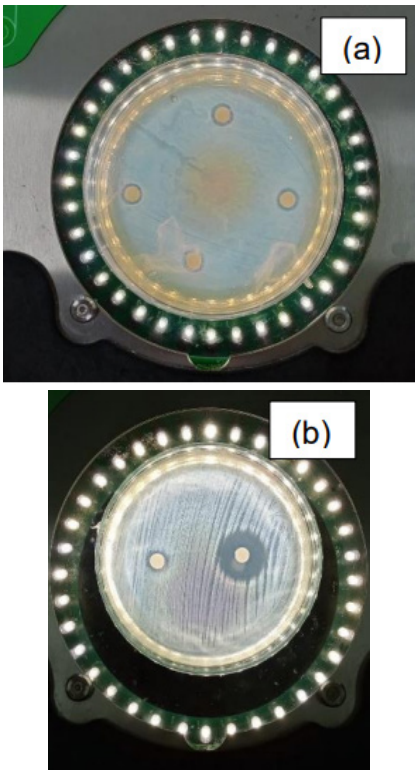


Figure 2. Bacterial inhibition zones of *Staphylococcus aureus*: (a) edible coatings with the addition of carrageenan and red ginger essential oil, and (b) positive and negative controls.

Both positive and negative controls were used in this study (Figure 2). The positive control used was chloramphenicol, with an inhibition zone of 19.67 mm, whereas the negative control, which involved sterile distilled water, was 0 mm. According to the results of tests conducted by Ali *et al*. (2013), antibacterial ginger essential oil served as a positive control in the form of the antibiotic chloramphenicol and as a negative control in distilled water. In the antibacterial test using chloramphenicol, the diameter of the clear zone produced by each test bacterium was greater than that produced by ginger essential oil. The size of the clear zone resulting from the antibiotic chloramphenicol was comparable to that of essential ginger oil. However, the active ingredient content in chloramphenicol was pure, whereas the active compound content in ginger essential oil was not pure and contained various other compounds. This results in less effective bacterial inhibition by ginger essential oil than chloramphenicol. Sterile distilled water was used as a negative control for the antibacterial test of ginger essential oil. No clear zone was obtained in this test because distilled water did not inhibit the test bacteria used in this study.

The results of the analysis of the antimicrobial response to *Escherichia coli* ranged from 1.95 to 4.35 mm (Table 3), with a significant influence ($p < 0.05$), specifically 0.0046. Therefore, the model can be used to predict optimal conditions for inhibiting *Escherichia coli* growth in the edible coating production process. The edible coating produced the highest antibacterial activity with the addition of 0.75 g of carrageenan and 1.74 mL of essential oil, resulting in an inhibition zone of 4.35 mm. This result was lower than the antibacterial activity observed against *Staphylococcus aureus*.

These results are consistent with those of Puteri *et al*. (2018), who showed that the reduction in the number of Staphylococcus aureus was relatively greater than that of *Escherichia coli*. This difference is influenced by the distinct cell wall structures of the two bacteria. The cell wall of *S. aureus*, a gram-positive bacterium, has a simpler structure composed of layers of peptidoglycan and teichoic acid, which dissolve in water, allowing antibacterial compounds to penetrate the cell. Red ginger essential oil predominantly comprises monoterpene (hydrocarbon and oxidized), sesquiterpenes (hydrocarbons and oxidized), alcohols, aldehydes, acids, and other compounds. The monoterpene and sesquiterpene components exhibit potent antibacterial activity. Research conducted by Saedi *et al*. (2020) indicated that the antimicrobial activity of carrageenan combined with SNP composites was faster than that of carrageenan alone. However, the growth of *E. coli* in the carrageenan edible film was lower than that of *L. monocytogenes* (a gram-positive bacterium).

The antibacterial activity of ginger extract depends on its chemical composition. Gingerol is a phenolic derivative that interacts with bacterial cells via an adsorption process involving hydrogen bonds. At low concentrations, the phenol interacts with proteins to form phenol-protein complexes. The bond between phenol and protein was weak and broke down quickly. Free phenols can penetrate cells and cause protein precipitation and denaturation. At higher concentrations, phenol causes protein coagulation and cell membrane lysis (Handriyanti, 2016). The results of another study conducted by Sukmawati (2021) on *E. cottonii* indicated that it contains secondary metabolites in flavonoid compounds, which can form complexes with bacterial cell proteins through hydrogen bonds. This interaction destabilizes the structure of the bacterial cell wall and cytoplasmic membrane, which contains proteins. As a result, bacterial cell proteins lose their balance in their metabolic processes. This disruption affects the permeability of the bacteria, leading to cell lysis, ultimately resulting in the death of bacterial cells.

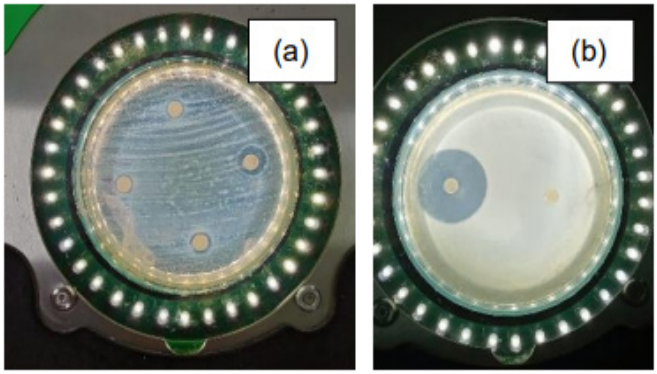


Figure 3. Bacterial inhibition zones of *Escherichia coli*: (a) edible coatings with the addition of carrageenan and red ginger essential oil, and (b) positive and negative controls.

In this study, the positive control used was chloramphenicol, with an inhibition zone of 20.79 mm, whereas the negative control using sterile distilled water was 0 mm (Figure 3). According to the results reported by Nurjanah & Fathia (2017), the antimicrobial activity of ginger extract can be assessed by measuring the diameter of the clear zone formed around the well in NA medium filled with the sample extract, positive control, and negative control. A chloramphenicol concentration of 100 µg/mL as the positive control demonstrated the largest inhibition diameter, ranging from 15.0 to 20.6 mm. In contrast, DMSO as a negative control did not exhibit a clear zone, indicating the absence of an inhibitory effect. The inhibition zones measured from the edible coating of gelatin from pangasian catfish fish skin with the addition of carrageenan and essential oils ranged from 1.95 to 4.35 mm, indicating that the strength of the inhibition zone was weak. This finding aligns with research conducted by Mahmudah & Atun (2017), who classified bacterial growth inhibition responses based on the diameter of the clear zone into four groups: weak (diameter ≤ 5 mm), medium (diameter 5-10 mm), strong (diameter 10-20 mm), and robust (diameter ≥ 20 mm).

Optimum value prediction

Optimum value prediction was performed by establishing criteria based on the results of the central composite design method. Confirmatory results were obtained by retesting the factors and response variables. These confirmation results predict the maximum value of the verified response parameters by checking the predicted values obtained from the optimization results. The optimization results obtained through Design Expert determine and provide the most optimal solution, represented by a desirable value closest to one. The desirability value indicates the accuracy of the optimization; as it approaches or reaches one, the optimization accuracy improves. At the optimization stage, the Design Expert program provided one solution with the highest desirability value of 0.953. This solution recommends using 1 g of kappa carrageenan and 1.5 mL of red ginger essential oil. The estimated antioxidant response (% inhibition) was 57.52%, while the antimicrobial activity against *Staphylococcus aureus* was 5.51 mm, and against *Escherichia coli* was 4.24 mm.

CONCLUSION AND RECOMMENDATION

Conclusion

Edible coating from Pangasius fish skin gelatin, with the addition of carrageenan and red ginger essential oil. The optimum formula was 1 gram of carrageenan, 1.5 mL of red ginger essential oil, 3 grams of gelatin, and 0.5 mL of Tween 80. The antioxidant activity, measured as % inhibition, was 57.52%, while the antibacterial activity against *Staphylococcus aureus* was 5.51 mm and against *Escherichia coli* was 4.24 mm.

Recommendation

Suggestions for further research include the application of edible coatings on food products, exploring combined gelatin formulations, and adding other essential oils to enhance antibacterial activity.

AUTHORS' CONTRIBUTIONS

Data collection and analysis: RN, OTN; writing-original draft: RN, PY, DAP; reviewing and editing: RTA, AAF, and BK. All the authors have read and approved the final manuscript.

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