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FULL PAPER

Characterization of tuna (*Thunnus albacares*) skin gelatin edible film incorporated with clove and ginger essential oils and different surfactants

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Abstract

Tuna skin gelatin has the ability to form a good film, transparent color, and a good barrier against oxygen, CO_2, and lipids. But the tuna skin gelatin edible film needs to be modified by adding hydrophobic materials and surfactants, to improve their physical and functional properties. The objectives were to determine the physical properties, antioxidant activity, and antimicrobial of tuna skin gelatin edible film were incorporated with ginger, clove essential oils, and surfactants. The stage 1) the extraction of gelatin from the tuna fish skin, 2) making edible films: ginger-tween edible film (GTF), ginger-soy lecithin edible film(GSF), clove-Tween® 20 edible films (CTF), and clove-soy lecithin edible film (CSF). The results showed an increase of thickness, **b* values, and the highest value (**b*) on GTF, but did not significantly affect **L* and **a* value. CTF and CSF have higher tensile strength compared to GTF, GSF, and control but not significantly different for elongation at break for all samples. Water vapor permeability was not significantly different amongst all edible films. Solubility decrease when clove essential oil was incorporated, in comparison with GTF, GSF, and control. Fourier transform infrared spectroscopy analyses spectra indicated that edible film added with clove essential oil and soy lecithin exhibited higher hydrophobicity than the control edible film. CTF showed the highest DPPH radical scavenging activities and the highest antimicrobial inhibitory activity. Therefore, clove essential oil and both surfactants could affect the physical and functional properties of resulting edible films.

1. Introduction

In the fisheries industry, fish filet processing produces large amounts (more than 75%) of by-products that are not edible, which are thrown away as waste or are underused in some parts of the world. The by-products of processing marine products are rich in useful sources of biomolecules such as collagen and gelatin (Newton *et al*., 2014). One type of fish whose byproducts have not been widely used is tuna skin. The Indonesian Ministry of Fisheries and Maritime Affairs in 2018 reported that tuna production in Indonesia amounted to 409,024.18 tons (Anonim, 2020). This large amount of production is also followed by large byproducts that are not used or thrown away.

Gelatin from fish can be an alternative film material. Gelatin has been widely used as a starting material for film formation. Among biopolymers, proteins have the good film-forming ability, transparent color, and oxygen, CO_2, and lipid barrier properties (Lacroix and Vu, 2014). However, films derived from pure gelatin also show weaknesses that have low water vapor barrier properties (Hoque *et al*., 2011).

The physical and functional properties of food product packaging are very important to maintain or increase the shelf life of a food product. Therefore we need food product packaging that has good physical and functional properties such as containing antioxidants, antimicrobials, and biodegradable properties (Gómez-

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Guillén *et al*., 2009).

The characteristics of a good edible film can be achieved by improving the process and adding ingredients that can improve physical and functional properties such as antioxidants and antimicrobials. Hydrophobic materials such as fat, wax, and fatty acids can be added to the film from fish gelatin to improve physical, mechanical properties including water resistance and water vapor barrier (Nor Amalini *et al*., 2018). Essential oil is a hydrophobic material that is thought to improve physical, mechanical properties including water resistance and water vapor barrier from films incorporated into films from protein material.

It is hoped that the incorporation of essential oils will not only improve physical properties The incorporation of essential oils not only improves physical properties but also increases the antioxidant and antimicrobial activity of the edible film produced. As a result, the film can function as an active packaging material for food protection and preservation. One of the wealth of spices in Indonesia is the clove. So far, clove is more widely used in cigarette production, and less is used in food, especially essential oils. The main component of clove essential oil is eugenol (56.06%) followed by caryophyllene (39.63%) and α-caryophyllene (4.31%), where this component has natural antioxidant potential based on the DPPH test (Radünz *et al*., 2019). While one type of herbs that are widely used in food is ginger. The main component of ginger essential oil is camphene, sabinene, α-curcumin, zingiberene, α-farnesene, β-sesquiphellandrene, neral, and geranial, where the active component is effective in counteracting free radicals and can be used as a natural antioxidant (Yeh *et al*., 2014). Besides having potential as an antioxidant, essential oils both clove and ginger also have the potential as an antibacterial. The antibacterial activity of essential oils is caused by essential oils containing compounds that can kill or inhibit bacterial growth. The content of antibacterial compounds in clove flowers are flavonoids, tannins, alkaloids, and eugenol (Rukmana dan Yudirachman, 2016). While ginger essential oil with the main content of zingiberene showed significant antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans,* and *Aspergillus niger* (Sharma *et al*., 2016).

In addition to essential oils to produce good edible film characteristics (emulsion films), effective surfactants are needed to stabilize the emulsion during the preparation of film-forming solutions and film casting (Hsu and Nacu, 2003). Some common types of surfactants added in film making are Tween® 20 and soy lecithin. The difference in surfactant addition will affect the structure or morphology and characteristics of edible films (Tongnuanchan *et al*., 2014). Several previous studies related to efforts to improve the physical-mechanical and functional properties of edible films from fish gelatin, (Tongnuanchan *et al*., 2012) examining films derived from tilapia skin incorporated with bergamot, kaffir lime, lemon, and lime with the addition of glycerol and surfactants (Tween® 20), films from the tilapia skin incorporated in volatile basil (*Ocimum basilicum*), plai (*Zingiber montanum*) and lemon (*Citrus limonum*) as well as with the addition of glycerol and surfactants (soy lecithin) (Tongnuanchan *et al*., 2014) and added edible film of silver carp fish with cinnamon, glycerol, and Tween® 80 essential oils (Wu *et al*., 2017). However, little information regarding the effect of the addition of cloves, ginger essential oils, and different surfactants (soy lecithin, Tween® 20) on physical properties, antioxidant and antibacterial activity of edible films from tuna skin gelatin. The objective of this investigation was to study the effect of adding clove and ginger essential oils and surfactant types (soy lecithin, Tween® 20) on physical properties, antioxidant activity, and antibacterial edible film of tuna skin gelatin.

2. Materials and methods

2.1 Materials

The material used in this research is tuna skin from Bantul Special Region of Yogyakarta, Indonesia. Clove and ginger essential oils were obtained from CV. Nusa Aroma Jakarta, Indonesia. The chemicals were glycerol, soy lecithin, Tween® 20, 2,2 - Diphenyl-1-Picryl Hidrazyl (DPPH), methanol, NaOH (Merck, Jerman), citric acid, sulfuric acid (Merck, Jerman), and aquadest, the culture of *Staphylococcus aureus, Escherichia coli*, nutrient broth, and nutrient agar media.

2.2 *Extraction of tuna skin gelatin*

First, fresh tuna skin was washed and removed the residual fat. Tuna skin was cut into small pieces (0.5 x 0.5 cm) and then demineralized by immersing in a 0.2% NaOH solution for 2 hrs at a ratio of 1:6 (w/v). Then, the tuna skin sample was drained and washed with clean water up to pH 7 and soaked again with 0.2% H_2SO^4 for 2 hrs with a sample ratio with 1: 6 (w/v) acid solution. Then the tuna skin sample was washed with water up to pH 7 and finally soaked with 0.1% citric acid solution for 2 hrs 1:6 (w/v) ratio and then washed again with water up to pH 7. Tuna skin that has been treated with NaOH, H_2SO^4, and citric acid then was extracted using aqua dest with a ratio of 1: 3 (w/v) at 60°C for 6 hrs and continued with filtration. The drying process was carried out at a temperature of 50 °C for 36-48 hrs with a cabinet

dryer to obtain a gelatin sheet. Then the gelatin was milled to obtain gelatin powder (Tkaczewska *et al*., 2018).

2.3 Preparation edible films

Edible film preparation was based on the previous method (Tongnuanchan *et al*., 2014) with some modifications. To make a film-forming solution or film-forming solution (FFS) of 3 g of gelatin powder was dissolved in distilled water to obtain a concentration of 3% (w/v). The solution was heated at 50°C for 30 mins. Then, glycerol was added as much as 20% (w/w) of gelatin as a plasticizer. Before being added to FFS, essential oils of cloves, ginger (100% v/w) from gelatin were mixed with surfactants (soy lecithin, Tween® 20) as much as 25% (w/v of essential oils). Then the solution obtained was homogenized with a homogenizer at 4,200 rpm for 5 mins. The film was then cast in a small plastic tray and dried in a cabinet dryer at a temperature of 50°C for 20-24 hrs then the edible film sheet was obtained.

2.4 Thickness

Film thickness was measured using a micrometer based on the method of (Tongnuanchan *et al*., 2013). Five points for each edible film sample were measured for thickness and then averaged as the thickness value of the edible film.

2.5 Color

This analysis is used to determine the values of L (brightness), a (red-green), b (yellow-blue) on edible film. The edible film was placed on a plate reader. Then the reader plate was placed on the chromameter. The edible film was analyzed using chromameter so that the color value will be measured as **L*, **a*, and **b* (Nur Hanani *et al*., 2019).

2.6 Tensile strength (TS) and *elongation at break* (EAB)

Tensile strength (TS) and elongation at break (EAB) were measured using UTM (*Universal Testing Machine*) Zwick/Z.0.5 Texture Analyzer with slight modification (Pranoto, 2008). Film specimens of size 5 cm x 0.5 cm were measured in mean thickness using a micrometer. The sample was placed between the grips with an initial distance of 50 mm and then pulled at a speed of 10 mm/ minute. TS was calculated by dividing the maximum force at breaking with the cross-sectional area of the edible film and was expressed in MPa. Whereas EAB was calculated based on the basis length extended as compared to the original length of the film (read from machine or chart).

2.7 The solubility of edible film

The water solubility of the films was based on the method describes elsewhere (Hosseini *et al*., 2013) with slight modification. Films were cut into 1 x 4 cm^2 size of 3 pieces, then weighed. After that, the sample is dried in an oven at 105°C for 24 hrs to determine the initial dry weight (Wi). After that, the film samples were immersed in 30 mL of distilled water in a tube which is stirred in a water bath at a speed of 100 rpm at room temperature (22-25°C) for 24 hrs. The samples were then passed through a filter paper (Whatman 1). Then the filter paper together insolubilized fraction was dried in an oven (105°C, 24 hrs), then the dry sample was weighed (Wf). The percentage of solubility of the sample in water (S) was calculated by the equation:

$$S = \frac{W_i - W_f}{W_i} \times 100\%$$

2.8 Water vapor permeability (WVP)

The water vapor permeability (WVP) of films was measured gravimetrically [Hosseini *et al*., 2013]. WVP edible film was measured using a bottle made of glass with an inner diameter of 1.3 cm and 5 cm height. The edible films without pinholes or any defects were measured thickness by using a micrometer. The vial bottles were filled with 6 mL of distilled water, and edible films were sealed to the cup mouth, placed in desiccators containing silica gel. The water was transferred through the film and absorbed by the desiccant was determined from the weight loss of the permeation cell. The cells were weighed at intervals of 1 hr for 7 hrs with an analytical balance. The slope of weight loss versus time was obtained by linear regression. Temperature and storage space were recorded and used to determine the atmospheric partial pressure using a steam table. The value of WVTR (Water Vapor Transfer Rate) was determined by the equation:

$$WVTR = \frac{\Delta w}{A\Delta t}$$

$$WVP = \frac{WVTR \times L}{\Delta P}$$

Where Δw is the weight of water vapor passing through the film (g), L is the thickness of the film (mm), A is the exposed film area and ΔP is the partial pressure difference of water vapor (Pa). WVP is expressed in g/ msPa.

2.9 Fourier transform infrared spectroscopy analyses

This analysis was carried out to identify the presence of specific chemical groups in a material and to test the efficiency of the crosslinking process. The film needed for this test was 5 mg. The film was made in pellets forms then analyzed, this analysis was carried out using

Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra were obtained between 4000 - 500 cm^{-1}(Sutono and Pranoto, 2013).

2.10 Antioxidant activity test

The DPPH radical scavenging activity was based on the method describes elsewhere (Maryam Adilah and Nur Hanani, 2016) with some modifications. 0.1 g of each film samples were dissolved in 2 mL methanol. The extract with 10 µL was diluted with 990 µL methanol (500 ppm). Samples (500 ppm) 100 µL were diluted with 900 µL methanol then added to 2 mL of 0.1 mM DPPH solution in 95% methanol. Followed by incubation in a dark room for 30 mins and then measured the absorbance at 517 nm on a spectrophotometer. Antioxidant activity is calculated by the equation:

Radical Scavenging Activity (%) = Abs DPPH – Abs sample/Abs DPPH x 100%

2.11 Antibacterial activities of edible film

Antibacterial activity of the edible films was tested by the method describes elsewhere (Pranoto *et al*., 2005) with slight modification. The edible film with a diameter of 10 mm was placed on the nutrient agar plates, which had been previously seeded 0.1 mL of a culture of test microorganisms containing 10^6 CFU/mL. Petri dishes were incubated at 37°C for 24 hrs. After going through the incubation period, the inhibition zone will appear and measurement of the diameter of the inhibition zone will be measured with the calipers in mm. The diameter of the inhibition zone is calculated as the diameter of the clear zone (including the diameter of the edible film) formed.

2.12 Statistical analysis

The measurement results of each variable were analyzed with the SPSS version 25 program. Data were tested statistically using ANOVA. If there were differences between treatments as indicated by the F count<F table then the DMRT test was continued at the 5% significance level to find out the level of further differences.

3. Results and discussion

3.1 Thickness

The addition of essential oils and surfactants increases the thickness of the edible film. in Table 1, shows the thickness of edible film added by 2 different types of essential oils and surfactants, having a thickness higher than the edible control film (P<0.05). This is due to an increase in solid material (solid content) in the edible gelatin film. The essential oil droplet enters and localizes it in the edible film network. As a result, the interaction between gelatin chains could be impeded and then the loss of a compact network of gelatin chains might bring about the protruded structure as indicated by the thickness of the increases. Guided by JIS (Japanese Industrial Standard), plastic films for food packaging that are categorized as films are those that have a maximum thickness of 0.25 mm (Nurindra *et al*., 2015). Edible film from tuna skin gelatin (*Thunnus albacares*) in this study still meets the standard to be categorized as a food packaging film, because the resulting film thickness ranges from 0.113±0.042 to 0.184±0.052 mm. From the results of the thickness value of an edible film, it can be seen that the addition of essential oils and surfactants can increase the thickness value and approach JIS criteria with a value of 0.137±0.038 - 0.184±0.052 mm. Increased film thickness was also reported by (Tongnuanchan *et al*., 2014) where the thickness of the tuna skin gelatin film incorporated with basil, plai, and lemon essential oil was higher than the control film.

3.2 Color

Edible film color is very influential on the appearance of the packaged product. Table 1 presents the color (**L*, **a,* and **b*) of edible films incorporated with two types of essential oils and surfactants. Duncan's follow-up test results showed that the addition of essential oils and surfactants had higher **b (*yellowness) value (P<0.05) compared with edible film control, but did not significantly affect **L* (lightness) and **a (*redness/greenness) value. The highest **b* value in the

Table 1. Color of edible film

Sample	Thickness (mm)	L*	a*	b*
Control	0.113±0.042 ^{Aa}	64.20±11.24 ^{Aa}	1.56±1.26 ^{Aa}	4.58±1.91 ^{Aa}
GTF	0.137±0.038 ^{Bb}	65.94±6.26 ^{Aa}	1.10±1.54 ^{Aa}	11.26±6.13 ^{Bb}
GSF	0.184±0.052 ^{Bb}	75.30±5.08 ^{Aa}	1.55±1.40 ^{Aa}	22.63±4.99 ^{Bb}
CTF	0.181±0.058 ^{Bb}	68.55±1.75 ^{Aa}	1.94±1.05 ^{Aa}	16.80±5.98 ^{Bb}
CSF	0.147±0.058 ^{Bb}	69.67±6.26 ^{Aa}	0.64±0.91 ^{Aa}	16.63±2.42 ^{Bb}

Values with different uppercase letter superscripts indicate significant difference (p<0.05) among essential oils while values with different lowercase letter superscripts indicate significant difference (p<0.05) in the treatment of surfactants. GTF = ginger-Tween® 20 edible film; GSF = ginger-soy lecithin edible film; CTF = clove-Tween® 20 edible film; CSF = clove-soy lecithin edible film.

edible film added with ginger essential oil and soy lecithin (GSF) is 22.63±4.99. The highest *b value is thought to be caused by carotene pigments from soy lecithin (brownish yellow) and curcumin(pale yellow to golden orange) from ginger. *L (lightness) and *a (redness/greenness) value does not significantly affect with edible film control. This is presumably due to the natural pigment from tuna skin gelatin is light yellow and 2 types of essential oils (ginger, cloves) are yellow-orange and brownish-yellow and 2 types of surfactants (Tween® 20, soy lecithin) tend to be bright yellow and brownish yellow so does not change the color of the edible film control. According to an earlier study (Tongnuanchan *et al*., 2014) reported that the tilapia gelatin film which was incorporated with basil, plai, and lemon essential oils and soy lecithin surfactant showed a decrease in *L value, an increase *a value, and an increase in the *b value. The differences in the components of pigments/compounds present in essential oils might determine the difference in the color of the resulting film (Tongnuanchan *et al*., 2014).

3.3 Tensile strength (TS) and elongation at break (EAB)

Tensile strength is the maximum pull that can be achieved until the edible film persists before breaking. Tensile strength is a mechanical property of the edible film. Tensile strength determines the strength of the edible film. The higher the tensile strength, the better edible film can withstand mechanical damage. Table 2 presents the TS and EAB values of edible films incorporated with two types of essential oils and surfactants. Duncan's follow-up test results showed that the value of tensile strength edible film added with soy lecithin was higher ($P<0.05$) than edible film added Tween® 20, but not significantly different from edible control films ($P>0.05$). This can be caused by soy lecithin which has (HLB = 4) and is included in the low Hydrophile-lipophile balance (HLB) surfactant. The lower the HLB value of a surfactant, it is more hydrophobic (more non-polar groups) (McClements, 2005). Thus the polar group will bind to gelatin while the non-polar group binds to essential oils because the more non-polar group will make the edible film matrix stiff. The addition of clove essential oil significantly affected the TS edible film ($P<0.05$), where the clove-soy lecithin edible film (CSF) had the highest tensile strength value. The addition of clove essential oil significantly affected edible film ($P<0.05$). This is due to the content of different essential oil components where the main content of clove essential oil is eugenol which is a group of phenolic compounds. The oxidation of phenolic compounds produces quinones, which can react with side chains of protein amino groups via covalent (C-N or C-S) or hydrogen bonding (Makishi *et al*., 2013). Intermolecular interactions result in cross-links between chains, leading to improved film properties (Choi *et al*., 2018). This is an agreement with the study earlier (Hoque *et al*., 2011) reported where essential oils that contain mainly aldehyde, ketone, and phenolic compounds when interacting with protein films will increase the tensile strength of the film.

Elongation at break is the maximum length change experienced by the film until it breaks. The highest elongation value in CTF while the lowest value in GTF. The addition of essential oils and surfactants did not significantly affect the elongation of edible films. The tilapia gelatin film which was incorporated with basil, plai, and lemon essential oils and soy lecithin surfactant showed a decrease in tensile strength and increased EAB (elongation at break) (Tongnuanchan *et al*., 2014). While the edible film of black tilapia skin added by seaweed extract (*Kappaphycus alvarezii)* that containing phenol compounds increased tensile strength (TS) and elongation at break properties (Sutono and Pranoto, 2013). But the overall tensile strength and the elongation at break value still conform to the JIS (Japanese Industrial Standard) 1975 which is at least 0.392266 MPa and at least 70%, respectively (Nurindra *et al*., 2015).

3.4 Water vapor permeability

WVP (Water Vapor Permeability) is a measure of the ease of water vapor passing through materials, such

Table 2. Tensile Strength (TS), Elongation at Break (EAB), WVP and solubility edible film

Sample	TS (Mpa)	EAB (%)	WVP (g/msPa)	Solubility (%)
Control	1.54 ± 1.09^{Aab}	463.74 ± 28.83^{Aa}	$4.63x10^{-12}\pm1.49x10^{-12\ Aa}$	79.63 ± 6.29^{Ba}
GTF	0.57 ± 0.05^{Aa}	463.16 ± 50.94^{Aa}	$4.23x10^{-12}\pm0.93x10^{-12\ Aa}$	62.11 ± 9.19^{ABa}
GSF	1.03 ± 0.07^{Ab}	547.17 ± 11.39^{Aa}	$3.79x10^{-12}\pm0.66x10^{-12\ Aa}$	67.17 ± 29.97^{ABa}
CTF	1.65 ± 0.99^{Ba}	557.52 ± 10.59^{Aa}	$3.47x10^{-12}\pm1.02x10^{-12\ Aa}$	52.63 ± 2.58^{Aa}
CSF	3.60 ± 0.37^{Bb}	454.41 ± 55.34^{Aa}	$2.84x10^{-12}\pm0.76x10^{-12\ Aa}$	43.44 ± 1.93^{Aa}

Values with different uppercase letter superscripts indicate significant difference ($p<0.05$) among essential oils while values with different lowercase letter superscripts indicate significant difference ($p<0.05$) in the treatment of surfactants. GTF = ginger-Tween® 20 edible film; GSF = ginger-soy lecithin edible film; CTF = clove-Tween® 20 edible film; CSF = clove-soy lecithin edible film.

as biopolymer films. Table 2 presents the WVP values of edible films incorporated with two types of essential oils and surfactants. The value of WVP edible film ranges from $2.84x10^{-12}$±$8.76x10^{-12}$ g/msPa to $4.63x10^{-12}$ ± $1.47x10^{-12}$ g/msPa. The highest value of WVP was in edible film control, while the lowest was in clove-soy lecithin edible film (CSF). From Duncan's follow-up test results it was found that the addition of essential oils and surfactants did not significantly affect (P>0.05) on WVP edible film. The addition of essential oils at high concentrations is thought to influence the suboptimal role of essential oil crosslinking, thus causing edible film control not significantly different from the treatment of adding essential oils (3%). This is an agreement with the study earlier (Nisaa, 2020) reported tilapia skin gelatin film added with 1-2% cinnamon essential oil was not significantly different from the control film, but at a concentration of 4% WVP increased (higher than the control film).

Although the WVP value was not significantly different between treatments, there was a decrease in the value of WVP edible control films from $4.63x10^{-12}$ ± $1.49x10^{-12}$ g/msPa to $2.84x10^{-12}$ ± $0.76x10^{-12}$ g/msPa (decreased 38.66%) on clove edible films - soy lecithin. The reduction in WVP or the increase in vapor barrier properties in edible gelatin films may be caused by the formation of winding pathways for the spread of steam through the film by reducing the free −OH / NH group or increasing the degree of crystallinity of biopolymers (Kanmani and Rhim, 2014). The impact of the lipid addition on the microstructure of the emulsified film is a determining factor in water barrier efficiency (Atares and Chiralt, 2016). The rate of edible film vapor transmission is influenced by several factors, namely the structure of edible films (homogeneity, emulsions, multilayers), crystal type, shape, size, and lipid distribution (Morillon, *et al*., 2002).

Tongnuanchan *et al*. (2014) reported the tilapia gelatin film which was incorporated with basil, plai, and lemon essential oil and soy lecithin surfactant had a WVP value of 0.71±0.01 x 10^{-11} g/msPa, 1.01±0.01 x 10^{-11} g/msPa, and 0.75±0.04 x 10^{-11} g/msPa or decreased in WVP by 61.4%, 41.1% and 59.2% of the control film. Besides, Wu *et al.* (2017) also reported that films from silver carp skins that were incorporated with cinnamon essential oil and tween 80 surfactants had WVP values of 1.52±0.05 x 10^{-10} g/msPa or decreased by 61.70% of control films. Incorporation of essential oil could enhance the water vapor barrier property of gelatin film, in which the capability was dependent on the types of essential oils used (Tongnuanchan *et al*., 2014).

3.5 Solubility

The solubility of edible films can be a parameter of the water-resistance of the film (Rhim *et al*., 2000). Table 2 presents the solubility of edible films incorporated with two types of essential oils and surfactants. The highest solubility value in the edible film control, while the lowest value in the clove-soy lecithin edible film (CSF). Duncan's further test results on the solubility of the edible film showed that the addition of clove essential oil significantly affected (P<0.05) on the decrease of film solubility, but the addition of ginger essential oil and surfactant did not significantly affect the solubility of the edible film (P>0.05%). This is due to the interaction of the hydrophobic component of the clove essential oil with the hydrophobic component of gelatin thereby increasing the hydrophobicity of the edible film. This causes the solubility of edible films to decrease. A decrease in the solubility of edible gelatin films was also reported Ahmad *et al*. (2012), where the bergamot essential oil and lemongrass added as much as 25% (w/w) film-forming solution can reduce the solubility of edible film. The greatest solubility in edible film control was 97.80%, while the lowest was in an edible film with lemongrass was 89.16%.

3.6 FTIR spectra of edible film

In this study the transmittance spectrum tested was from wavenumbers 400 - 4000 cm^{-1} which corresponds to the absorption of active functional groups of edible films. The vibrational mode that is often observed in infrared spectroscopy is a strain where the bond length changes when it vibrates and requires more energy.

The FTIR spectra of edible films are depicted in Figure 1. In the spectra image, it can be identified that in the range of wave numbers 3273-3306 cm^{-1} is the spectrum of amide A, while in wave number 2922-2933 cm^{-1} is the region of amide B. Amide A represents the absorption of the NH stretching coupled with hydrogen bonds while the amide B corresponds with CH strain. While the spectra in the range of 1635-1651 cm^{-1} (amide I) is related to the vibrations of C=O stretching/hydrogen bonding coupled with COO. The addition of essential oils generally gives a higher amplitude to amide A amide 1 than control edible films. The addition of clove essential oil and soy lecithin showed the highest increase in amplitude. This indicates an interaction between eugenol compounds in clove essential oil and hydrophobic groups in gelatin which causes gelatin to experience cross-linking, thus increasing the hydrophobicity of edible films. Besides the increase in hydrophobicity of edible film was also shown in the solubility test, where the addition of clove essential oil

significantly affected the edible film's solubility or increased hydrophobicity of edible film.

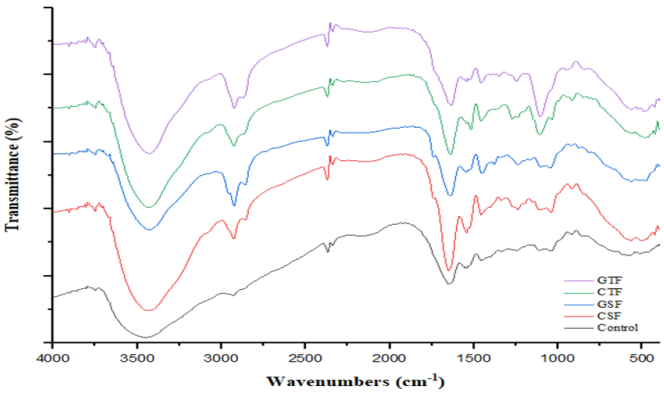

Figure 1. FTIR spectra of edible films. GTF = ginger-Tween® 20 edible film; CTF = clove-Tween® 20 edible film; GSF = ginger-soy lecithin edible film; CSF = clove-soy lecithin edible film; control = control edible film

3.7 Antioxidants activity

Table 3 presents the antioxidant activity of edible films incorporated with two types of essential oils and surfactants. From Duncan's continued test results, edible film plus clove essential oil significantly affected ($P<0.05$) its antioxidant activity with an edible film. The edible film added with surfactant type (Tween® 20, soy lecithin) significantly different antioxidant activity with edible film control and between edible film added with different surfactant types ($P<0.05$), where edible film added clove essential oil and Tween® 20 (CTF) had the highest antioxidant activity of 52.22±14.81% with the DPPH free radical immersion method at a concentration of 50 ppm. Antioxidant activity is related to the main active component in essential oils. According to Radünz *et al*. (2019), the main component of clove essential oil is eugenol (56.06%) followed by caryophyllene (39.63%) and α-caryophyllene (4.31%), where this component has natural antioxidant potential. The antioxidant activity of clove essential oil was higher than BHT>α-tocopherol>BHA>Trolox which was 83.6, 67.8, 64.9, 62.5, and 29.4% at concentrations of 45 µg/mL. While based on Bellik *et al*. (2013), the antioxidant activity of ginger essential oil was 19.92±2.80% to 68.31±3.32% with concentrations ranging from 1.2 to 19.17 mg/mL. Based on this, it will affect the antioxidant activity of edible films, where clove edible films are higher than ginger or control edible films.

The addition of Tween® 20 had a higher effect than soy lecithin on edible film, although statistically not significantly different. This is thought to be related to the film's emulsion system in the form of oil-in-water emulsions. The oil-in-water emulsion system is more stable when an emulsifier with a high HLB value is added. Tween® 20 (HLB: 16.7) has a higher HLB than soy lecithin (HLB: 4). In the clove-Tween® 20 film emulsion, the functional group of the essential oils is completely dispersed (forming droplets), giving a higher antioxidant effect. However, edible control films also showed antioxidant activity against DPPH free radicals of 3.07±2.60%. This is due to the peptide fraction of gelatin containing amino acids such as glycine and proline which have antioxidant activity (Kim *et al*., 2001).

As a comparison material (positive control) BHT (butylated hydroxytoluene) antioxidant activity was also obtained IC_{50} value (effective concentration value that can inhibit 50% oxidation) of 9.06 ppm while at a concentration of 50 ppm the antioxidant activity value of CTF 52.22%. It can be stated that the antioxidant activity of BHT is equivalent to 5.5 times the antioxidant activity of edible film added with clove essential oil and tween surfactant 20. IC_{50} BHT is calculated by comparing the concentration of BHT with the value (%) of antioxidant activity in a regression linear.

3.9 Antibacterial activity

Gelatin films incorporated with different essential oils and surfactants are tested against the selected bacteria (*E. coli* and *S. aureus*) to determine antibacterial activity (Table 3). Based on the results of the study, the

Table 3. Antioxidant activity and antibacterial edible film

Sample	Antioxidants	Antibacterial	
	Radical Scavenging Activity (%)	Inhibitory zone *E. coli* (mm)	Inhibitory zone *S. aureus* (mm)
Control	$3.07±2.60^{Aa}$	0.00^{Aa}(ND)	0.00^{Aa}(ND)
GTF	$5.55±1.40^{Ab}$	$5.87±0.70^{Bb}$	$3.97±0.28^{Bb}$
GSF	$4.61±1.04^{Aab}$	$6.84±0.28^{Bc}$	$4.84±1.16^{Bb}$
CTF	$52.22±14.81^{Bb}$	$12.85±1.05^{Cb}$	$19.33±4.01^{Cb}$
CSF	$19.91±4.87^{Bab}$	$8.93±0.74^{Cc}$	$14.97±2.34^{Cb}$

Values with different uppercase letter superscripts indicate significant difference ($p<0.05$) among essential oils while values with different lowercase letter superscripts indicate significant difference ($p<0.05$) in the treatment of surfactants. GTF = ginger-Tween® 20 edible film; GSF = ginger-soy lecithin edible film; CTF = clove-Tween® 20 edible film; CSF = clove-soy lecithin edible film.

edible film added with ginger and clove essential oils can inhibit the growth of *E. coli* and *S. aureus*. Based on the results of Duncan's further tests on the antibacterial activity of edible films showed the addition of essential oils significantly (P<0.05) to increase the antibacterial activity of edible films both against *E. coli* and *S. aureus*. The addition of clove essential oil has the effect of inhibiting higher antimicrobial activity than the addition of ginger essential oil. This is because the MIC (minimum inhibitory concentration) value of clove essential oil is lower than ginger essential oil. Based on research by Lee *et al*. (2009), the MIC value of cloves was 0.015 µg/mL - 0.062 µg/mL while the MIC value of ginger essential oils was 3.9-62.5 µL/mL (3,900-62,500 µg/mL) (Debbarma *et al*., 2012) .

Likewise, the addition of Tween® 20 surfactants and soy lecithin had a significant effect (P<0.05) on the increased antibacterial activity of edible films. Tween® 20 exerts an increased effect on antibacterial activity than soy lecithin. This is related to the stabilization of the film emulsion system in the form of an oil-in-water emulsion. The oil-in-water emulsion system is more stable when an emulsifier with a high HLB value is added. Tween® 20 (HLB: 16.7) has a higher HLB than soy lecithin (HLB: 4). In the clove-Tween® 20 film emulsion, the functional group of the essential oil is completely dispersed (forming a droplet), giving a higher antibacterial effect. Uniformity of droplet shape (smaller diameter) of the emulsion film will increase the ability of penetration through bacterial cell wall membranes (Farshi *et al*., 2019).

Based on the results obtained clear zone areas are categorized into several categories, namely weak (< = 5 mm), moderate (6-10 mm), strong (11-20 mm), and very strong (>20 mm) (Permadani, 2015). The highest antibacterial activity in CTF which can inhibit *E. coli* with a clear zone diameter of 12.85 mm and *S. aureus* of 19.33 mm, and included in the category of strong antibacterial inhibition. The clear zone in *S. aureus* is higher than *E. coli*. This is because the two types of bacteria are different, where *S. aureus* bacteria are gram-positive bacteria while *E. coli* bacteria are gram-negative bacteria. The cell wall structure of gram-negative bacteria is more complex compared to gram-positive bacteria, so that clove essential oil (eugenol) is easier to inhibit the growth of *S. aureus* bacteria compared to *E.coli* bacteria (Oliver *et al*., 2004). Lestari (2017) also reported that MID (minimum inhibitory dose) of clove essential oil in *S. aureus* bacteria was 25 µL/L while in *E. coli* bacteria was 50 µL/L. The MID value needed by clove flower essential oil to inhibit the growth of *S. aureus* bacteria is smaller than *E. coli*. The lower MID value indicates stronger antibacterial activity, which means clove flower essential oil has greater antibacterial activity against gram-positive bacteria than gram-negative bacteria. The zone of inhibition by essential oils in fish gelatin edible films was also reported by Wu *et al*. (2017), were at a concentration of 2% cinnamon essential oil had given an inhibition zone of *E. coli* bacterial growth of 18.78 mm and *S. aureus* bacteria of 19.95 mm.

When essential oils are added to edible films, essential oils are diffused into agar media and produce clear zones on microbial growth media. Factors affecting the size of the inhibitory area, namely the sensitivity of the organism, culture medium, incubation conditions, and the speed of agar diffusion (Utami *et al*., 2013).

The antibacterial ability of the clove essential oil obtained from the lipophilic nature of eugenol can cause bacterial cell membranes to undergo adhesion which causes inhibited bacterial respiration. This will disrupt transport in cells so that bacteria experience death. Besides, phenol groups contained in eugenol when attached to bacterial cells will make bacteria undergo lysis, then die (Kumala *et al*., 2008).

4. Conclusion

The addition of essential oils and surfactants has a significant effect on physical properties: thickness, color **b* edible film, but not significantly different in color values **L* and **a*, no significant effect on elongation at break, however, the addition of clove essential oil increased the mechanical properties of the edible film (tensile strength), had no significant effect on WVP, and the addition of clove essential oil had a significant effect on the decrease in the solubility of the edible film of tuna skin gelatin. For the functional properties of tuna skin gelatin edible film, the addition of clove essential oil and surfactant increased the antioxidant activity of edible film, where the addition of clove essential oil and Tween® 20 gives the highest increase in antioxidant activity on edible film. The antioxidant activity of edible film added with clove essential oil and Tween® 20 surfactant (50 ppm concentration) is equivalent to the antioxidant activity of BHT at 5.5 ppm concentration. The addition of essential oils (cloves and ginger) and surfactants (Tween® 20 and soy lecithin) can inhibit the growth of *E. coli* and *S. aureus*. The highest antimicrobial activity in edible clove tween film 20 which can inhibit *E. coli* with a clear zone diameter of 12.85 mm and *S. aureus* of 19.33 mm, and included in the category of strong antimicrobial inhibition. Therefore, tuna skin gelatin edible film incorporated with clove essential oil and surfactants could be used as an alternative active film packaging.

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