p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Strengthening for Research and Development No: 30/E/KPT/2018

Tropical Animal Science Journal, September 2021, 44(3):369-376
DOI: https://doi.org/10.5398/tasj.2021.44.3.369
Available online at http://journal.ipb.ac.id/index.php/tasj

Characterization and Antimicrobial Activity of Whey Edible Film Composite Enriched with Clove Essential Oil

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(Received 20-11-2020; Revised 29-03-2021; Accepted 20-04-2021)

ABSTRACT

Antimicrobial edible films made from clove essential oil play a role in reducing, inhibiting, or slowing the growth of bacteria that may be existed in food packaging or packaging materials to extend the shelf life of packaged foods. This study aims to produce whey and clove oil films with film thickness, elongation, tensile strength, water vapor transmission rate, optical properties, antimicrobial properties of clove oil, and its inhibition against pathogenic microbes. The physical and antimicrobial characteristics of edible film were investigated using an experimental method in a completely randomized design with four treatments. The treatment used different concentrations of clove oil (C0: 0% clove essential oil, C1: 5% clove essential oil, C2: 10% clove essential oil, and C3: 15% clove essential oil). The percentage value of elongation and microbial inhibitory activity were significantly affected ($p<0.01$) by the concentration of clove essential oil. Based on all variables, 10% clove essential oil concentration is the best treatment with a thickness of 0.035 mm, elongation of 78%, tensile strength of 8.82 N, water vapor transmission rate of 7.80 $g.mm^{-2}.day^{-1}$, and inhibitions of *E. coli* and *S. aureus* resulted in high rates. The results conclude that the addition of clove essential oil has a promising potential to improve the antimicrobial properties of whey edible film composites. The use of clove essential oil at the level of 10% is the best treatment.

Keywords: edible film composite; clove essential oil; antimicrobial activity; package food; shelf life

INTRODUCTION

Edible film began to grow rapidly in the application of livestock food-products to produce quality and guaranteed foods. Nowadays, consumers are smart in choosing foods that are not only to meet their needs but also beneficial for their health. This fact requires that foodstuffs no longer meet the basic needs of the body and must support the body's function.

Whey protein is a by-product of the dairy industry in great demand in the nutrition and polymer industry, such as a whey protein concentrate and a whey protein isolate containing 20% to 90% protein. Whey protein has the advantage of excellent mechanical and barrier properties, as were reviewed by various researchers (Ramos et al., 2012). Many studies investigated the potential use of whey protein to form edible films and coatings. Thermal degradation denatures whey protein, resulting in a more cohesive and stronger film than native proteins (Chakravartula et al., 2019). Whey protein produces films with barrier properties to the aroma, oil, oxygen, and adequate mechanical properties to provide resistance when used as a film or coating for packaging applications (Chen et al., 2019; Shendurse et al., 2018).

Edible films made from a mixture of whey and konjac (composite) can produce a new property as a result of the interaction of the two materials. The interaction between whey and konjac has an important role in forming protein structures and functional properties, such as the ability to form gels (Leuangsukrerk *et al*., 2014). The composition of the ingredients is an important factor in the manufacture of edible film because it can affect carbohydrate-protein interactions. The interactions of carbohydrates and protein in gel formation can affect the physical properties of edible-film gels produced. The interaction of carbohydrates and proteins in gels is influenced by the ratios of the basic ingredients that eventually determine the quality of the gel produced (Fahrullah *et al*., 2020b).

Polyol such as glycerol, sorbitol, and polyethylene glycol are materials commonly used as a plasticizer to improve the mechanical properties of protein layers (Fahrullah *et al*., 2020a). Baldwin *et al*. (2011) argue that welders (connectors) can modify the style of intermolecular connections that will increase the flexibility of edible films by widening the molecular-free space. The use of glycerol as a plasticizer is better than sorbitol in increasing film flexibility, lowering the strength of the bland, and the rate of moisture. The use of plasticizers can decrease modulus properties and tensile strength and increase film lengthening.

Antimicrobial edible films play a role in reducing, inhibiting, or slowing the growth of bacteria that may be existed in food packaging or packaging materials themselves to extend the shelf life of packaged foods (Sasaki *et al*., 2016). Antimicrobial materials usually come from chemicals or natural materials. The use of chemicals as antimicrobials still raises various doubts, especially in terms of health and if their use exceeds the prescribed dose. The high consumer demands for foods free from the addition of synthetic chemical compounds lead to the development of preservation methods by adding natural components or preservatives. One of the abundant natural antimicrobial ingredients and easy to obtain and considered to have antibacterial properties is clove oil. In this study, essential oils (clove oil) have been included as antimicrobial agents in whey-composite films for applications in food packaging (Bassanetti *et al*., 2017).

The properties of the clove-oil composite of edible film and its characteristics depend not only on the type of polymer used but also on its compatibility, preparation technique, and drying process (Galus & Kadzinska, 2016). This study is different from the other film studies that only dry the edible films at room temperature without oven drying. There is little information about using clove oil to prepare coatings or edible films in the literature. Previous research on the addition of edible film using clove oil (Sharma *et al*., 2020) shows that the structure of emulsion in the formation of the film changes during the drying process with the combination of polymer and clove oil. Besides that, the addition of clove essential oil results in the decreased tensile strength. However, along with the increase of clove oil concentration, the elongation value also increases. Therefore, this research aims to produce whey and clove oil films with film thickness, elongation, tensile strength, water vapor transmission rate, optical properties, antimicrobial properties of clove oil, and its inhibition against pathogenic microbes. In addition, film microstructure was also monitored.

MATERIALS AND METHODS

This experiment used clove oil obtained from CV Makmur Sejahtera, East Java, Indonesia. The cultures of *Escherichia coli* and *Staphylococcus aureus* bacteria were obtained from the Microbiology Laboratory of Faculty Animal Science, Brawijaya University, Malang, Indonesia.

Preparation of Edible Film

The whey powder at a concentration of 8% (w / v) was mixed with 0.5 g (w / v) konjac and then distilled water was added until the solution reached the final volume of 25 mL. The whey + konjac solution was added with 30% glycerol, then the film solution was heated at 90°C ± 2°C on a hot plate and stirred using a magnetic stirrer with a speed of 250 rpm for 30 minutes. The film solution was poured into a petri dish and then left to stand at room temperature for 24 hours. The finished edible film was packaged using a paper wrap for 2 days before being tested (Fahrullah *et al*., 2020b).

Thickness. The thickness of the edible film was measured by using a micrometer screw model MDC-25M (Mitutoyo, MFG, Japan), which has an accuracy of 0.001 mm. The film thickness was assessed by calculating the average thickness of five different film areas, namely 4 in the edges and 1 in the middle (Maruddin *et al*., 2018).

Elongation at break. Elongation values were measured using a Universal Instrument Tensile Strength Meter by cutting the edible film with an area of 10x5 cm and then stretching it at a speed of 50 mm/minute. The formula used for length measurement was adopted from Wardana & Widyaningsih (2017):

$$\text{Elongation (\%)} = L/L0 \times 100\%$$

where L was length of the film at break (mm) and L0 was initial length (mm).

Tensile strength. Measurement of tensile strength was carried out by cutting an 8x3 cm edible film with a diameter of 1.5 cm and attached horizontally to the clamp, and the maximum tensile strength was measured when the film showed signs of damage during the pulling process (Wittaya, 2014).

The rate of water vapor transmission. The rate of water vapor transmission was measured by cutting the film in a circle with a diameter of 2.8 cm. The cut film was stored in a glass filled with 3 g of silica gel, and it put in a desiccator, then the measurements were conducted every 24 hours for 5 days. The rates of water vapor transmission (WVTR) were expressed in units of $g/mm^{-2}.day^{-1}$ by using the following formula (ASTM E96-95):

$$WVTR = n / (t \times A)$$

where n was weight change (gram), t was time (day), and A was the area of edible-film surface (mm^2).

The microstructure of edible film. The observation of microstructures of the edible film were conducted using a scanning electron microscope SEM JEOL JSM 5310 LV model. The edible film was cut to a size of 0.5x0.5 cm; then the cut film was placed on a plate that had been coated with carbon and gold. The ready sample was then placed on the SEM device for microstructural observations.

Microbicide Characteristics

This observation was conducted to determine the presence or absence of *E. coli*, *Salmonella, S. aureus*, *coliform*, and molds. The series of dilution were made from a sample. To make a dilution from the sample, 0.1 mL liquid of the desired dilution was placed in the center of the surface of the agar plate. The L-shaped glass spreader was immersed in alcohol, then heated on a bunsen burner. The sample was spread evenly over the agar surface using a sterile glass spreader, and carefully the petri dish underneath was turned at the same time. The plates were incubated at 37°C for 24 hours. The sample CFU value was calculated. Once the colonies were counted, the counting number was multiplied by the appropriate dilution factor to determine the number of CFU/mL in the original sample (Ayu *et al*., 2020).

Antimicrobial Activity

The antibacterial activity of the edible film was tested by using the agar diffusion method. The edible film was cut with a diameter of 9.5 mm and then placed on a nutrient plate that had previously been inoculated with as much as 0.1 mL solution containing about 10^5 CFU/mL of tested bacteria. Then the incubation process was carried out at a temperature of 37°C ± 24 hours. The diameter of the zone of resistance around the film disc and the contact area of the edible film with the agar surface was observed (Tooraj *et al*., 2012).

Film Color Measurement

The measurements of film color were performed using the Minolta CR 300 Chromameter. The edible film sample was placed on a white mat. The measurement yielded the values of L*, a*, and b*. L* represented the brightness parameter (a chromatic color, black= 0 to white= 100), a* and b * are the chroma coordinate colors, a (-60: green to +60: red, b (-60: blue to +60: yellow) (Bourtoom *et al*., 2008).

Statistical Analysis

The research used a completely randomized design with five replications. The treatment used different concentrations of clove oil (C0= 0% clove essential oil, C1= 5% clove essential oil, C2= 10% clove essential oil, and C3= clove essential oil 15%). The data were analyzed using analysis of variance (ANOVA). If the treatment gave a difference, it was followed by the least significant difference (LSD).

RESULTS

Physical Characteristics of Composite of Whey Edible Film

The analysis of variance showed that the addition of clove essential oil at different levels did not provide a significant difference in the thickness of the composite of whey edible film. The value of thickness with the addition of clove essential oil at doses of 0%, 5%, 10%, and 15% were 0.0357 mm, 0.0348 mm, 0.0353 mm, and 0.0357 mm, respectively (Table 1).

Analysis of variance showed that the addition of clove essential oil showed a significant difference ($p<0.01$) in the elongation value of whey edible film composite. The average percentage of film elongation produced was 67.64%. The values of elongation of whey edible film composite with the addition of clove essential oil at doses of 0%, 5%, 10%, and 15%, were 53.21%, 76.00%, 78.00%, and 63.33%, respectively (Table 1).

Analysis of variance showed that the addition of clove essential oil at different levels did not give a significant difference in the tensile strength of the whey edible film composite. The resulting average tensile strength was 7.96 N. The value of tensile strength with the addition of clove essential oil at doses of 0%, 5%, 10%, and 15% were 8.86 N, 6.90 N, 8.82 N, and 7.26 N, respectively (Table 1).

Analysis of variance showed that the addition of clove essential oil at different levels did not significantly affect the WVTR of the whey edible film composite. However, although the results were not statistically different, the higher the addition of clove essential oil tended to increase the WVTR of the whey edible film composites as well.

Observation of the microstructure aims to determine the characteristics of the edible film. The microstructure of whey edible film composites added with clove essential oil was observed using the Scanning Electron Microscope method (Figure 1). Whey edible film added with clove essential oil at a concentration of 5% showed a less homogeneous microstructure between the polymer and clove essential oil, and the use of clove essential oil at a concentration of 10% showed that the concentration had a rough surface and a looser texture with an elongated structure like a stem and had an empty cavity. The structure of the edible film with a concentration of 10% clove essential oil began to show a unifying structure between polymer films. Likewise, the addition of clove essential oil at the level of 15% showed the structure that was starting to flatten. This result showed that the clove essential oil was trapped homogeneously in the mixture of film solution.

Microbicide Characteristics

The addition of clove essential oil at different levels did not show the growth of *E. coli*, *Salmonella*, and *S. aureus*, and the total mold was only found in the film without the addition of clove oil with a value of $1.0\text{x}10^1$. The amount of coliform at a concentration of 3.6 MPN/g was found in the control treatment and with the addi-

Table 1. Physical characteristics of whey edible film composite with the addition of clove essential oil

Characterization	Clove essential oil			
	C0	C1	C2	C3
Thickness (mm)	0.0357 ± 0.0015	0.0348 ± 0.0017	0.0353 ± 0.0012	0.0357 ± 0.0010
Elongation (%)	53.21 ± 12.77^c	76.00 ± 18.01^a	78.00 ± 18.94^a	63.33 ± 14.90^b
Tensile strength (N)	8.86 ± 3.60	6.90 ± 2.66	8.82 ± 1.39	7.26 ± 0.97
WVTR (g/m^2.day^{-1})	7.15 ± 0.65	8.45 ± 0.44	7.80 ± 0.44	8.61 ± 0.92

Note: C1= 0% clove essential oil, C2= 5% clove essential oil, C3= 10% clove essential oil, C4= 15% clove essential oil, WVTR= water vapor transmission rate. Means in the same row with different superscripts differ significantly ($p<0.01$).

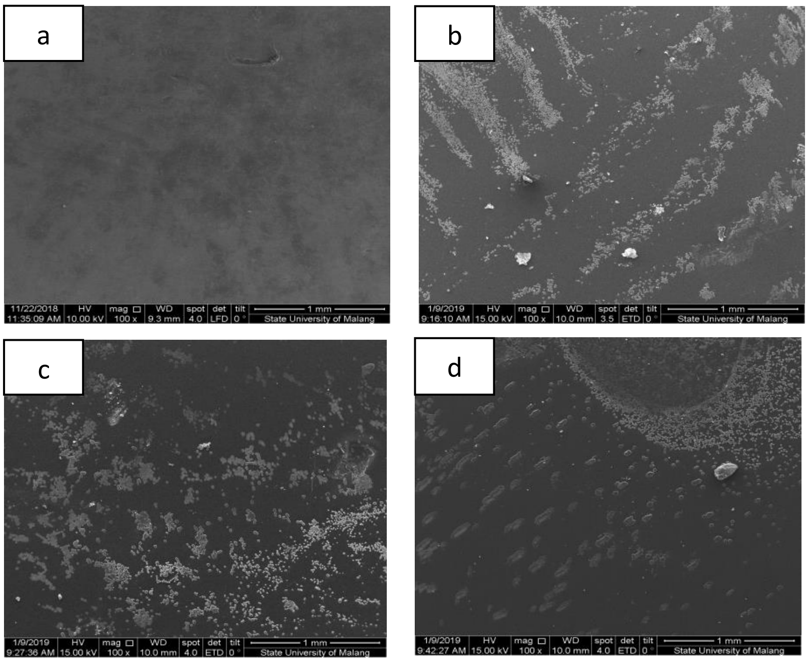

Figure 1. Microstructure of the whey composite film with clove essential oil at 0% (a), 5% (b), 10% (c), and 15% (d).

tion of clove oil, the concentration of coliform was ≤ 3 MPN/g (Table 2).

Antibacterial Activity

The analysis of variance shows that the use of clove oil concentration results in the very significant differences ($p<0.01$) in the inhibition of *E. coli* and *S. aureus*. Furthermore, the LSD test showed that the addition of clove essential oil at different concentrations gave significant effects ($p<0.01$) on the inhibitions of *E. coli* and *S. aureus* (Table 3).

Film Color Measurement

The addition of different concentrations of clove essential oil to the whey-based edible film did not give significant effects on the color, with the overall color was seen as a transparent color (Table 4). The color measurement in this research was based on the Hunter system, by using L* (0= black, 100= white), a* (-60= green, +60= red), and b* (-60= blue, +60= yellow) coordinates.

Table 2. Microbicide characteristics of the whey edible film composite with the addition of clove essential oil

Microbes	Clove essential oil			
	C0	C1	C2	C3
Escherichia coli (CFU/g)	-	-	-	-
Salmonella (CFU/g)	-	-	-	-
Coliform (MPN/g)	3.6	≤3	≤3	≤3
Staphylococcus aureus (CFU/g)	-	-	-	-
Molds (CFU/g)	$1.0x10^1$	-	-	-

Note: C1= 0% clove essential oil, C2= 5% clove essential oil, C3= 10% clove essential oil, C4= 15% clove essential oil.

DISCUSSION

Physical Characteristics of Composite Whey Edible Film

Thickness. The thickness of the edible film in this research was proven to be affected by the increase in film

Table 3. The average diameter of the resistance zone (mm) of clove essential oil against *Escherichia coli* and *Staphylococcus aureus*

Clove essential oil (%)	Inhibition (mm)	
	Escherichia coli	*Staphylococcus aureus*
C1	20.88 ± 0.31^b	16.60 ± 0.29^c
C2	21.58 ± 0.98^b	17.40 ± 0.35^b
C3	22.76 ± 0.83^a	18.42 ± 0.83^a

Note: C1= 0% clove essential oil, C2= 5% clove essential oil, C3= 10% clove essential oil, C4= 15% clove essential oil. Means in the same column with different superscripts differ significantly ($p<0.01$).

Table 4. Color measurement of whey edible film composite with the addition of clove essential oil

Color	Clove essential oil			
	C0	C1	C2	C3
L*	45.5 ± 1.93	45.9 ± 1.00	45.5 ± 0.97	46.7 ± 1.66
a*	8.8 ± 0.34	8.8 ± 0.25	8.9 ± 0.43	9.5 ± 0.57
b*	22.5 ± 0.89	22.3 ± 0.70	22.6 ± 1.52	23.2 ± 1.68

Note: C1= 0% clove essential oil, C2= 5% clove essential oil, C3= 10% clove essential oil, C4= 15% clove essential oil.

components along with the increase in the concentration of clove essential oil. The mixing of the composite film (a mixture of whey and konjac) with the addition of clove essential oil each contributed to the thickness of the film produced. In addition, the addition of a glycerol plasticizer also affects the thickness of the film produced. This result agrees with Nugroho *et al*. (2013), which state that increasing the total amount of solution will increase the total solids in the solution. Increasing the number of solids in the solution results in a higher number of polymers forming an edible film matrix. In addition to the total solids in the solution, the thicker the edible film is influenced by the viscosity and the content of constituent polymer. In addition, the glucomannan content in konjac can form a thick mass (gel) that can form a film layer (Fahrullah *et al*., 2020b).

Elongation. The percentage value of elongation found in the present study does not meet the standard edible film elongation, which is at least 70% (Japanese Industrial Standard, 1975). The high elongation indicates that the film is more flexible when subjected to stress or mechanical stress. Heat treatment can separate the quaternary structure of the protein and desaturate the molecular protein chains and cause intermolecular interactions that are not possible in the original protein form. Clove essential oil can directly enter into the film matrix. This result has a good effect because the film is biodegradable due to the increased concentration of antimicrobial agents, promoting more effective action against microbes (Chen *et al*., 2018). Benavides *et al*. (2012) state that in the increasing lengthening of the film with the addition of oil droplets, it can be deformed to increase the film's flexibility. Materials with high elongation values provide good flexibility due to the cohesion between molecular polymers (Lorevice et al., 2016). Fahrullah *et al*. (2020a) revealed that the addition of sorbitol plasticizer would result in a higher percent elongation of whey edible film than glycerol and PEG plasticizers.

Tensile strength. The tensile strength results in this study with the addition of 15% clove essential oil to the film-forming mixture showed a decrease in tensile strength compared to the addition of 5% clove essential oil. This result is due to the hydrophobic nature of clove essential oil, resulting in a low tensile strength of the film. The addition of compounds having hydrophobic properties to the film solution causes a weakening of the tensile strength but can increase the percentage of film elongation (Sharma *et al*., 2020). The tensile and elongation forces are usually also related to the microstructure of the film and the intermolecular forces between them (Atarés *et al*., 2010). Many studies have shown that the incorporation of essential oils affects tensile strength depending on the interaction of the composite film matrix with the added oil (Atarés & Chiralt, 2016). Chen *et al.* (2018) reported a decrease in tensile strength by 14.13% and an increase in elongation by 26.64% after using clove essential oil in an active polyvinyl alcohol film. The destabilization process can occur during film drying and can cause a decrease in tensile strength (Hosseini *at al*., 2015).

Water vapor transmission rate. The addition of clove essential oil at a concentration of 15% gave the largest rate of water vapor transmission compared to the other concentrations, namely 8.61 g/mm^{-2}.day^{-1}. The average rate of water vapor transmission produced was 8 g/m^{2}.day^{1}, and this result met the Japanese Industrial Standard (1975), which required a maximum of 10 g/m^{2}. day^{-1}. Analysis of variance showed that the addition of clove essential oil did not give a significant difference ($p>0.05$) in the values of the water vapor transmission rates in the whey edible film composite. The protein content in the whey has an impact on higher water absorption so that in essence, the value of the water vapor transmission rate produced by the whey edible film composite is more hygroscopic than the edible film with low protein content. Several research results related to this have varied, such as Fahrullah *et al*. (2020a) yielded 7.63-7.96 g/mm^{-2}.day^{-1} and Fahrullah *et al*. (2020b) yields 7.64-8.45 g/mm^{-2}.day^{-1}.

Microstructure of film. Figure 1 shows the SEM of clove oil at concentrations of 0%; 5%; 10%, and 15%. The microstructures of the films reveal the structural arrangement of its components that influence both the physical and mechanical properties of the films (Cofelice *et al*., 2019). Microstructure of edible films surface shows continuous, compact, and homogenous structures, without any irregularities such as cracks or air bubbles. Film microstructure was affected by the homogenization method, emulsion composition, and structural arrangement of the different components obtained at the end of the drying process.

Whey edible film added with clove essential oil at a concentration of 5% showed a less homogeneous microstructure between the polymer and clove essential oil. To get the best results, it is necessary to add lecithin. As an emulsifier, lecithin is water and oil-soluble. The workability of the emulsifier is mainly due to the shape of the molecules that can be bound to oil or water. If the emulsifier is more water-bound or more water-soluble (polar), then it can help the dispersion of oil in the water so that it will occur oil-in-water emulsion. In the edible film, the use of clove essential oil at a concentration of 10% produces a rough surface and a looser texture with an elongated structure like a stem and has an empty cavity. This result is related to forming two phases (polymer and oil) in the film matrix with a higher percentage of clove essential oil. Increasing the concentration of clove essential oil in the edible film solution led to an increase in the interaction between oil particles and the polymer matrix. This increase tends to produce or form large pores. Unlike emulsifiers with small molecular weight, which can diffuse rapidly into the interface to form a good emulsion, proteins tend to occupy large spaces and have a slow diffusion rate. The hydrophobic surface affects the ability of the protein to adsorb oil on the surface, where the greater the hydrophobic nature of the protein, the higher the emulsion capacity. The surface of the protein charge affects the rate of diffusion of the protein towards the surface of the oil grain. The structure of the edible film with the addition of clove essential oil at a concentration of 10% began to show a unifying structure between polymer films.

Likewise, the addition of the clove essential oil at a concentration of 15% shows the structure that is starting to flatten. This result shows that the clove essential oil is trapped homogeneously in the mixture of film solution. The formation of lipid droplets and their development during film drying entail the interruption of the polymer matrix, increasing the internal heterogeneity and surface roughness of the film (Fabra *et al*., 2009).

Whey protein plays a role in emulsion stabilization by forming a stabilizing layer around the fat droplets. Whey protein must form a protective layer around the fat droplets during the homogenization process and emulsion stability obtained from the homogeneous distribution of fat in the film (Kurek *et al*., 2014).

Microbicide Characteristics

Whey edible film composite containing antimicrobial agents can inhibit the release of antimicrobial substances into the food. The main inhibiting components of clove essential oil for microbes are eugenol, ß-caryophyllene, and acettaugenol. Clove essential oil has the potential to interact with bacterial cell membranes or the other intracellular components, and in turn, will result in disruption of cell configuration, ion exchange, leakage, changes in permeability, inhibition of respiration, and microbial death (Tajkarimi *et al*., 2010). The antimicrobial mechanisms of eugenol vary, including degrading bacterial cell walls, damaging cell membranes and membrane proteins, and then infiltration by intracellular substances (Bassanetti *et al*., 2017). Eugenol also inhibits the activity of several enzymes, such as L-asparaginase, which involves Salmonella virulence mechanisms (Vimal *et al*., 2018). Numerous studies have reported the ability of eugenol to inhibit pathogenic microbes, including Salmonella (Gutierrez *et al*., 2017).

Research by Sharma *et al*. (2020) showed that the edible film of whey composite added with the addition of clove oil at a concentration of 1% showed an antibacterial effect against *E. coli* and *S. aureus* in the first 4 hours, and a concentration of 5% clove oil showed an inhibitory effect on *E. coli* for up to 12 hours and the rest did not show this effect. Edible film of whey composite with clove essential oil at a concentration of 10% showed that the growth of *E. coli* bacteria was reduced from 6.5 log CFU/mL to 4.4 log CFU/mL. Inhibition of *S. aureus* bacteria at 5% clove essential oil concentration decreased from 6.5 log CFU/mL to 4.5 log CFU/mL. The composite film with clove essential oil at a concentration of 10% showed total *S. aureus* killing by reducing growth from 6.5 log CFU/mL to 0 log CFU/mL. The incorporation of clove essential oil has higher bacterial inhibition efficacy against Gram-positive bacteria (Song *et al*., 2014).

Antibacterial Activity

Antibacterial active packaging prevents bacterial growth on food surfaces due to direct contact with packaging materials (Sharma *et al*., 2020). The average inhibition power of clove essential oil against *E. coli* resulted in a bright zone diameter of > 20 mm (strong growth inhibition response). In contrast, the average inhibition power of clove essential oil against *S. aureus* growth resulted in a diameter of 16-18 mm (growth inhibition response moderate). The difference in inhibitory power between *E. coli* and *S. aureus* is caused by the characteristics of the cell walls between the two bacteria. *E. coli* has only one wall, whereas *S. aureus* has cell walls that are multilayered. The addition of clove essential oil in a higher concentration produces the higher the diameter of the inhibition zone related to the eugenol content found in clove essential oil. The ability of clove essential oil to inhibit bacterial growth is due to its high eugenol content (Sharma *et al*., 2020). This property can enter the lipopolysaccharide found in the cell membrane of gram-negative bacteria and damage the cell structure. Tajkarimi *et al*. (2010) reported that the increasing concentration of clove essential oil increases the inhibition of *B. cereus*, *E. coli*, and *S. aureus*. Cloves contain phenolic components such as thymol and eugenol, which are bactericidal against *E. coli*. Clove oil is more effective because it contains a higher concentration of eugenol, i.e., 180 mg (Khalil *et al*., 2017).

The mechanism of antimicrobial activity of clove is related to the attacking of the phospholipids present in the cell membrane, which increases the permeability and leakage of the cytoplasm or its interaction with enzymes present in the cell wall. In other words, phenolic compounds in cloves inhibit the growth of microorganisms by sensitizing the phospholipid bilayer of the cell membrane (Hosseini *et al*., 2019).

Film Color Measurement

The highest brightness (L*) was found in edible film produced by adding clove essential oil at a concentration of 15%. Furthermore, the highest a* and b* values were still at the addition of olive oil at a concentration of 15%. This result is due to the natural color of the clove oil itself. This phenomenon may indicate light absorption at higher wavelengths. The transparency and color of the edible film are the two most important things in the acceptance and appearance of consumers as a whole (Atarés *et al*., 2010). The addition of oil affects the color and transparency of the edible film (Atarés *et al*., 2010). The main factors that influence the formation of color (L*, a*, and b*) are whey edible film composite and the use of plasticizers that are influenced by the nature of the material of edible film formulation that covers it.

Best Treatment

The procedure of selection for the best treatment implements a method introduced by Zeleny (1982) in which a test parameter determines the ideal value. An ideal value is a value that is hoped indicated by the maximum or minimum value in the test parameter. To determine the best treatment, a multiple attribute method is applied. The parameters valued are thickness (mm), tensile strength (N), elongation (%), water vapor transmission rate ($g/m^2.day^{-1}$.), microbicide characteristics, and antibacterial activity. The use of clove essential oil at a concentration of 10% is the best treatment.

In this study, the best treatment of the low thickness value was at 0.035 mm; high tensile strength value at 78%; high elongation value at 8.82 N; low WVTR value at 7.80 g/mm-2.day^{-1}. While for microbial testing, it produced high antimicrobial activity strong growth inhibition response.

CONCLUSION

The present study concluded that different levels of clove oil in the film significantly affected the elongation and antimicrobial activity, except for thickness, tensile strength, WVTR, and colors. The addition of clove essential oil has a promising potential to improve the antimicrobial properties of whey edible film composites. The use of clove essential oil at a concentration of 10% is the best treatment with a thickness of 0.035 mm, 78% elongation, 8.82 N tensile strength, and 7.80 g.mm^{-2}.day^{-1} water vapor transmission rate

CONFLICT OF INTEREST

The author does not have a conflict of interest with personal and other relationships related to this research.

ACKNOWLEDGEMENT

The author would like to thank the BUDI-DN Scholarship and the Educational Fund Institution, the Ministry of Finance of the Republic of Indonesia who funded this research

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