

Development of Antimicrobial Edible Film Enriched with Double Emulsion of Cinnamon (*Cinnamomum burmannii*) Essential Oil

Pembuatan Edible Film dengan Penambahan Antimikroba dari Emulsi Minyak Atsiri Kayu Manis (Cinnamomum burmannii)

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ABSTRACT

The edible film can be used as a carrier of bioactive compounds that contributed to the shelf life or nutritional benefit of food products; however, the addition of bioactive compounds relied greatly on the compatibility of the bioactive compound toward the edible film matrix. Most of the bioactive compounds are nonpolar which are incompatible with the polar nature of the edible film. In this research, the nonpolar essential oil of cinnamon, a potent antimicrobial agent, was made into a double emulsion. The double emulsions were made through a two-step emulsification stage, with CaCl₂ as the inner water phase and guar gum as the outer water phase. The physicochemical characteristics (stability, viscosity, and droplet size) and the antimicrobial activity of the double emulsion were observed. The double emulsion showed stability up to 7 days of storage at room temperature with high antimicrobial activity; MBC values of 0.86, 1.37, 0.31, and 0.51 mg/mL against *E. coli*, *S. aureus*, *R. stolonifera*, and *A. niger*, respectively. Different concentrations (5%, 10%, 15%) of both emulsions were added into edible film suspension. The formation of double emulsion showed a promising result as a means to incorporate nonpolar compounds into basic edible film formulation to increase its functional properties while retaining their physicochemical characteristic. All formulations showed good edible film characteristics, with edible film with 8% essential oil showing a high inhibition zone (15.81 dan 6.92 mm) toward *E. coli* and *R. stolonifer*, 0.0052 mm thickness, the tensile strength of 6.32 MPa, 13% elongation and WVTR of 1.06 g/cm².h.

Keywords: Edible Film; Double Emulsion; Cinnamon; Essential Oil; Antimicrobial Activity

ABSTRAK

Kemasan edible dapat digabungkan dengan berbagai senyawa bioaktif yang dapat meningkatkan umur simpan maupun memberikan fungsi nutrisi tambahan pada produk pangan. Sebagian besar dari senyawa bioaktif alam ini bersifat non-polar sedangkan polimer bahan kemasan edible umumnya bersifat polar sehingga penambahan senyawa bioaktif ini akan bergantung dari tingkat kecocokan dari bahan aktif dengan polimer dari bahan kemasan. Dalam penelitian ini minyak atsiri dari kayu manis akan dimasukkan kedalam kemasan edible dengan proses emulsi ganda, menggunakan kalsium klorida dan guar gum. Sifat fisikokimia dari emulsi ganda minyak kayu manis (stabilitas, kekentalan dan ukuran droplet) serta aktivitas antimikroba dari emulsi minyak kayu manis akan dianalisa. Emulsi ganda minyak kayu manis menunjukkan kestabilan dari segi ukuran droplet dan kekentalan selama 7 hari penyimpanan dan memiliki nilai inhibisi sebesar 0.86, 1.37, 0.31, and 0.51 mg/mL terhadap mikroorganisme *Escherichia coli*, *Staphylococcus aureus*, *Rhizopus stolonifer* dan *Aspergillus niger*. Penambahan emulsi ganda minyak kayu manis kedalam kemasan edible terlihat meningkatkan sifat fungsional (antimikroba) dari kemasan dan tetap mempertahankan sifat fisik kemasan yang baik. Edible film dengan 8% penambahan minyak atsiri kayu manis, memiliki diameter inhibisi sebesar 15.81 dan 6.92 mm terhadap *E. coli* dan *R. stolonifer*, memiliki ketebalan 0.052 mm, kuat tarik 6.32 MPa, kemuluran sebesar 13% dan kemampuan serap air sebesar 1.06 g/cm².jam.

Kata kunci: Edible film; Emulsi ganda; Minyak Atsiri; Kayumanis; Aktivitas Antimikroba

INTRODUCTION

The Edible film has been applied to the food surface, to replace conventional packaging. The edible film has the advantage of being a protective barrier that did not alter the character of the food product. The edible film also has superiority in carrying biochemical compounds such as essential oil with antimicrobial and antioxidant properties that might prolong the shelf life of perishable food products.

However, the incorporation of essential oil into the edible coating has some limitations as an essential oil has a low solubility, which contributes to a phase separation between the immiscible component, resulting in a nonhomogeneous film (Bahram et al., 2014). To overcome the immiscibility between the polar nature of the edible film and the non-polar nature of the essential oil, in this research the essential oil will be formed into double emulsion droplets.

Double emulsion is defined as an emulsion of emulsion in which the droplet of the dispersed phase contained a smaller droplet of miscible substance. In the W/O/W emulsion, the small-sized water droplets (W_1) are contained within the larger oil droplets (O), and both are dispersed within another aqueous continuous phase (W_2). Double emulsion droplets have been known to have better dissolution ability, better releasing ability, and better protection toward sensitive molecules from external factors (Ding et al., 2019). The double emulsion techniques in this research were done to improve the immiscibility of essential oil into the edible film.

Cinnamon (*Cinnamomum burmannii*) is a native plant that originated from Sri Lanka and South India and is widely cultivated in Indonesia (El Atki et al., 2019). The cinnamon essential oil is usually obtained from distillation of the dried cinnamon tree bark and yields around 0.5-2.0%. The major component of cinnamon essential oil is cinnamaldehyde (80%) and other phenolic compounds (12%) such as α -terpineol, benzaldehyde, and coumarin (Nabavi et al., 2015; Noshirvani et al., 2017; Tarek et al., 2014). Cinnamon oil has many biological activities such as antimicrobial activities, antioxidant activity, and many other functional properties. Cinnamon oil's antimicrobial activity is effective toward both Gram-positive and Gram-negative bacteria such as *S. Aureus* and *E. Coli*, respectively, and also toward fungi such as *Aspergillus* and *R. stolonifer*. In this research, the cinnamon essential oil will be encapsulated in the form of a double emulsion and incorporated into the edible film base.

The edible film will then be tested for its physicochemical and functional ability.

METHODS

The main materials used in the research were cinnamon barks obtained from Balitro (Balai Penelitian Tanaman Rempah dan Obat), and PGPR (PT. Tegar Inti Sentosa), CaCl_2 , guar gum, distilled water, corn starch (maizenaku) and glycerol. Other materials needed were dimethyl sulfoxide (DMSO), nutrient agar (NA), nutrient broth (NB), potato dextrose agar (PDA), and potato dextrose broth (PDB), tartaric acid, immersion, oil, and gram stains. The microorganisms used for the determination of antimicrobial activity were pure cultures of *E. coli*, *S. aureus*, *R. stolonifer*, and *A. niger*. The equipment used was distillation apparatus, centrifuge (Hettich Zentrifugen EBA 20 and Hermle), centrifuge tubes, graduated cylinders, beaker glasses, desiccator, film applicator, oven (Mettler), petri dish (Gosselin), caliper, microscope (Olympus), mixer (Philips HR1538), food processor (Philips HR2115), heater (Cimarec), magnetic stirrer, analytical balance, cuvettes, spectrophotometer (Genesys 20), viscometer (Brookfield DV-1+), water bath (Mettler WB14) and texture analyzer (Lloyd Instruments LR 50K).

Table 1. Double emulsion formulations

Component	Percentage (w/w)	
	Formulation 1 (4% CEO)	Formulation 2 (6% CEO)
Cinnamon oil	4.00	6.00
PGPR Solution	0.08	0.12
1% CaCl_2 solution	5.92	3.88
1% Guar gum solution	90.00	90.00

Table 2. Edible film formulations

Formulations	DE (%)	CS (%)	Gly (%)	DW (%)
4%CEO-2%DE	2.0	4.0	1.5	92.5
4%CEO-4%DE	4.0	4.0	1.5	90.5
4%CEO-8%DE	8.0	4.0	1.5	86.5
6%CEO-2%DE	2.0	4.0	1.5	92.5
6%CEO-4%DE	4.0	4.0	1.5	90.5
6%CEO-8%DE	8.0	4.0	1.5	86.5

Note: CEO: cinnamon essential oil; DE: double emulsion; CS: corn starch; Gly: glycerol; DW: distilled water

Extraction of Cinnamon Essential Oil

The extraction of cinnamon essential oil was conducted following (Wong et al., 2014) the steps with 100 g of cinnamon barks were placed into a 1 L distillation flask (round bottom flask) connected to the Bidwell-sterling and cold-finger condenser to retrieve the oil, with distilled water added into the same flask. The essential oil was extracted with boiling distilled water at 100 °C for 3 hours. After the distillation process, the mixture of cinnamon essential oil and water was collected and separated using a centrifugation process at 5000 rpm for 3.5 min.

Double Emulsion Formation

The double emulsion was formulated following the procedures from (Paula et al., 2018). The first step was the making of the inner water phase (W_1) which was a 1% CaCl_2

solution and the outer water phase (W_2) which was a 1% guar gum. The oil phase (O) was made by mixing 1 g Polyglycerol-polyricinoleate (PGPR) and cinnamon oil at 1200 rpm and further equilibrated in a 50°C water bath for 15 min with constant stirring. The first emulsion was made by mixing an equal amount of inner water phase (W_1) and oil phase (O) with constant stirring at 1000 rpm to form (W_1/O) emulsion. The second emulsification method was done by combining the first (W_1/O) emulsion with (W_2) guar gum solution in a ratio of 1:9 with 5 minutes of homogenization.

Edible Film Formulation

Edible films were made with formulations shown in Table 2 following the procedure from (Sabari et al., 2017). Each mixture was homogenized and heated until 85 °C with constant stirring conducted for 30 minutes. The suspension

was cooled to 50 °C, followed by the addition of double emulsion into the cooled edible film solutions. The edible film solutions were placed on a film applicator to form the thin film. The films were left to dry at 55-60 °C for 24 h.

Physicochemical Analysis

The physicochemical analysis of the cinnamon essential oil obtained (Wong et al., 2014) includes yield and antimicrobial activity. For the double emulsions, the physicochemical analysis that is performed includes droplet size, viscosity, and stability by centrifugation following the research conducted by (Tekin Pulatsu et al., 2018) and creaming index (Yildirim et al., 2017). As for edible films, the physicochemical analysis included thickness value, opacity value, tensile strength, elongation, swelling properties, and water vapor transmission rate (Šuput et al., 2016; Saberi et al., 2017) and antimicrobial activity (Lixandru et al., 2010). The mechanical properties will be compared with the Japanese International Standard for Film Packaging (Nurindra et al., 2019).

RESULTS AND DISCUSSION

Cinnamon Essential Oil Extraction

The yield (% w/w) of cinnamon essential oil obtained through the distillation process was 0.79±0.05% which was higher than several other research 0.45-0.86% (Khumpirapang et al., 2021; Lixandru et al., 2010; Tarek et al., 2014). The difference in yield of the cinnamon essential oil was due to some factors, one of them being the distillation condition which includes pressure, temperature, and contact time. The size of the cinnamon barks will also be affected, to which the smaller the size of the cinnamon barks, the higher the yield is as more surface area of the barks are being exposed and the reduction in the size of cinnamon barks will open the structure inside the cinnamon barks, hence more oil are extracted. The part from where the cinnamon barks are taken will also affect the yield which is associated with the plant's metabolism. The closer the part is to the leaves and the higher the location of the part is, the higher the yield of the cinnamon essential oil will be.

Antimicrobial Activity Determination of Cinnamon Essential Oil

Table 3 showed the Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) value of cinnamon essential oil obtained for *E. coli*, *S. aureus*, *R. stolonifer*, and *A. niger*.

The cinnamon essential oil was shown to have antimicrobial activity toward all test microorganisms. *Cinnamomum burmannii* is known as a major bioactive component that possesses significant antibacterial activities against *S. Aureus* and *E. Coli*. Cinnamon oil contains a phenolic compound that attacks the cell membrane due to its hydrophobic nature leading to the depletion of the cell content (Nabavi et al., 2015). The main component of cinnamon oil, cinnamaldehyde, is a compound containing an aldehyde group conjugated to a carbon double bond with a highly electronegative arrangement. This high electronegativity will disrupt many biological processes that rely on electron transfer between molecules. This compound will also bind with nitrogen-containing structures of the bacteria such as DNA and protein thus interfering with other biological functions (Liu et al., 2017; Nabavi et al., 2015). Cinnamon essential oils have high antifungal activity toward *R. Stolonifer* and *A. niger* through the destruction of mitochondrial and plasma membranes causing the cytoplasm to coagulate, folding of the cell wall, and the loss

of cell wall regularity. Cinnamaldehyde will also inhibit the cell wall synthesizing enzymes (i.e. β -(1,3)-glucan synthase, chitin synthase, and ergosterol biosynthesis of the fungal membrane, leading to membrane damage and leakage of cell content (Dibazar et al., 2015; Li et al., 2014).

Table 3. MIC and MBC value of cinnamon essential oil

Test microorganisms	MIC (mg/mL)	MBC (mg/mL)
<i>E. coli</i>	0.22	0.86
<i>S. aureus</i>	0.09	0.37
<i>R. stolonifer</i>	0.33	1.31
<i>A. niger</i>	0.12	0.50

As could be seen in Table 3, for both MIC and MBC, the highest value was observed in *R. stolonifer* followed by *E. coli*, *A. niger*, and *S. aureus* as the microorganism with the least MIC and MBC value. *E. coli* had a higher MIC (0.22 mg/mL) value compared to *S. aureus* (0.09 mg/mL). Gram-positive bacteria such as *S. aureus* are more sensitive to essential oil than Gram-negative bacteria such as *E. coli*. This is due to the existence of an outer membrane that surrounds the peptidoglycan layer of the Gram-negative bacteria. Lipopolysaccharide outer membrane of Gram-negative bacteria restricts diffusion of hydrophobic compounds, preventing the accumulation of essential oils on the membrane, resulting in the need for having a higher concentration of essential oil to inhibit the growth of Gram-negative bacteria. The outer membrane outside of the thin peptidoglycan layer is composed of a double layer of phospholipids that is linked to the inner membrane by lipopolysaccharides. The lipopolysaccharide structure provides the protection which allows Gram-negative bacteria to be more resistant to essential oils as well as other natural extracts exhibiting antimicrobial activity. Vice versa, the structure of the Gram-Positive bacteria cell wall allows hydrophobic molecules to easily penetrate the cells, acting on their cell wall and even into the cytoplasm.

R. stolonifer had a higher MIC value compared to the test bacteria. Molds are more complex than bacteria, with an internal membrane system dividing the cell into different regions, membrane-enclosed organelles, and having the DNA contained in a membrane-bound nucleus, and essential oils can also exist in the potentially highly bioactive vapor phase. This vapor phase is often more effective especially against fungi than the liquid form, as lipophilic molecules responsible for some parts of the activity may associate in the aqueous phase to form micelles, which will suppress the molecules' attachment to the organism (Noshirvani et al., 2017). This is also associated with the fact that fungal strains tend to grow more on the agar surface than bacteria. Hence, molds will tend to be more exposed to the vapor, unlike bacteria which will be mainly affected by essential oil compounds present in the substrate. *A. niger* showed a lower MIC value compared to *R. stolonifer*. *A. niger* is one of the molds that are more sensitive and susceptible to an essential oil (Li et al., 2014; Lixandru et al., 2010; Noshirvani et al., 2017). Different essential oils may have a great variety of active compounds responsible for either larger or smaller antimicrobial activity, depending on the constituents of the essential oil and the characteristics of the microorganism.

Table 4. Double Emulsion Stability

The concentration of oil in double emulsion	4% cinnamon oil		6% cinnamon oil	
	Day 0	Day 7	Day0	Day 7
Stability by centrifugation (%)	100±0	100±0	100±0	100±0
Droplet size (µm)	37.07±2.37	46.81±4.73	50.70±5.32	52.63±3.56
Viscosity (cP)	7034.88±30.17	7071.38±21.34	8150.00±56.82	8131.25±46.21

Double Emulsions Characteristic

The double emulsions were analyzed for their stability characterization including droplet size and also viscosity during day 0 and day 7 of storage as shown in Table 4. The stability of a double emulsion is affected mainly by the concentration of the essential oil. It can be seen that the increase in cinnamon oil concentration affected the droplet size, viscosity, volume of the phases, and the osmotic gradient (Paula et al., 2018). Both double emulsions with 4% and 6% oil showed 100% stability after centrifugation, implying that separation did not occur. Separation might occur if the oil phase droplets in the dispersed phase accumulate on the top of the aqueous phase. This happens due to the effect of floatation or buoyancy as the oil droplets are less dense (Ding et al., 2019; Ghasemi et al., 2020). However, with the presence of gum as a stabilizer, the thickening ability of guar gum results in a more viscous emulsion system, which then also increases the stability of the emulsions. Emulsions with higher viscosity values will tend to exhibit higher stability values as at the same time, the creaming process is slowed down (Tekin Pulatsü et al., 2018).

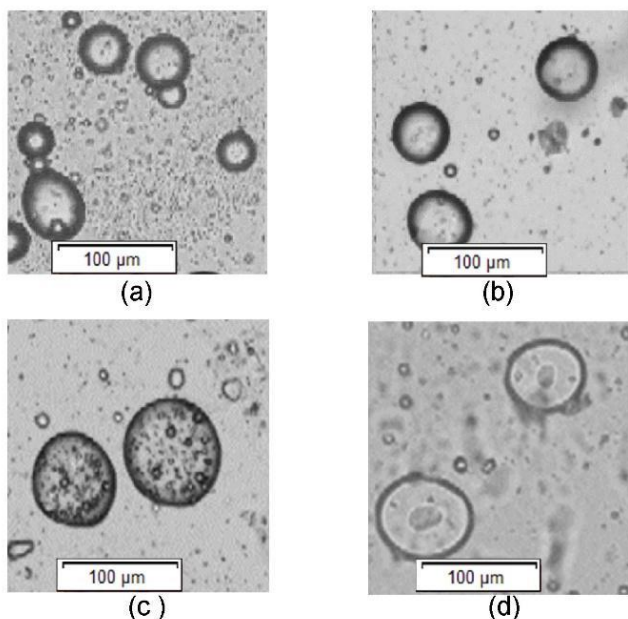


Figure 1. Double emulsion droplets are seen unmicroscoperope with 1000x magnification. Note: (a) 4% oil (day 0); (b) 4% oil (day 7); (c) 6% oil (day 0); (d) 6% oil (day 7)

The droplet size of double emulsion is affected by various factors, such as the type and concentration of emulsifiers, phase volume fractions, interfacial properties, viscosity ratios, and composition of the phases. The droplet size of 4% oil double emulsions ranged from 19.36 µm to 61.60 µm and from 24.64 µm to 98.56 µm during day 0 and day 7 of storage, respectively. While the droplet size of 6% oil double emulsions ranged from 22.88 µm to 96.80 µm and from 24.64 µm to 96.80 µm during day 0 and day 7 of storage, respectively. Double emulsion with 6% oil had larger droplets

compared to double emulsion with 4% oil, to which there was a significant difference ($p \leq 0.05$) in the droplet size between double emulsions with 4% oil and 6% oil. This correlated to the literature mentioning that higher oil content can inhibit the activity of the stirrer to dissipate oil droplets, resulting in larger droplets which are shown in the higher droplet size in double emulsion with 6% oil (Niknam et al., 2020; Terjung et al., 2012).

As seen through statistical analysis, there was a significant increase ($p \leq 0.05$) in droplet size of the double emulsion made with 4% cinnamon essential oil between day 0 and day 7 of storage time. However, there was no significant increase ($p > 0.05$) in droplet size of the double emulsion made with 6% cinnamon essential oil after 7 days of storage time. In general, a higher concentration of oil phase will increase emulsion instability through the flocculation process which is followed by coalescence to form bigger droplets. A double emulsion made with 4% cinnamon oil had more water phase compared to the double emulsion with 6% oil thus may induce greater instability due to increased incidence of coalescence and bridging at higher water volume (Ghannam, 2005).

Statistically, there was no significant ($p > 0.05$) difference in terms of viscosity for both double emulsions containing 4% and 6% oil. However, storage time did not significantly ($p > 0.05$) affect the viscosity of the double emulsions which might be due to the high concentration of guar gum involved. A significant characteristic of guar gum is its ability to hydrate rapidly in cold systems, giving highly viscous solutions. The thickening ability of guar gum will maintain the viscosity of the double emulsions (Tekin Pulatsü et al., 2018) explaining why both double emulsions did not show a significant change in viscosity. There was a significant difference ($p \leq 0.05$) between the viscosity of double emulsion with 4% oil and 6% oil. This was in correlation with other research stating that a higher concentration of oil will lead to higher interfacial area, which leads to higher resistance to flow and energy dissipation, hence causing higher viscosity of the system, which was found in a double emulsion containing 6% oil compared to a double emulsion containing 4% oil (Paula et al., 2018). The double emulsion was then applied to the edible film.

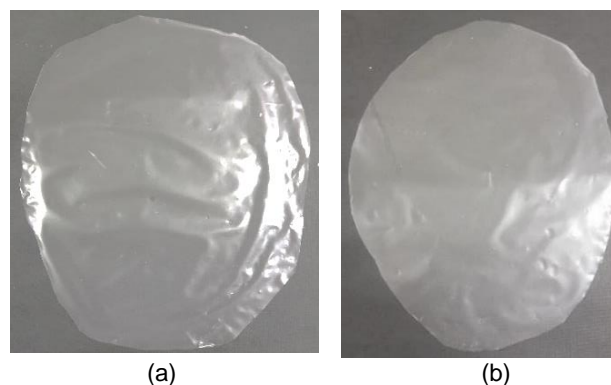


Figure 2. Edible Film made with 4% (a) and 6% (b) Double Emulsion

Edible Film Characteristic

The appearance of the edible films was varying depending on the formulations. The visual appearance of the edible films was seen to still have adequate transparency with a smooth surface, however, the incorporation of more double emulsions will produce a more opaque color (Figure 2).

Table 5 shows the thickness of edible film with increasing double emulsion concentration, to which there was a significant effect ($p \leq 0.05$) of double emulsion concentration on film's thickness. The edible film with 8% double emulsion was the thickest with a thickness of 0.053 ± 0.004 mm, while the thinner film was observed with the addition of 2% double emulsion and 4% double emulsion. The thickness of the edible film will increase due to increasing material concentration with the same volume of solution poured on each film applicator. This increases the total solids of the film after the drying process. The increasing thickness of the edible film was associated with the development of intermolecular hydrogen bonds between guar gum of the double emulsion and starch of the edible film (Saber et al., 2017). According to the Japanese Industrial Standard, the

maximum value of edible thickness should not exceed 0.25 mm (Nurindra et al., 2019). The thickened the edible film, the less the palatability of the edible film. In this research, the thickness of the edible film was lower than the maximum thickness of 0.25 mm which can be considered an acceptable thickness.

Statistically, there was a significant effect ($p \leq 0.05$) of both oil concentration and double emulsion concentration on the opacity of the film. The higher the oil concentration, the higher the opacity value was. The presence of oil that is dispersed and non-miscible will promote opacity as a function of the differences in the refractive index of the phases and the concentration as well as the particle size of the dispersed phase (Galus & Kadzińska, 2016). Increasing double emulsion concentration will also increase the film's opacity because the components of the double emulsion may prevent light transmission through the film. The major component of the double emulsion, which was guar gum, is a high molecular weight polysaccharide known to be white to yellowish-white in color, hence will also increase the opacity level (Mudgil et al., 2014).

Table 5. Textural properties of the single and double emulsion edible film

Treatment	Thickness (mm)	Opacity abs/mm	Tensile Strength (MPa)	Elongation %	WTVR g/hour	Swelling Index
Control	0.046 ± 0.002	2.23 ± 0.03	3.74 ± 0.04	9.38 ± 0.71	2.06 ± 0.09	116.16 ± 1.69
4% oil - 2 % double emulsion	0.045 ± 0.004	2.55 ± 0.08	3.43 ± 0.30	16.03 ± 0.52	1.64 ± 0.01	120.72 ± 2.24
4% oil - 4 % double emulsion	0.046 ± 0.005	2.62 ± 0.07	5.70 ± 0.35	14.97 ± 0.74	1.74 ± 0.03	127.64 ± 2.14
4% oil - 8 % double emulsion	0.054 ± 0.005	2.72 ± 0.05	7.74 ± 0.29	13.53 ± 0.66	2.06 ± 0.08	153.09 ± 2.76
6% oil - 2% double emulsion	0.042 ± 0.003	2.60 ± 0.01	3.34 ± 0.23	16.72 ± 0.30	1.22 ± 0.07	114.22 ± 2.37
6% oil - 4 % double emulsion	0.046 ± 0.005	2.68 ± 0.04	5.22 ± 0.30	15.46 ± 0.79	1.49 ± 0.03	122.19 ± 1.32
6% oil - 8 % double emulsion	0.052 ± 0.003	2.81 ± 0.04	6.32 ± 0.26	13.92 ± 0.38	1.64 ± 0.03	137.04 ± 1.92

All the edible films in this research are shown to have good tensile strength, above 0.3 MPa (according to the Japanese industrial standard) (Nurindra et al., 2019). The lowest tensile value was observed on edible films with 6% oil-2% double emulsion (3.43 MPa) with edible film with 4% oil-8% double emulsion having the highest tensile strength (7.14 MPa). The tensile strength of the edible film was significantly affected ($p \leq 0.05$) by both oil concentration and double emulsion concentration. While increasing oil concentration was found to result in decreased tensile strength, increasing double emulsion concentration was observed to result in increased tensile strength. The double emulsion, mainly composed of guar gum, affects the tensile strength as there is the development of intermolecular hydrogen bonds between guar gum and starch, leading to a more cohesive molecular structure of the films, resulting in improved tensile strength.

The organized crystalline structure of the starch molecules is disordered through the gelatinization process during film making, which exposes the hydroxyl groups of the starch that will form hydrogen bonds with guar gum. This will result in increasing the mechanical properties of the edible film (Saber et al., 2017). Vice versa, the reduction in tensile strength is associated with the presence of oily compounds such as essential oils in the film, as this will result in a heterogeneous structure of the film. Cinnamon oil will also lead to a cracked structure which will decrease the tensile strength of the film (Bahram et al., 2014). A decrease in

tensile strength occurs through partial replacement of stronger polymer-polymer interactions by weaker polymer-oil interactions (Noshirvani et al., 2017).

All double emulsion-added edible films had higher elongation at break value compared to an edible film made without the addition of double emulsion. The synergistic interaction between guar gum and starch in edible film affects the film's elongation at break as amylose-amylose interaction is inhibited. The more unfolded the network is, the weaker the interaction forces are, and hence also the lower the elongation at break will be (Saber et al., 2017). However, the addition of higher cinnamon oil concentration resulted in higher elongation at break value, due to the strong plasticizing effect of the oil, causing higher stretch-ability (Šuput et al., 2016). According to the Japanese Industrial Standard (1975), a good edible film should have percent elongation at a minimum of 10% elongation and can be categorized as an ideal edible film if it can reach elongation above 50% (Nurindra et al., 2019). In this research, the percent elongation value of all the edible film was in the range of 13-16% which still can be considered acceptable.

There was significant interaction ($p \leq 0.05$) between oil and double emulsion concentration towards the water vapor transmission rate (WVTR) of the edible films. Both oil concentration and double emulsion concentration significantly ($p \leq 0.05$) affected the WVTR. The highest WVTR value was observed on edible film with 4% oil-8% double emulsion, while the lowest WVTR value was found on edible

film with 6%oil-2% double emulsion. Higher oil concentration resulted in lower WVTR, while vice versa, increasing double emulsion concentration led to higher WVTR. While the hydrophobic nature of essential oil allows reduction of WVTR as water vapor permeation generally occurs through a hydrophilic portion of the film, guar gum, the component of double emulsion, contributes to an increase of hydrophilic nature and to more accessible hydroxyl groups capable of interacting with water through hydrogen bonds, allowing water molecules to be more freely absorbed into the surface of the films and also permeate through the film's network (Saber et al., 2017). All the edible film exceeded the maximum value of edible film WVTR according to the Japanese Industrial Standard (10 g / m².day). The starch-based edible film, although having good superiority in mechanical properties, usually has inferior WVTR quality due to the polar nature of starch. This result can also be seen in the swelling index of the edible film, in which all the edible films have a considerable high swelling index. Higher oil concentration led to a lower swelling index, while higher double emulsion concentration led to a higher swelling index. With the increasing volume of lipids, swelling capacity decreases because of their hydrophobic character. A high volume fraction of oil changes the property of the films as the affinity towards water is decreased (Galus & Kadzińska, 2016). However, the presence of guar gum in double emulsion will increase the swelling capacity due to its high water retention characteristic (Oprea, 2013).

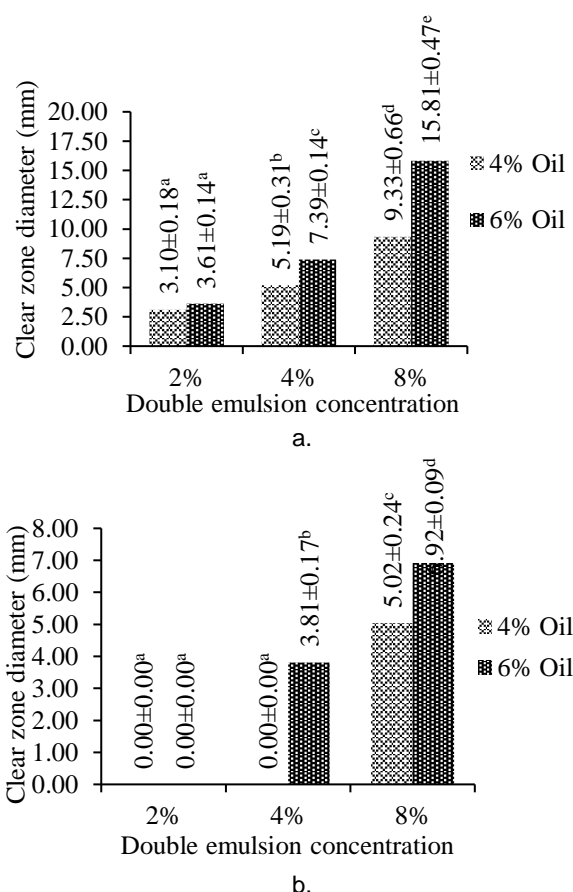


Figure 3. Antibacterial activity of edible films against *E. coli* (a) and antifungal activity against *R. stolonifer* (b). Note: different superscripts indicate there is a significant difference ($p < 0.05$)

Edible Film Antimicrobial Activity

There was significant interaction ($p \leq 0.05$) between oil concentration and double emulsion concentration towards

the antimicrobial activity of the edible films towards both test microorganisms. Both oil concentration and double emulsion concentration also did significantly affect ($p \leq 0.05$) the antimicrobial activity of the edible films. Generally, increasing oil concentration and double emulsion concentration will increase the antimicrobial activity while controlling edible film without any double emulsion addition didn't exhibit any antimicrobial activity.

The antimicrobial activity of the cinnamon essential oil can increase the diameter of the clear zone (inhibition of microbial growth) as oil concentration is increased. The cinnamon essential oil can attack the cell membrane and disrupt it due to its hydrophobic structure, leading to depletion of cell content and inhibiting ergosterol biosynthesis of the fungal membrane (Li et al., 2014).

The high viscosity of the guar gum solutions and the antimicrobial activity of the cinnamon essential oil resulted in an increase in the diameter of the clear zone (inhibition of microbial growth) when both oil and double emulsion concentrations are increased. Generally, increasing oil concentration and double emulsion concentration containing the cinnamon essential oil will increase the antimicrobial activity. Control edible film without any double emulsion addition did not exhibit any antimicrobial activity. Some media did not show any clear zone around the edible film solutions. Despite not having any clear zone surrounding the edible film solution, there was no growth of any microorganisms below the edible film solution.

CONCLUSION

The cinnamon essential oil was obtained from cinnamon barks with a yield of $0.79 \pm 0.05\%$ (w/w) with Minimum Bactericidal Concentration (MBC) of the cinnamon essential oil obtained were of 0.86 mg/mL, 0.37 mg/mL, 1.31 mg/mL and 0.51 mg/mL, respectively for *E. coli*, *S. aureus*, *R. stolonifer*, and *A. niger*. The Edible films were incorporated with double emulsions containing 4% and 6% of cinnamon essential oil. A higher concentration of essential oil increases the droplet size and viscosity of the double emulsions significantly ($p \leq 0.05$). All double emulsions were stable in regards to centrifugation and storage at refrigeration temperature although lower oil concentration resulted in lower stability, the droplet size getting larger upon storage time. An edible film with higher cinnamon essential oil concentration is shown to have good mechanical properties (low thickness, good tensile strength properties, and moderate elongation capacity) with superior antimicrobial activity against challenging microorganisms. The incorporation of double emulsion is proven to increase the functional properties and stability of edible film, which gave a promising future application in a food product. Future studies should cover the challenge test of the edible film in prolonging the shelf life of food products should be explored.

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