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Characterization of Antimicrobial Edible Films with Single and Double Emulsions from Clove (*Syzygium aromaticum*) Oil

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Abstract

Clove oil as a potent antimicrobial agent was added to enhance the properties of edible films. Clove oil was converted to single and double emulsion emulsions for homogenous dispersion in a starch based edible film suspension. Double emulsion was made with two steps emulsification with $CaCl_2$ as inner water phase and guar gum as outer water phase. Single emulsion was prepared similarly without inner water phase. The physico-chemical characteristics and the antimicrobial activity of the of starch-based edible film added with the emulsion were observed. MBC/MFC of clove oil was determined against E. coli, S. aureus, R. stolonifer, and A. niger which gives value of 1.95, 1.46, 0.52, and 0.35 mg/ml respectively. Incorporation of different emulsions on starch-based edible films affect the properties of resulting edible films by increasing thickness, opacity, elongation at break, water vapor transmission rate, and swelling index. Both emulsions showed comparable physicochemical characteristics such as thickness, WVTR, and swelling index value. However, double emulsion produced more superior edible films in terms of tensile strength and antimicrobial activity. 15% addition of double emulsion were able to show strong antimicrobial activity with inhibition zone of more than 8.0 mm for E. coli and 24.0 mm for R. stolonifer.

Keywords: *clove oil; edible film; single and double emulsion*

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INTRODUCTION

The potential of edible coatings to improve food quality and to prolong shelf life has received increasing attention. Edible film is a thin layered structures of biopolymer composition that enhances the quality of food products and act as barrier from physical, chemical, and microbial contamination. It may also act as carriers of active ingredients such as antioxidants, flavors, colorants, or antimicrobial agents (Guilbert *et al*., 1996).

Essential oils are among many natural antimicrobial agents that have been widely incorporated into edible films. Clove oil is one of the essential oils that exhibited strong antimicrobial activity (Sheeladevi and Ramanathan, 2012). Clove itself is abundant in Indonesia as the country contributes to 71% of total clove production worldwide (Siagian, 2014). Clove oil contains high content of eugenol which contributes to wide spectrum of

antibacterial and antifungal properties (Packyanaathan and Prakasa, 2017). However, essential oil has low solubility and high volatility and needs to be converted into emulsion to be incorporated into hydrophilic starch based edible film suspension for homogenous distribution as well as to protect the bioactive compounds.

Double emulsion emerges as potential system that possesses numerous potential benefits including the encapsulation of bioactive compounds such as vitamins, minerals, and flavoring agents (Yildrim, 2015; McClements, 2016).

Considering the potential use of clove oil as antimicrobial agent, it is expected that by making it into single emulsion and double emulsion and incorporating the emulsions into edible film suspension would enhance the characteristics of edible films in terms of antimicrobial activity. The aim of this research was to determine physicochemical characteristics of edible films incorporated with two types of emulsions from clove oil.

RESEARCH METHODOLOGY

Materials and Equipment

Materials needed for this research were dried clove buds obtained from *Balai Penelitian Tanaman Rempah dan Obat* (Balittro, Bogor), Polyglycerol polyricinoleate (PGPR) from PT. Tegar Inti Sentosa, corn starch (Maizenaku), $CaCl_2$, guar gum, glycerol (brataco), Nutrient Agar (Merck), Potato Dextrose Agar (Merck), pure cultures of *Escherichia. Coli BTCC B-609, Staphylococcus aureus BTCC B-613. Rhizopus stolonifer*, and *Aspergillus niger* (Probiotic Microbial).

Equipment used were Bidwell-sterling extraction apparatus, film applicator, oven (Memmert UNB 100-500), microscope OlympusCX31, viscometer (Brookfield Digital Model DV-II), cuvettes, spectrophotometer (thermos Scientific genesys20), Vernier caliper, texture analyzer (Lloyd Instruments LR 50K).

Clove Oil Preparation

Clove oil extraction process was done by distillation method adopted from Amelia *et al*. (2017). Clove buds were first milled and sieved with 60 mesh in the sieve shaker, weighed, and transferred to the Bidwell-sterling extraction apparatus. Water is used as the extraction medium, and the extract were boiled for 6 hours.

Clove Oil Antimicrobial Activity Test

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC) determination was conducted using agar well diffusion method adopted from Sheeladevi and Ramanathan (2012) and Tarek *et al*. (2014) with modifications.

Nutrient agar and Nutrient broth were used for preparation of bacteria stock culture and working culture. While Potato Dextrose agar and Potato dextrose broth are used were used for preparation of fungi stock culture and working culture. Firstly, the stock culture was made by inoculating one ose of pure culture into 10 ml of medium broth and then incubated at 37ºC for 24-48 hours. After incubation, one ose of culture from medium broth was taken and spread on slant agar, then incubated at 37ºC for 24 hours, and the microbial suspension was obtained.

Microbial suspension (OD_{600}: 0.3-0.4) 0.2% v/v from the media was inoculated and poured into petri dish up to 30mm. Four wells with diameter of 6mm were made and then filled with different concentrations of clove oil (1.25, 2.5, 5.0 and 10 mg/ml). After incubation, the inhibition zones indicated by the presence of clear zones were measured using digital Vernier calipers. MIC, MBC and MFC towards the four microorganisms were calculated by using method of Bloomfield (1991).

Single and Double Emulsion Preparation

The double emulsion formulation referred to the work of Pulatsu *et al*. (2017) with modifications. The single emulsion, the emulsion was prepared using 1% $CaCl_2$ solution as inner water phase and 1% guar gum solution as second water phase. 1 g of PGPR was mixed with 20 g clove oil and homogenized with magnetic stirrer at 1200 rpm for 15 s followed by placing it into water bath at 50ºC for 15 min. For double emulsion, the first emulsion was done by mixing an equal amount of $CaCl_2$ and oil phase was mixed for 10 min using mixer at 1000 rpm. Afterward, the mixture was mixed with 90% guar gum solution (W2) and homogenized in food processor for 5 min at speed 3 to form the double layer emulsion.

The physical characteristics (droplet size, viscosity and stability) of both emulsions type were observed. T-test analysis was used to compare the characteristics of single and double emulsion.

Edible Film Preparation

Edible films were made with corn starch referring to the work of Aisyah *et al*. (2017). The formulations of edible films were tabulated in Table 1. Corn starch, glycerol, and water were weighed in beaker glass and the suspension was heated up to 85ºC and kept at the temperature for 15 min under constant stirring. The suspension was then cooled to 50ºC before adding the clove oil emulsion. The mixture was stirred until homogenous and then casted into thin

Table 1. Formulation of edible films added with single and double Emulsion

Formulation	St (%)	Gly (%)	Water (%)	Emulsion (%)
Control	4	1.5	94.5	0
5% SE	4	1.5	89.5	5
10% SE	4	1.5	84.5	10
15% SE	4	1.5	79.5	15
5% DE	4	1.5	89.5	5
10% DE	4	1.5	84.5	10
15% DE	4	1.5	79.5	15

Note: St = Starch, Gly=Glycerol, SE= Single Emulsion, DE= Double Emulsion

film by using film applicator. The films were dried in the oven at 55-60^{o}C.

A completely randomized two-factorial design, using two-ways ANOVA and a Duncan multiple range test is used to compare the characteristic of the edible film. All analysis was done in four replications and statistical analysis were done using SPSS Statistics 25.

Analysis

In this research the analysis conducted were yield (Amelia *et al*., 2017), MBC/MFC (Bloomfield, 1991), droplet size (Frascareli *et al*., 2011 with modifications), viscosity (Ali *et al*., 2014 with modification), stability by centrifugation (Pulatsu *et al*., 2017), creaming index (Hadnadev *et al*., 2013 with modification). Analysis for edible films include thickness, opacity (Galus and Kadzinska, 2015), tensile strength and elongation at break (Dashipour *et al*., 2014), water vapor transmission rate (ASTM, 1995 with modifications), swelling index (Galus and Kadzinska, 2015), and antimicrobial activity (Bahram *et al*., 2013 with modifications).

RESULTS AND DISCUSSION

Clove Oil Extraction

Clove buds used in this research were *Syzygium aromaticum* (L.) Merr. & L.MPerry. that yielded an average of 4.73±0.23% w/w clove essential oil. The result is comparable to the previous research done by Amelia *et al*. (2017) that performed similar extraction technique and yielded 4.99% and 4.58% essential oil.

a. Antimicrobial and Antifungal Activity

The extracted oil was tested for its ability to inhibit or to kill microorganisms. The test organisms were *E. coli*, *S. aureus*, *R. stolonifer*, and *A. niger*. The results of MIC, MBC, and MFC determination can be seen in Table 2.

It can be concluded that clove essential oil is effective against all test microorganisms, hence, having wide range of antibacterial and antifungal properties. These properties are attributed to eugenol which is the major component of clove essential oil. Essential oil interacts with cell wall and membrane first, then cause the losses of vital intracellular materials.

Table 2. Results of MIC, MBC, MFC Determination

Test Microorganisms	MIC (mg/ml)	MBC/MFC (mg/ml)
E. coli	0.49 ±0.10	1.95 ±0.13
S. aureus	0.37 ±0.02	1.46 ±0.07
R. stolonifer	0.13 ±0.02	0.52 ±0.06
A. niger	0.09 ±0.03	0.35 ±0.03

It penetrated to cytoplasmic membrane and inhibited the normal synthesis of DNA and proteins necessary for growth (Xu *et al*., 2016).

Eugenol's antimicrobial activity is linked to its ability to permeabilize cell membrane demonstrated in various studies as increased transport of potassium and ATP out of the cells (Hyldgaard et al., 2012). The eugenol content in the essential oil is able to reduce the amount of ergosterol in fungal membrane. Ergosterol is the major sterol component of the fungal membrane responsible to maintain cell function and integrity and is specific to fungi (Pinto *et al*., 2009) thus, repression of ergosterol will weaken the cell wall of fungi.

It can be seen in Table 2 that bacteria had higher MBC compared to MFC of molds. *E. coli* had higher MBC value compared to *S. aureus*, indicating *E. coli* is the least susceptible microorganisms among the other test organisms. *A. niger* is the most sensitive towards clove essential oil. The results resembled the previous work done by Fu *et al.* (2017) in which *2.5 mg/ml* eugenol is known to inhibit A. niger well compared to other fungi and inhibit *S. aureus* better than *E. coli* . It is however, difficult to compare the MIC, MBC or MFC value as differences in antibacterial effects of essential oil against microorganisms may be linked to experimental conditions such as inoculation amount of bacteria, incubation time, and different source of clove oil and many other factors (Xu *et al*., 2016).

Single and Double Emulsion Characterization

The oil extracted was used to make single and double emulsion. The making of double emulsion involved multiple emulsification procedure using $CaCl_2$ as inner water phase and guar gum as outer water phase.

Single emulsion was made similarly without inner water phase. Concentration of oil speed and time of mixing was made constant. The physical characteristics (droplet size, viscosity and stability) of both emulsions can be seen in Table 3.

a. Droplet Size of Emulsion

Droplet size of emulsions is an important parameter as emulsion properties such as rheology, stability, texture and appearance are largely determined by droplet size and the distribution (Hadnadev *et al*., 2013). The droplet size of freshly made emulsions were measured and after 7 days of storage in refrigeration temperature, it was re-measured.

The average droplet size of single emulsion was 26.86 µm ranging from 14.19- 40.13 µm. The average droplet size of double emulsion was 54.25 µm with the range of 32.12-77.69 µm. The droplet size of single emulsion is significantly smaller than that of double emulsion ($p<0.05$), this might be due to smaller interfacial tension between clove oil and guar gum solution.

There was a significant increase ($p<0.05$) in droplet size from day 0 to day 7 of storage. The average droplet size of single emulsion on day 7 was 34.04 ± 1.94 µm. This is according to the literature which states that droplet size increases over time due to coalescence and Ostwald Ripening (Tadros, 2013). The average droplet size of double emulsion on day 7 was 55.90 ± 2.20 µm, which was not significantly different from the droplet size on day 0.

Table 3. Physical Characteristic of The Single and Double Emulsion

Types of Emulsion	Average Droplet Size (μm)		Viscosity (cP)		Stability 7 Days (%)	Stability 14 Days (%)
	Day 0	Day 7	Day 0	Day 7		
Single Emulsion	26.86 ± 2.85	34.04 ± 1.94	8469 ± 383	8400 ± 148	100 ± 0.00	98.45 ± 0.25
Double Emulsion	54.25 ± 3.69	55.90 ± 2.20	7475 ± 145	7375 ± 129	100 ± 0.00	100 0.00

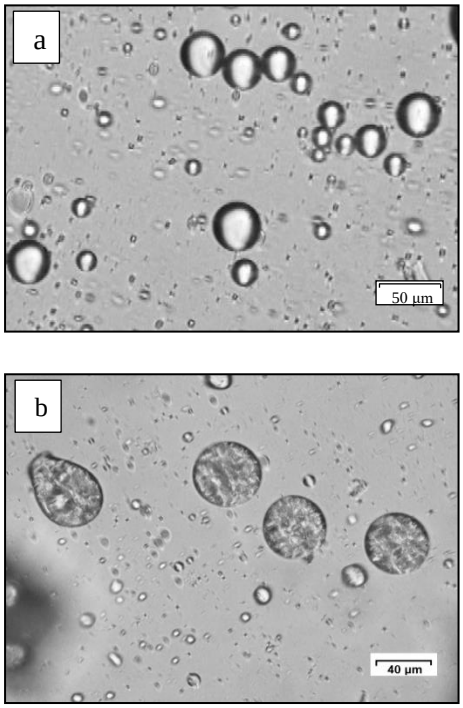


Figure 1. Droplets of single emulsion (a) and double emulsion (b)

The oil globules neither increase nor decrease significantly. However, there seemed to be a breakup of double emulsion drops and coalescence of the inner droplets. The inner water globules were larger in size and less in numbers.

b. Viscosity of the Emulsion

The freshly made emulsions were stored in the refrigerator before measuring the viscosity on the same day. After 7 days of storage, the viscosity was re-measured. The temperature of emulsions was about 10ºC when subjected into viscometer. Results of viscosity can be seen in Table 3.

There was a significant difference between the viscosity of single and double emulsion. Single emulsion possessed higher viscosity due to single emulsion having smaller oil droplets. This is supported by Chanamai and McClements (2006) that states with decreasing droplet size, the apparent viscosity of the emulsions increases, as they become more stable and lighter. Higher viscosity of single emulsion can also be contributed to it having higher concentration of guar gum solution. There was no significant difference of viscosity on day 0 and day 7 for both single and double emulsion. This suggests that the emulsions were stable.

c. Stability of the Emulsion

In this research, stability of emulsions was analyzed using two methods: by centrifugation and by measuring creaming index. The first method utilized centrifugal force where the emulsions were loaded into centrifuge tube and subjected to the force at 15000 rpm for 5 minutes. The second method was done by placing the emulsions into the graduated cylinders and let it stand for 15 days and kept in refrigeration temperature. There was no separation observed after the emulsions were centrifuged, hence they were all 100% stable. There was also no creaming or separation seen after the emulsions were stored for 15 days in graduated cylinders, showing 0% creaming index, hence both single and double emulsions were stable. This could be because the emulsions were stored in refrigerator at low temperature. Temperature can affect emulsion stability significantly. Temperature affects the physical properties of oil, water, interfacial films, which in turn, affect the stability of the emulsion. Viscosity of emulsion increases with decreasing temperatures (Tadros, 2013).

Characterization of Edible Films

The single and double emulsions from the previous research stage was incorporated into starch based edible film suspension with three different emulsion concentration (5%, 10%, and 15%) for each emulsion. There was no apparent difference among control edible film and edible films made from both types of emulsions. All edible film showed a semi-transparent form with different degree of opacity. The appearances of all edible films were smooth and free from lumps. All formulations were relatively thin, but easy to peel.

Thickness of edible films can be seen in Figure 1. The thickness of edible films increased as the concentration of emulsions increased. The thinnest films were the ones added with only 5% emulsions. The thickest films were the ones added with 15% emulsions. However, they were not significantly different than those films added with 10% emulsions. This is in accordance with the investigation conducted by Kusnadi and Budyanto (2015) that states the thickness of edible film is affected by the amount of total dissolved solids. Addition of emulsions whose major component were guar gum solution, increase the total dissolved solids. More emulsions added would increase the edible polymer films, increasing the viscosity of the suspension, hence will increase the thickness of films during drying process.

Opacity

Edible films and coatings should be as close to colorless or transparent as possible so that the appearance of food product would not be much affected by the color of the edible films/coatings (Galus and Lenart, 2013). Opacity of edible films can be seen in Table 4.

Increasing emulsion concentration elevates the opacity value. There is a significant effect of emulsion type and emulsion concentration towards the opacity of edible films ($p<0.05$). Control edible film without any addition of emulsion resulted in less opaque edible film with the value of 2.17± 0.07 mm^{-1}. The opaquest edible film consisted of 15% single emulsion with the opacity value of 3.61±0.14 mm^{-1}.

Table 4. Textural Properties of The Single and Double Emulsion Edible Film

	Thickness (mm)	Opacity abs/mm	Tensile Strength (Mpa)	Elongation %	WTVR	Swelling Index
Control	0.049 ± 0.001	2.17 ± 0.07	3.75 ± 0.11	9.11 ± 0.46	1.98 ± 0.04	117.7 ± 2.20
SE – 5%	0.048 ± 0.003^{a}	2.48 ± 0.12^{c}	3.63 ± 0.21^{f}	12.13 ± 0.86^{i}	2.42 ± 0.08^{l}	126.6 ± 1.12^{o}
SE – 10%	0.055 ± 0.005^{b}	2.87 ± 0.13^{d}	3.36 ± 0.29^{g}	16.79 ± 0.46^{j}	2.53 ± 0.05^{m}	150.4 ± 1.10^{p}
SE – 15%	0.059 ± 0.003^{b}	3.61 ± 0.14^{e}	2.84 ± 0.11^{h}	22.64 ± 0.44^{k}	1.95 ± 0.03^{n}	188.2 ± 2.08^{q}
DE – 5%	0.046 ± 0.005^{a}	2.53 ± 0.11^{c}	5.58 ± 0.32^{f}	11.29 ± 0.51^{i}	2.42 ± 0.03^{l}	127.2 ± 2.21^{o}
DE – 10%	0.056 ± 0.004^{b}	2.73 ± 0.13^{d}	5.20 ± 0.05^{g}	11.89 ± 0.96^{j}	2.59 ± 0.03^{m}	155.8 ± 7.87^{p}
DE – 15%	0.058 ± 0.002^{b}	3.36 ± 0.08^{e}	4.98 ± 0.27^{h}	15.84 ± 0.58^{k}	2.26 ± 0.06^{n}	182.6 ± 1.83^{q}

The appearance of both types of emulsion is milky white, hence addition of more emulsions would increase the opacity of the edible film. The result is also in agreement with the previous studies conducted by Suput *et al*. (2016) and Galus and Kadzinska (2016) that states higher additional content of essential oil results in less transparency of the film samples, hence increasing opacity value. Droplets dispersed in the starch matrix affect the transparency by preventing light transmission through the film.

There is a significant difference between opacity of edible films added with single emulsion and double emulsion ($p<0.05$) as each emulsion type possessed different droplet size and distribution. The transparency of emulsion-based films is related to their internal structure, which is affected by the oil volume fraction and droplet size distribution in film-forming emulsions and its rearrangement during drying (Villalobos *et al*., 2005).

Tensile Strength

Table 4. shows the tensile strength of edible films. There is a significant effect of types of emulsion and concentration of emulsion added towards the tensile strength of edible films ($p<0.05$). Tensile strength of edible films incorporated with single emulsion ranged from 2.84 to 3.63 MPa, with the lowest value possessed by addition of 15% single emulsion and highest value possessed by addition of 5% single emulsion.

For both types of emulsion, increasing concentration of emulsion added, lowers the tensile strength value. This effect can be attributed to the development of discontinuities in the starch polymer network resulting from essential oil addition. The formed complex structure reduces the cohesion forces of a starch network, hence reducing the tensile strength. The same findings are also shared by Suput *et al*. (2016) and Jimenez *et al*. (2013). Tensile strengths of double emulsion-added edible films were significantly higher than single emulsion-added edible films. This can be attributed to the addition of $CaCl_2$ in the double emulsion formulation which increases ionic strength in the double emulsion system.

Elongation at Break

Elongation at break of edible films is a part of mechanical properties. It is the maximum change in length of edible film sample before breaking. Table 4 Shows the percent elongation of edible films.

Edible films incorporated with emulsions had higher percentage of elongation compared to control edible film with the value of 9.11±0.46%. This is due to the plasticizing effect of essential oil which can increase percent elongation value (Galus and Kadzinska, 2016). For both types of emulsions, the percentage elongation at break increases as the concentration of emulsion added increase.

The result is supported by the theory that states addition of essential oil decreased elongation value due to its plasticizing effect (Benavides *et al*., 2012; Suput *et al*., 2016). Single emulsion-added edible films generally had higher percent elongation value than double emulsion-added films this can be due to the difference in tensile strength. Tensile strength values are inversely proportional to the elongation value, which is the common observation of essential oil

incorporation in biopolymer matrices (Suput *et al.*, 2016).

Water vapor transmission rate (WVTR) is an important parameter for biodegradable films which can show their barrier properties against water vapor. Since the major purpose of food packaging or coating is to keep away from or at least to minimize moisture transfer between the food and the environment, water vapor transmission rate should be as low as possible (Farahnaky *et al.*, 2013). Table 4 Shows the average WVTR of edible films.

The lowest WVTR value was 1.95±0.08 g.mm/m^{-2}.h which resulted from addition of 5% double emulsion. The highest WVTR value was 2.59±0.03 g.mm/m^{-2}h which resulted from addition of 15% double emulsion.

There is a general increase in WVTR value as the concentration of emulsions increases. There is no significant difference between addition of single and double emulsions and there is no interaction between types of emulsion and concentration of emulsions added ($p>0.05$).

The incorporation of essential oil into a polymeric matrix can improve the WVTR of the edible films by increasing the hydrophobic compound in the film (Bahram *et al.*, 2013). However, from the result, average WVTR increases as concentration of emulsion increase. It should be taken into account that the emulsions consist of mostly guar gum solution, which is hydrophilic in nature. The addition of essential oil increases the hydrophobic hydrophilic ratio of edible film, probably hydrophilic nature of guar gum outweighs the increase in the ratio. Furthermore, increasing essential oil increase film permeability since presence of oil droplets lead to loosening of polymer film compactness, increase irregularity microstructure in which oil droplets are dispersed and have impact on film hydrophobicity, therefore increasing moisture that passed through the edible film (Galus and Kadzinska, 2016).

Swelling Index

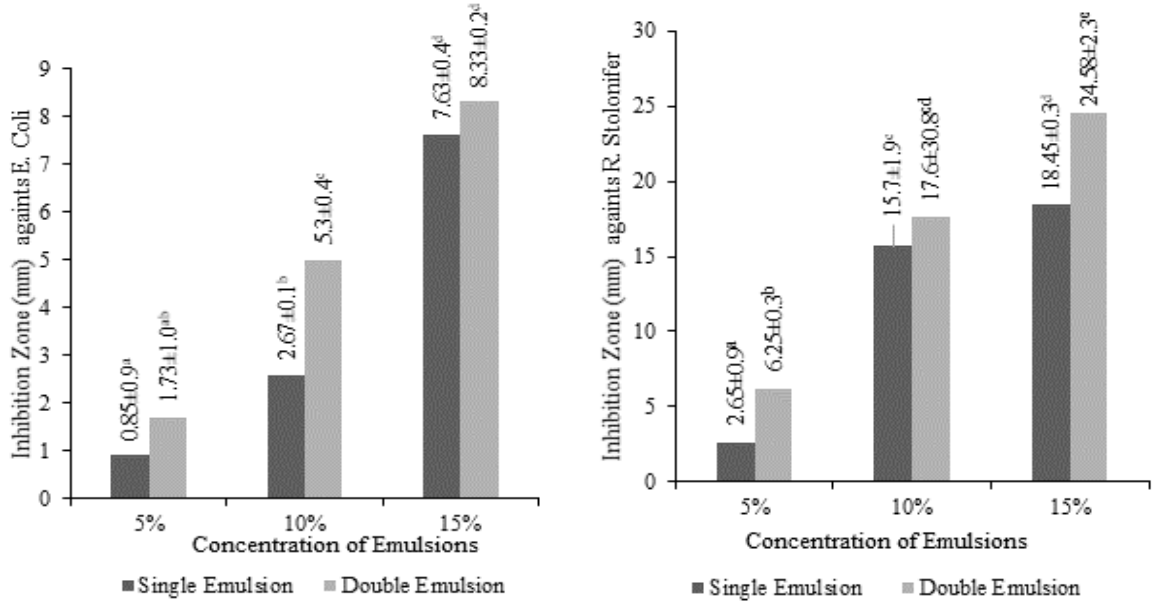
Swelling capacity of edible films indicates their biodegradation and applicability in packaging food with high water content such as peeled fruits (Galus and Kadzinska, 2016). The swelling index of edible films is displayed in Table 4

There was a significant increase in swelling index as concentration of emulsion increases ($p<0.05$). There was no significant effect of type of emulsions and interaction between the two factors towards swelling index value ($p>0.05$). Swelling index value of edible films ranged from 126.6 ± 1.1 to 188.2 ± 7.8 %, with the lowest value resulted from addition of 5% single emulsion and highest value resulted from addition of 15% single emulsion.

Increasing concentration increases the swelling index of edible films due to the hydrophilic characteristic of guar gum in the emulsion system. According to Galus and Kadzinska (2016), the higher degree of swelling of the film with the addition of oil may be related to the modification of microstructure during drying. Oil droplets change the internal structure of the films, leading to migration of water molecules. Therefore, higher concentration of emulsions added would result to higher percentage of swelling index.

Antimicrobial Activity

Antimicrobial activity of edible films was analyzed to know the effectivity of clove oil when made into emulsions and further incorporated to edible film suspension against bacteria and fungi.



Note: different superscripts indicate there is a significant difference ($p<0.05$)

Figure 2. Antibacterial activity of edible films against E.coli and antifungal activity againts R. Stolonifer.

In this research *E. coli* and *R. stolonifer* were the test microorganisms as they both have higher MBC and MFC value among the other test microorganisms. The result of inhibition zone of edible film suspension against *E. coli* and *R. Stolonifer* an be seen in Figure 2. Edible film suspensions showed inhibition against both test microorganisms. Clove oil in the edible film suspension diffuse through the agar gel and provided a clear zone surrounding the film suspensions. The statistical result showed significant increase in the inhibition diameter as the concentration of edible film increases. This is due to the higher concentration of clove essential oil responsible for the antimicrobial activity which have been previously determined in preliminary research.

Comparing the inhibition zone of bacteria and fungi, inhibition zone in *R. stolonifer* is higher than in *E. coli*. This also coincides with the previous analysis which demonstrates that fungi are more sensitive towards clove essential oil, thus having lower MFC value. the inhibition diameter of edible film suspension added with double emulsions were significantly higher than edible film added with single emulsion. This means that double emulsion is more effective in dissolving clove oil as antimicrobial agent. This can also be explained by the droplet size of double emulsion, which is significantly bigger than droplet size of single emulsion. According to Terjung *et al*. (2012), emulsions with larger droplet sizes were more effective in inhibiting growth and inactivating cells than smaller ones.

CONCLUSIONS

Clove essential oil has been successfully extracted by distillation method having wide range of antimicrobial activity and is effective against all four test microorganisms. Single (O/W) and double (W/O/W) emulsions prepared from guar gum both have relatively high viscosity and had 100% stability at refrigeration temperature.

Incorporation of clove essential oil emulsions on starch-based edible films affect the properties of resulting edible films by increasing thickness, opacity, elongation at break, water vapor transmission rate, and swelling index. Both emulsions showed comparable physicochemical characteristics such as thickness, WVTR, and swelling index value. However, addition of double emulsion produced more superior edible films in terms of tensile strength and antimicrobial activity. 15% addition of double emulsion were able to show strong antimicrobial activity with inhibition zone of more than 8.0mm for *E. coli* and 24.0mm for *R stolonifer*.

SUGGESTIONS

Further research needs to be conducted on the application of edible films as edible coating to food products. Clove essential oil has strong odor which may decrease consumer acceptance. Application to food products is necessary to study the efficacy of double emulsion on masking the unwanted flavors and aroma, as well as to analyze the efficacy of edible coating in extending the shelf life of food products.

REFERENCES

Aisyah, Y., Irwanda, L. P., Haryani, S., and Safriani, N., (2017), Characterization of Corn Starch-Based Edible Film Incorporated with Nutmeg Oil Nanoemulsion, *IOP Conf. Series: Materials Science and Engineering*, 352, 012050.

Ali, M. S., Alam, M. S., Alam, N., and Siddiqui, M. R., (2014), Preparation, Characterization and Stability Study of Dutasteride Loaded Nanoemulsion for Treatment of Benign Prostatic Hypertrophy, *Iranian Journal of Pharmaceutical*, 13 (4), pp. 1125-1140.

American Society for Testing and Materials (ASTM), (1995), Standard Test Method for Water Vapor Transmission of Material, *ASTM Book of Standard*.

Amelia, B., Saepudin, E., Cahyana, A. H., Rahayu, D. U., Sulistyoningrum, A. S., and Haib, J., (2017), GC-MS Analysis of Clove (*Syzygium aromaticum*) Bud Essential Oil from Java and Manado, *AIP Conference Proceedings*, 1862, 030082.

Bahram, S., Rezaei, M., Soltani, M., Abdolghasem, K., Ojagh, S. M., and Abdollahi, M., (2013), Whey Protein Concentrate Edible Film Activated with Cinnamon Essential Oil, *Journal of Food Processing and Preservation* 38 (3).

Benavides, S., Villalobos, C. R., and Reyes, J. E., (2012), Physical, Mechanical and Antibacterial Properties of Alginate Film: Effect of the Crosslinking Degree and Oregano Essential Oil Concentration, *Journal of Food Engineering*, 110 (2), pp. 232-239.

Bloomfield, S. F., (1991), Methods for Assessing Antimicrobial Activity in *Mechanisms of Action of Chemical Biocides Their Study and Exploitation*, editor Denyer, S. P. and Hugo, W. B., Blackwell Scientific Publication, London.

Chanamai, R. and McClements, D. J., (2006), Dependence of Creaming and Rheology of Monodisperse Oil-in-Water Emulsions on Droplet Size and Concentration, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 172(1-3), pp. 79-86.

Dashipour, A., Hosseini, H., Shojoae-Aliabadi, S., and Ghaanati, K., (2014), Physical, Antioxidant and Antimicrobial Characteristics of Carboxymethyl Cellulose Edible Film Cooperated with Clove Essential Oil, *Zahedan Journal of Research in Medical Sciences*, 16 (8), pp. 34-42.

Farahnaky, A., Saberi, B., and Majzoobi, M., (2012), Effect of Glycerol on Physical and Mechanical Properties of Wheat Starch Edible Films, *Journal of Texture Studies*, 44 (3), pp. 176-186.

Frascareli, E. C., Silva, V. M., Tonon, R. V., and Hubinger, M. D., (2011), Physicochemical Properties of Coffee Oil Microcapsules Produced by Spray Drying, *III Jornadas Internacionais*.

Fu,Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., and Efferth, T., (2007), Antimicrobial Activity of Clove and Rosemary Essential Oils Alone and in Combination, *Phyto therapy Research*, 21 (10), pp. 989-994.

Galus, S. and Lenart, A., (2013), Development and Characterization of Composite Edible Films Based on Sodium Alginate and Pectin, *Journal of Food Engineering*, 115 (4), pp. 459-465.

Galus, S. and Kadzinska, J., (2015), Moisture Sensitivity, Optical, Mechanical and Structural Properties of Whey Protein-Based Edible Films Incorporated with Rapeseed Oil, *Food Technology and Biotechnology*, 54 (1), pp. 75-89.

Guilbert, S., Gontard, N., and Gorris, L. G. M., (1996), Prolongation of the Shelf Life of Perishable Food Products Using Biodegradable Films and Coatings, *Lebensmitten-Wissenschaft und Technologie*, 29 (1-2), pp. 10-17.

Hadnadev, T. D., Dokic, P., Krstonosi, V., and Hadnadev, M., (2013), Influence of Oil Phase Concentration on Droplet Size Distribution and Stability of Oil-in Water Emulsions, *European Journal of Lipid Science and Technology*, 115(3), pp. 313-321.

Hyldgaard, M., Mygind, T., and Meyer, R. K., (2012), Essential Oils in Food Preservation: Mode of Action, Synergies, and Interactions with Food Matrix Components, *Frontiers in Microbiology*, 3 (12).

Jimenez, A., Fabra, M. J., Talens, P., and Chiralt, A., (2013), Phase Transitions in Starch-Based Films Containing Fatty Acids, Effect on Water Sorption and Mechanical Behavior, *Food hydrocolloids*, 30(1), pp. 408-418.

Kusnadi, J. and Budyanto, P., (2015). Antibacterial Active Packaging Edible Film Formulation with Addition Teak (*Tectona grandis*) Leaf Extract, *International Journal of Life Sciences Biotechnology Pharma Research*, (4) 2, pp. 79-84.

McClements, D. J., (2016), *Food Emulsions: Principle, Practices, and Techniques.* 3rd, CRC Press, Boca Raton.

Packyanathan, J. S. and Prakasam, G, (2017), Antibacterial Effect of Clove Oil against Clinical Strains of *Escherichia coli*, *Journal of Pharmaceutical Sciences and Research*, 9 (7), pp. 1203-1204.

Parthasarathy, V. A., Chempakam, B., and Zachariah, T. J., (2008). *Chemistry of Spices*, CAB International, Oxfordshire.

Pinto, E., Vale-Silva, L., Cavalein, C., and Salgueiro, L., (2009), Antifungal Activity of the Clove Essential Oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and Dermatophyte Species, *Journal of Medical Microbiology*, 58 (11), pp. 1454-1462.

Pulatsu, E. T., Sahin, S., and Sumnu, G., (2017), Characterization of Different Double Emulsion Formulation Based on Food Grade Emulsifiers and Stabilizers, *Journal of Dispersion Science and Technology*, 39 (7), pp. 996-1002.

Sanla-Ead, N. Jangchud, A., Chohenchob, V., and Suppakul, P., (2011), Antimicrobial Activity of Cinnamaldehyde and Eugenol and Their Activity After Incorporation into Cellulose-Based Packaging Films, *Packaging Technology and Science*, 25 (1), pp. 7-17.

Sheeladevi, A. and Ramanathan, N., (2012), Antibacterial Activity of Plant Essential Oils against Food Borne Bacteria, *International Journal of Pharmaceutical & Biological Archives*, 3 (5), pp. 1106-1109.

Siagian, V. J., (2014), *Outlook Komoditi Cengkeh*, Pusat Data dan Sistem Informasi Pertanian Kementrian Pertanian, Jakarta.

Suput, D., Lazic, V., Pezo, L., Markov, S., Vastag, Z., and Popovic, L., (2016), Characterization of Starch Edible Films with Different Essential Oils Addition, *Polish Journal of Food Nutrition Sciences*, 66 (4), pp. 277-285.

Tadros, T., (2013), *Emulsion Formation and Stability*, Wiley VCH, Weinheim.

Tarek, N., Hassan, H. M., AbdelGhani, S., Radwan, I. A., and Hammouda, O., (2014), Comparative Chemical and Antimicrobial Study of Nine Essential Oils Obtained from Medicinal Plants Growing in Egypt, *Beni-Suef University Journal of Basic and Applied Sciences*, 3 (2), pp. 1-8.

Terjung, N., Loffler, M., Gibis, M., Hinrichs, J., and Weiss, J., (2012), Influence of Droplet Size on the Efficacy of Oil-in Water Emulsions Loaded with Phenolic Antimicrobials, *Food and Function*, 3 (3), pp. 290-301.

Villalobos, R., Chanona, J., Hernandez, P., Gutierrez, G., and Chiralt, A., (2005), Gloss and Transparency of

Hydroxypropyl Methylcellulose Films Containing Surfactants as Affected by Their Microstructure, *Food Hydrocolloids*, 19 (1), pp. 53-61.

Xu, J. G., Liu, T., Hu, Q. P., and Cao, X. M., (2016), Chemical Composition, Antibacterial Properties and Mechanism of Action of Essential Oil from Clove buds against *Staphylococcus aureus*, *Molecules*, 21 (9), 1194.

Yildrim, M., (2015), Stability of Double Emulsions for Food Applications, *Thesis*, Middle East Technical University, Turkey.