Release Profile of the Antimicrobial Agent from Clove Oil Encapsulated in a Polyurethane Shell

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Keywords: Clove Oil, Encapsulation, Polyurethane Shell, Release Profile.

Abstract: The essential oil has been known for its antimicrobial properties and has the potency to be utilized as an active agent in food preservative, packaging, and textile. Eugenol and caryophyllene are a major antimicrobial component in the clove oil, which is proved against several bacterial and fungal strains. Due to the clove oil is easily oxidized and have a strong smell, it needs to be encapsulated so it can be used for long-term application. The encapsulation of the clove oil in the polyurethane shell was prepared by polymerization in an oil-in-water emulsion. The FTIR spectra of the microcapsules showed that the clove oil was successfully encapsulated. The release profile of the antimicrobial agent from the microcapsules was measured using Headscape GC. From the prediction based on the release profile showed that the microcapsules could emit the eugenol for 59 days and the caryophyllene for 15 days. Therefore, it could be concluded that the microcapsules of clove oil in the polyurethane shell is suitable for long term application.

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1 INTRODUCTION

Clove (*Syzygium aromaticum*) is one of the native Indonesian plants that are well known worldwide. Clove oils collected from the distillation of the clove's leaves have proved against several bacterial and fungal strains (Cortés-Rojas et al., 2014). The antimicrobial agent from natural plants such as the clove oil is considered safe, so it has more consumer preference than the chemical antimicrobial agent (Han, 2003). Therefore, the oils have been developed for widespread applications such as food preservatives (Cui et al., 2015), active packaging (Hosseini et al., 2009), and textiles (Kim and Sharma, 2011). However, clove oils are easily oxidized and have a strong smell, so they are not suitable for long-term use. The encapsulation process had been known could reduce those weaknesses (Kfoury et al., 2016).

Encapsulation is the process through which one substance or a combination of materials is coated or trapped in another material or system. The coated material is referred to as active or core material, and the coating material is referred to as a shell, wall material, carrier, or encapsulant (Madene et al., 2006). Chemical encapsulation using polymerization technique has been known as easy to be scaled-up, generically fast, and provides high encapsulation efficiency (Carvalho et al., 2016).

Many lists of researches regarding encapsulation by polymerization method, but only a few were using the essential oil, especially clove oil, as core material. Scarfato et al. (2007) provided encapsulation of essential oils by interfacial polymerization in o/w emulsion between polyfunctional isocyanates and diamines. They used essential oils from lemon balm (*Melissa officinalis L.*), lavender (*Lavandula angustifolia Miller*), sage (*Salvia officinalis L.*), and thyme (*Thymus vulgaris L.*). Liu et al. (2015) proposed a process for nanocapsules containing cologne essential oil for textile applications. Methyl methacrylate (MMA)–styrene (St) copolymer was used as a shell material to prepare nanocapsules containing cologne essential oil as a core material by miniemulsion polymerization. Chung et al. (2013) encapsulated thyme oil using melamine–formaldehyde prepolymer.

Nuraeni, C., Rahmi, D., Yunilawati, R., Ratnawati, E., Mailisa, T., Andrianty, T., Irwinanita, ., Amalia, B. and Riyanto, A.
Release Profile of the Antimicrobial Agent from Clove Oil Encapsulated in a Polyurethane Shell.
DOI: 10.5220/0009954900290036
In *Proceedings of the 2nd International Conference of Essential Oils (ICEO 2019)*, pages 29-36
ISBN: 978-989-758-456-5
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Gezundhait and Pelah (2017) used polyurethane as the shell for the encapsulation of essential oils in which the polyurethane shell made from the reaction of TDI (toluene diisocyanate) and polyethylene glycol 4000. In this paper, polyurethane from methylene diphenyl diisocyanate (MDI) was used as an encapsulation shell for the clove oil. According to Allport et al. (2003), MDI is less hazardous compared to TDI. Polyurethane was chosen as shell material because it is inexpensive and has high durability, then it is possible for a broad application (Engels et al., 2013).

The release profile is an important thing for the application of the encapsulated clove oil as an antimicrobial agent. The active compounds are expected can be released at a specific rate for a long time period as possible. The aim of this research is to study the release of antimicrobial compounds from clove oil encapsulated in a polyurethane shell. According to the literature, antimicrobial agents in clove oil are eugenol and caryophyllene. Marchese et al. (2017) described the mechanism of action of eugenol on bacteria and fungi. The antimicrobial activity of eugenol can be attributed to the presence of a free hydroxyl group in the molecule that able to bind to proteins, preventing enzyme action. The eugenol also disrupts the cytoplasmatic membrane and alters the permeability of the membrane, which leads to cell death. Dahham et al. (2015) proved that β-caryophyllene showed antimicrobial activity against *Staphylococcus aureus* and showed better antifungal activity than Kanamycin (a common antifungal drug on the market).

2 MATERIAL AND METHOD

Materials used in this study were methylene diphenyl diisocyanate (MDI) prepolymer, obtained from PT Covestro Polymers Indonesia; polyethylene glycol 400 (PEG) “Bratachem”; sodium lauryl sulphate (SLS) “Emal 10 N” Kao Chemicals; xanthan gum and clove oil. All materials were used without further purification.

Clove oil was obtained from Java area. The composition of the clove oil was analysed using GC-MS (Gas Chromatography–Mass Spectrometry) “Agilent” 7890B coupled to “Agilent” 5977. The analysis was performed using a non-polar capillary column (DB-5MS, 30 m × 250μm, film thickness 0.25 µm). Then, the compounds were identified by matching their mass spectra with GC-MS libraries (Wiley Registry).

Encapsulated clove oils were prepared by polymerization of polyurethane in an oil-in-water emulsion. At room temperature, the mixture of 150 mL of water and 25 grams of PEG were mixed at 400 rpm. Simultaneously, 8 grams of MDI and 25 grams of clove oil were mixed and then was added to the mixture. After the “clump” of polyurethane-clove oil was formed, add SLS and xanthan gum as much as 1 gram, respectively, while continuously stirring for 2 hours. The resultant microcapsules were strained from the liquid phase and then were rinsed with water twice. The process was conducted at room temperature. At last, the microcapsule powders were stored in chiller around 10 ^{o}C before analysed.

The microcapsules were examined its morphology using the microscope “Olympus BX53” and were characterized using FTIR (Fourier transform infrared) “Nicolet iS5” with an iD5 ATR diamond tip adapter.

The release properties of the clove oil encapsulated in polyurethane shells were qualitatively and quantitatively analysed by headspace-analysis technique using a Perkin Elmer Headspace GC Clarus® 680 (column 30.0m x 250μm). Sample as much as 2 grams of microcapsules was equilibrated at 40°C in the headspace unit before the injection. The carrier gas was helium; the detector temperature was 300 °C; the oven temperature was programmed from 40 °C (5 min hold) to 250 °C (10 min hold) increasing at 20°C/min. A split injector was used at 200° in split mode at a ratio of 1:50. The measurements were conducted in 0 day, 1st day, 3rd day, 8th day, and 10th day.

3 RESULTS AND DISCUSSION

3.1 The Composition of the Clove Oil

The clove oil analysis performed by GC-MS shows 10 (ten) peaks (Figure 1). From the chromatogram, was obtained major components clove oil that are 81.64% eugenol, 15.86% trans-caryophyllene, 1.15% alpha-caryophyllene, 0.47% caryophyllene oxide, 0.29% trans-anethole (Table 1).

3.2 The Morphology of the Clove Oil Encapsulated in a Polyurethane Shell

The creation of microcapsules of clove oil in polyurethane shell due to the reaction of a diol with a diisocyanate (Figure 2). In this study, a diol is referred to as PEG, whereas the diisocyanate is referred to as MDI. The diol is dissolved in the aqueous phase and the diisocyanate in the organic. The reaction at the oil–water interface produces the encapsulating shell (Yow & Routh, 2006).

In the process of encapsulation by emulsion polymerization, surfactants play major roles such as solubilising of highly water-insoluble monomers, determining the mechanism of particle nucleation, determining the number of particles nucleated and therefore the rate of polymerization, maintaining colloidal stability during the particle growth stage, and controlling average particle size and the size distribution of the final system (El-Aasser, 1990). In this study, sodium lauryl sulphate (SLS) was chosen because SLS is an anionic surfactant and a very strong type of surfactant and is a common emulsifier for most heterogeneous systems. SLS also helps reduce the size of the capsules by lowering the surface tension in the matrix (Lakkis, 2016)

Figure 3 shows the appearance of clove oil encapsulated in polyurethane shell. At four times magnification, it shows that the capsules gave spherical shape with size range from 246 μm to 832 μm. The size is larger compared to other researches, because this study uses a lower stirring rate. The increase in the stirring rate led to the formation of smaller particles and narrower distributions (Leimann et al., 2009), but the higher stirring rate might less efficient in scale-up manufacturing. Mamaghani and Naghib (2017) demonstrated that the stirring rate at 400 rpm is affordable for production regarding the energy consumed.

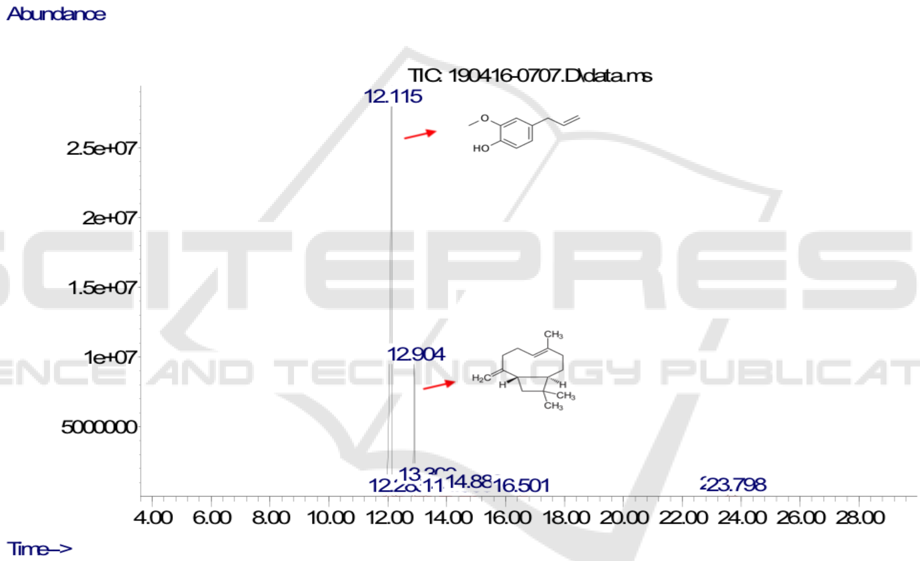

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Figure 1: Chromatogram of the clove oil.

Table 1: Compounds identified from clove oil using GC-MS.

Peak No.	RT (min)	Area %	Name	CAS	% Sim
1	12.115	81.64	Eugenol	97-53-0	98
2	12.283	0.14	Alpha-copaene	3856-25-5	98
3	12.904	15.86	trans-caryophyllene	87-44-5	99
4	13.304	1.15	alpha-caryophyllene	753-98-6	99
5	14.111	0.14	1-S-cis-calamenen	483-77-2	97
6	14.501	0.07	cis-jasmone	488-10-8	64
7	14.879	0.47	Caryophyllene oxide	1139-30-6	81
8	16.505	0.12	3-methoxycinnamic acid	6099-04-3	46
9	23.563	0.29	Trans-anethole	4180-23-8	46
10	23.802	0.13	6-Nitro-2,4-diphenylquinoline	138432-74-3	53

RT (min): retention times in minutes; Area%: relative area counts; CAS: CAS numbers; %Sim: % similarities to reference library spectrum

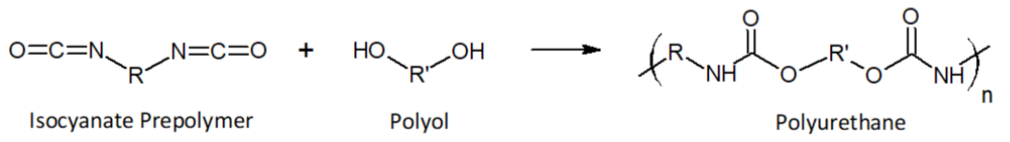

Figure 2: Synthesis of polyurethane.

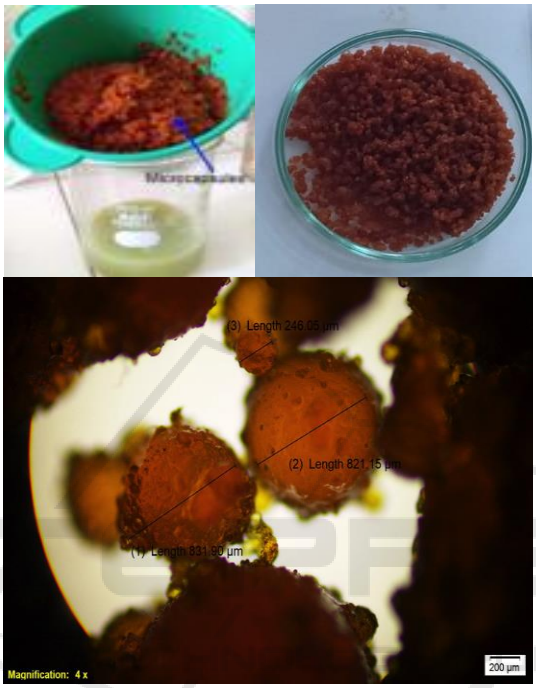

Figure 3: The morphology of clove oil microcapsule in a polyurethane shell.

The parameters that affect the morphology and size of the microcapsules have been reported by previous researches. Bouchemal et al. (2004) reported that the increase in the molecular weight of polyol tends to increase the mean size of capsules. Zhenxing et al. (2011) showed that the microcapsules from emulsion polymerization were influenced by the concentration of surfactant SLS. The higher concentrations of the surfactant, the smaller particle size would be created.

The increase of the SLS concentration means more surfactants can be adsorbed, and hence the surface charge density should increase. Therefore, it will lead to an increase in the particle number density, along with the decrease of particle size.

3.3 The IR Spectrum of the Microcapsules

The FTIR spectra of the clove oil encapsulated in polyurethane shell, clove oil, and polyurethane as the shell material are presented in Figure 4. As is shown in Figure 4, all the absorption peaks in the curve (b) could be found in the curve (a), it means that the clove oil was successfully encapsulated by polyurethane.

The peaks at ≈1700 cm^{-1}, 2250 cm^{-1}, and 3310 cm^{-1} correspond to C=O, excess isocyanate C=N=O, and -NH, respectively, are associated group in polyurethane. A small amount of polyurethane existed in clove oil microcapsule, which is showed by C=O and –NH in both curves (a) and (c).

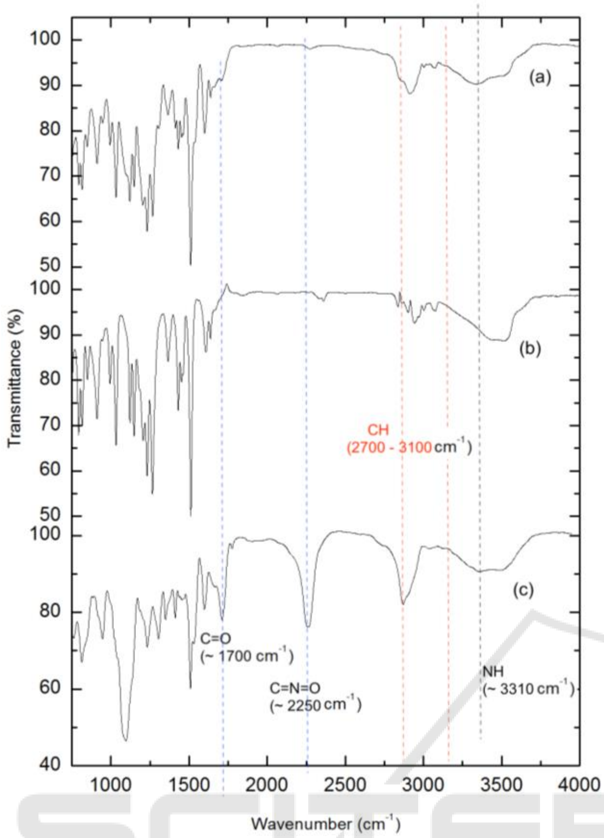

Figure 4: IR spectrum: (a) clove oil encapsulated in polyurethane shell, (b) clove oil, and (c) polyurethane.

3.4 The Release Profile of Antimicrobial Agent from the Microcapsules

According to Attaei (2017), the release of active ingredients can occur due to diffusion or rupture (due to thermal or mechanical) or dissolution. In this study, the release of antimicrobial agent due to thermal activity in which the microcapsule was heated 40 ^{o}C prior to the measurement of release using GC headscape.

GC headspace chromatogram of clove oil encapsulated in polyurethane shell is presented in

Figure 5. The chromatogram indicated the major compounds that are eugenol, caryophyllene and some fatty acid (hexadecanoid acid, octadecenoid acid and oleic acid). The fatty acid was not detected through the GC-MS analysis of the clove oil but occurred in the headspace analysis of the microcapsule. The reason is might due to the reaction of fatty acid with polyol resulting fatty acid in an ester form during the process of polymerization. Free fatty acids in the essential oil suspected because of the hydrolysis reaction during storage (Minhal et al., 2017)

In this case, the occurrence of fatty acid can be an advantage because fatty acid and fatty acid ester had been identified their antimicrobial bioactivities (Arora et al., 2017; Nakayama et al., 2015). Fatty acids have known modulate immune responses by acting directly on T cells so they have antibacterial and antifungal properties (Aparna et al., 2012).

The release of eugenol and caryophyllene in each day was summarized then were plotted and added with a trend line using Microsoft Excel®. The equation and R-square (R^2) were calculated based on the trend line. R^2 higher than 0.98 indicates the equation fits with the data. As presented in Figure 6, the concentration of caryophyllene were released based on the equation $C = 0.1922x^3 - 2.698x^2 + 11.372x$, while the equation of released eugenol is $C = 0.0436x^3 - 0.5741x^2 + 2.3489x - 0.1681$ with x refers to number of days.

Based on the equation of the release profile, it can be predicted the percentage of weight ratio for the next days (after the 10^{th} day). The release concentrations were compared with concentration of eugenol or caryophyllene in the clove oil that is 81.64% and 17.01% respectively (Table 1). The prediction is presented in Figure 7. It shows that the eugenol could release for 59 days but the caryophyllene only for 15 days.

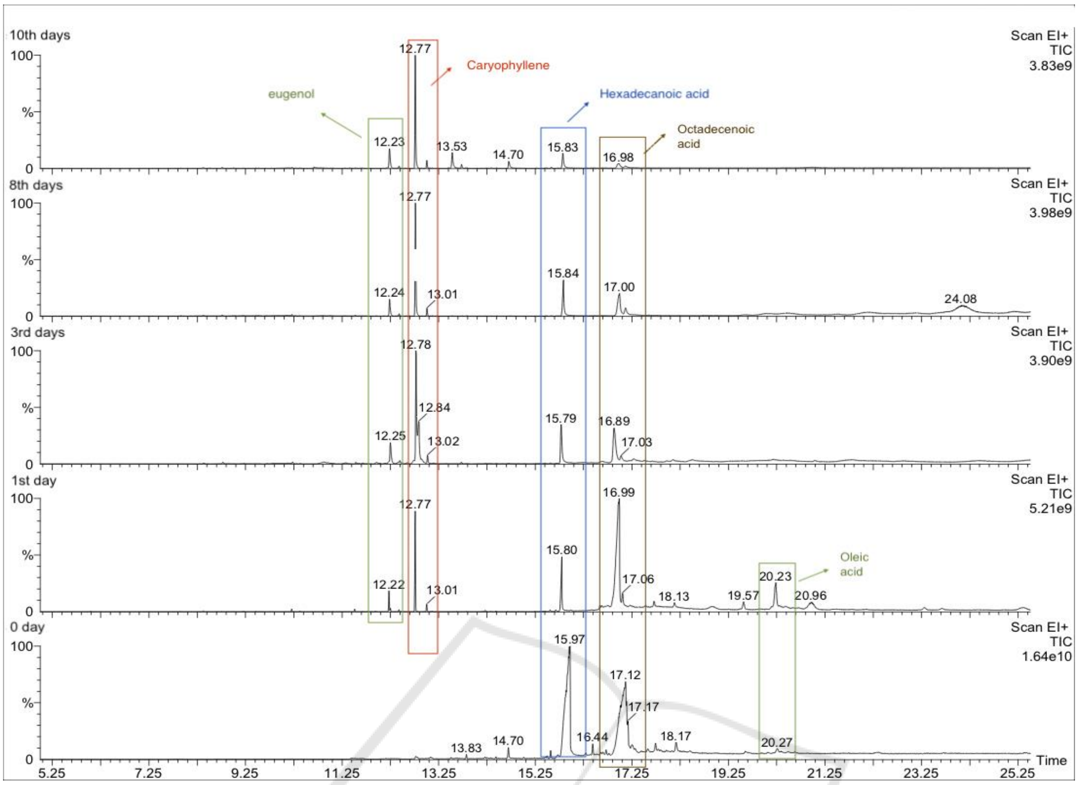

Figure 5: GC headspace chromatogram of clove oil encapsulated in polyurethane shell.

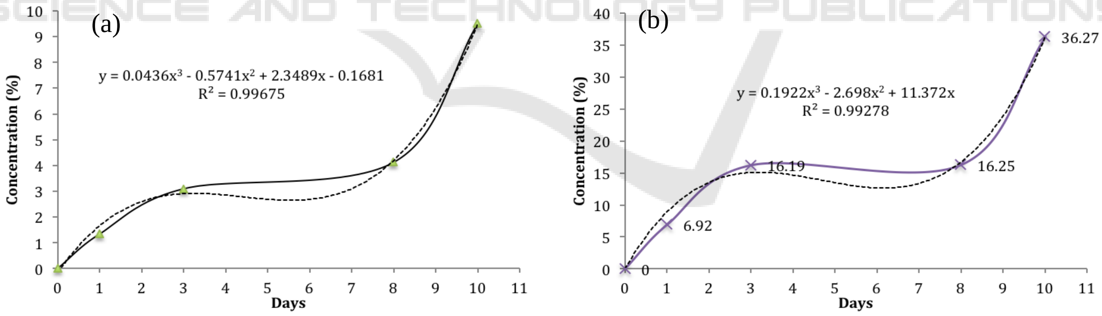

Figure 6: Release profile of clove oil encapsulated in polyurethane shell: (a) eugenol, (b) caryophyllene.

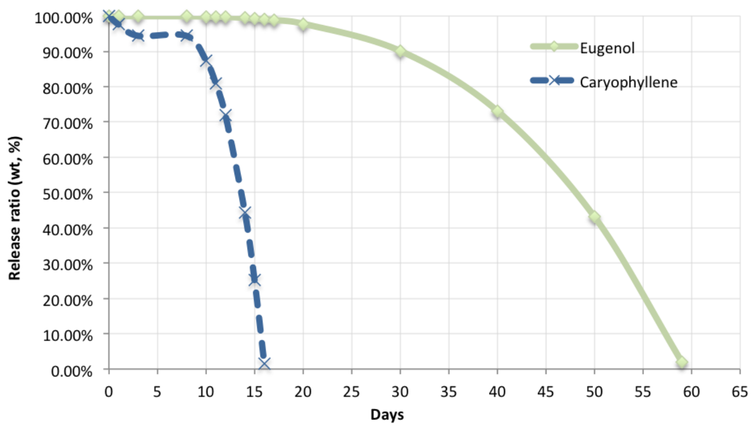

Figure 7: Predicted release of eugenol and caryophyllene.

4 CONCLUSIONS

The concentration of eugenol was released based on the equation $C = 0.0436x^3 - 0.5741x^2 + 2.3489x - 0.1681$, whereas the equation of released caryophyllene is $C = 0.1922x^3 - 2.698x^2 + 11.372x$ with x refers to number of days. From this release profile, it was found that the clove oil encapsulated in polyurethane shell could emit eugenol for 59 days and caryophyllene for 15 days. Therefore, it could be concluded that the microcapsules of clove oil in polyurethane shell is suitable for long term application.

ACKNOWLEDGEMENTS

We would like to thank to PT Covestro Polymers Indonesia who gave us the sample of MDI as material for this research.

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