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Cite as: AIP Conference Proceedings **2193**, 030006 (2019); https://doi.org/10.1063/1.5139343
Published Online: 10 December 2019

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Microencapsulation of Clove Oil using Spray Dry with Casein Encapsulator and Activity Test towards *Streptococcus mutans*

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Abstract. Clove oil originates from leaves, flower, and trunks of clove plant (*Syzigium aromaticum*) that is volatile and prone to both temperature and light. Clove oil generally uses as medicine due to properties such as analgesic, antibacterial, antioxidant, antifungal, and antivirus. This research aims to decrease the rate of clove oil evaporation by making it into a microcapsule. This research starts with the isolation of clove with a spray dryer using a casein encapsulator. Microcapsule resulted is evaluated in both physically and chemically as it is also tested for activity towards *Streptococcus mutans.* The result of clove oil microcapsule is in the shape of white-yellowish color crystal powder with a unique smell of clove. The particle shape is round with pores in the size of 1.16μm, encapsulation efficiency of 97.78%, loading capacity 62.24%, water content 12.59%, solubility in water 91.65%. Microcapsule activity test of clove oil shows inhibition as an antibacterial towards *Streptococcus mutans* of 1.663 CFU with a minimum inhibition concentration of 0.25g/mL and an inhibitory rate of 11.8±1.0 mm. Clove oil could be made into microcapsule with casein as an en which also having antibacterial activity towards *Streptococcus mutans*.

Keywords: Casein, Clove Oil, Microcapsule, Spray Dryer, *Streptococcus mutans*

INTRODUCTION

Clove oil is a form of essential oil, a colorless or pale-yellow liquid, that is in contact with sunlight would turn into black-brown in color and have a specific smell [1]. There are several kinds of clove oils, namely a clove oil that origins from its leaf (clove leaf oil), flower (clove bud oil), trunk (clove stem oil). Properties of clove oil include: antibacterial, antioxidant, and analgesic [2,3] Several forms of clove oil that have been used in the pharmaceutical industries are soap, perfume, toothpaste, dental fillings and mouthwash [4,5].

Mouthwash that is using clove oil could inhibit the growth of the *Streptococcus mutans* bacteria [6]. Clove oil in mouthwash is in its liquid form, that in a case of temperature and light exposure would cause it to easily evaporate [7]. Therefore, this research will focus on making a microcapsule form of clove oil to reduce the rate of its evaporation.

The 4th Biomedical Engineering's Recent Progress in Biomaterials, Drugs Development, Health, and Medical Devices
AIP Conf. Proc. 2193, 030006-1–030006-8; https://doi.org/10.1063/1.5139343
Published by AIP Publishing. 978-0-7354-1944-5/$30.00

A microcapsule is a solid-powdered form with micron size that consists of active ingredients coated with an encapsulator material using encapsulation method [8]. The purpose of making microencapsulation is to turn the liquid form into solid, perform a controlled release of active ingredients and to cover unpleasant taste or odor. There are various methods of encapsulation: spray dry, spray chilling, freeze-dry, fluid bed coating and coacervation [9].

Spray drying method that will produce a dry-powdered form from its original liquid form is done by spray drying at an inlet temperature of 110-220°C and outlet temperature of 50-80°C [9]. This drying method is widely used for active ingredients that are prone to heat like food and medicine [10]. The previous research produces clove oil microcapsule with casein and maltodextrin encapsulate for vegetal pesticide using spray dry with an encapsulation efficiency of 99.14%.

Casein is a biopolymer with hydrophilic and hydrophobic properties. Isolation of casein could produce approximately 3kg / 100kg or about 3% of casein from fat-free milk. casein is one protein in milk with excellent heat stability reaching to 140°C and is safe to use as a food product, thus it's widely used as an encapsulator [11]. Based on this information, this research will focus on making a microcapsule form of trunk clove oil using spray dry method with casein encapsulator and performing activity test towards *Streptococcus mutans*.

MATERIALS AND METHODS

Materials

Clove stem oil was purchased from CV Pavettia (West Java, Indonesia), fat-free milk Diamond brand was purchased from Superindo Supermarket (Jakarta, Indonesia), and rennet was purchased from Emmi (Lucerne, Switzerland). Buffer phosphate solution pH 10, HCl, NaOH, and $CaCl_2$ solution 10% were purchased from Merck Company (Darmstadt, Germany). Agar nutrient and Brain Heart Infusion were purchased from Oxoid (Basingstoke, United Kingdom).

Casein Isolation

The isolation of casein using Sahlan methods (2012) with slight modification [12]. One liter of "Diamond" brand cow milk is stored in a refrigerator 4 °C. The pH is reduced to achieve its iso-electric point in pH 5 by adding 0.1N HCl while being stirred and heated to 35 °C. Then after it, casein was coagulated using 50 mg of rennet solution. Then after aging for 45 minutes, coagulated casein was formed, it was washed using free mineral water at 70 °C. Coagulated casein was separated from the supernatant (whey solution) by decantation, washing the casein with 1 liter of aquadest 3 times using a filter paper until whey solution has disappeared. Coagulated casein that has been filtered. Then, the coagulated casein will be freeze-dried. The dry weight is then measured and used to calculate soakings (%) as well as the organoleptic test using Equation 1.

$$Soaking(\%) = \frac{Weight\ after\ freeze\ dry}{Weight\ before\ freeze\ dry}\ X\ 100\ \% \quad (1)$$

Clove Oil Suspension Preparation

Clove oil suspension preparation starts with the formulation as Yeshinta (2019) method shown in Table 1 [1]. 13.5g of casein was added with 50.1g of buffer phosphate solution stirred for 15 minutes. Then, it was added 27g of clove oil followed by gradually adding 49.2g of $CaCl_2$ 10%. The pH is maintained at 7 by adding HCl 0.1N and NaOH 0.1N. The mixture was homogenized with an Ultraturrax Homogenizer (IKA® T18 Switzerland) at 15,000 rpm for 15 minutes. The suspension of clove oil and casein was measured its viscosity using a viscometer (cole palmer) with a spindle L1 and speed of 100 rpm with 3-time repetitions.

TABLE 1. Microcapsule Formulation of Clove Oil

Component	Formula (%)
Clove Oil	9.09
Casein	4.65
$CaCl_2$ Solution (10%)	16.70
Buffer Phosphate Solution	69.57

Clove Oil Microencapsulation

Clove oil and casein suspension are dried using a mini spray dryer B-290 (Buchi, Switzerland) with inlet temperature between 110 °C – 120 °C and outlet temperature between 55 °C – 65 °C as the method described by Pratami (2018) with some modification [13]. During the spray drying process, clove oil and casein suspension are flowed through a hole into the nozzle thus allowing a microcapsule of clove oil and spray dried casein to formed. The result is observed with the organoleptic test, and soaking measurement is calculated by the clove oil microcapsule produced. Clove oil microcapsule soaking Equation 2 is shown on the below:

$$Soaking\ (\%) = \frac{weight\ after\ spray\ drying}{weight\ before\ spray\ drying}\ x\ 100\ \% \quad (2)$$

Particle Size Distribution Test

Particle size distribution test is performed using the particle size analyzer from DelsaTM Nano C Beckman Coulter. One gram of clove oil microcapsule was dissolved into 10 mL of aquadest. The mixture is then taken for 3 drops to be analyzed. The test is performed at Research Centre of Physics LIPI Serpong Indonesia.

Particle Morphology Analysis

Particle morphology test was done for clove oil microcapsule and spray dried casein. Samples were observed using SEM JMS-T20 (Japan) with voltage acceleration of 20kV. The magnification used is between 500x-10,000x. The morphology test was performed in the Research Laboratory of Material and Fire Engineering, Faculty of Engineering, State University Jakarta.

GC-FID and Encapsulation Efficiency Test

The analyzed was performed with Gas Chromatography Shimadzu GC (Japan) at the Laboratory of Department of Chemical Engineering, Universitas Indonesia. 20 µL of clove oil was dissolved into 1 mL diethyl ether then injected into GC. The oven temperature was 50 °C – 230 °C. Injector temperature and each of the detectors were 230 °C and 250 °C. Helium gas was used as a carrier for clove oil with a flow ratio 1 mL/minute.

0.5 g of clove oil microcapsule in the headspace was analyzed through an exit vapor from clove oil microcapsule. The components of the clove oil and the clove oil microcapsule were identified by comparing the retention time with the calibration curve of standard eugenol. Encapsulation efficiency was calculated using Equation 3 below:

$$Encapsulation\ Efficiency(EE) = \frac{Total\ unencapsulated\ eugenol}{Total\ encapsulated\ eugenol}\ X\ 100\ \% \quad (3)$$

Loading Capacity

The loading capacity was done with gravimetric analysis using an oven for 15 minutes at 105 °C and chilled into a desiccator. Clove oil microcapsule and spray dried casein were weighed for 1gram each, then put into the weighting bottle before being weighted to calculate weight before drying. Then it's dried using an oven at 105 °C for 3 hours, chilled at a desiccator for 30 minutes for then being weight again to measure its weight after being dried, after that its dried every 1 hour for 3 times to obtain its constant weight. The value of loading capacity is obtained by using the below Equation 4:

$$Loading\ Capacity = \frac{W\ Clove\ Oil\ Microcapsule - W\ Spray\ dried\ casein}{W\ Clove\ Oil\ Microcapsule}\ X\ 100\ \% \quad (4)$$

FTIR Analysis

Clove oil, clove oil microcapsule, spray-dried casein were characterized using spectrophotometer with infrared from Shimadzu IR Prestige. FTIR spectrum was read at infrared wave between 4000 to 500cm^{-1}. Functional groups were determined based on what has been identified from Clove oil, clove oil microcapsule, spray-dried casein. The test was performed at the Laboratory of Department of Chemical Engineering, Universitas Indonesia.

Water Content Test

Measurement of water content was performed by dried one gram of clove oil microcapsule into an oven at 105°C for 30 minutes. Then measured the water content after the water content has been constant.

Water Solubility Test

Measurement of water solubility was performed using gravimetry method. 250mg of clove oil microcapsule powder was dissolved into 25mL of aquadest with aid from ultrasonic cleaner sonification. The solution was filtered using filter paper (that has been weighted before). Filter paper and the residue were then dried in an oven at 105°C for 60 minutes. Solubility (%) was measured using the Equation 5 below:

$$\%\ Solubility = \left[1 - \left(\frac{Pc - Pb}{\frac{100 - \%\ MCxPa}{100}}\right)\right] X\ 100\ \% \tag{5}$$

Annotation:
Pc (g) : Filter paper and residue weight after drying,
Pb (g) : Filter paperweight before drying,
Pa (g) : Sample weight
MC (%) : Water content of the sample used

Antibacterial Test for Clove Oil Microcapsule

The antibacterial analysis was used method described by SA Soekanto (2017) with some modification [14]. All of the equipment used are firstly sterilized using an autoclave from Alp at 121 °C for 15 minutes. Materials used are firstly sterilized using tantalization method at 100 °C for 30 minutes before used for 3 straight days. 7.4g of Brain Heart Infusion (BHI) from Oxoid and 3g of ara were dissolved in 200 mL of aquadest, then heated until boiling before being put into a reaction tube and closed with cotton. Sterilization using autoclave was done at 121 °C for 15 minutes, after that the BHA media was sterilized by pouring it into a petri dish with a thickness of 6 mm and left to harden.

Bacteria that have been cultured for 1x24 hours was then diluted by putting 1mL of the bacteria into an empty reaction tube, then add 9 mL of sterile NaCl 0.9%, after that the turbidity of the suspension as compared to the turbidity of the Mc. Farlan 0.3.

Bacteria were spread onto the object-glass and fixated using fire, then dropped with 1-2 drops of violet crystal solution (Gram A) and left for 1 minute, before being washed using flowing water, followed by 1-2 drops of iodine solution (Gram B) and left again for 1 minute. After that, the solution was washed again with a flowing water, followed by decolorization with 1 drop of alcohol (Gram C) and left for 15 seconds, then its washed again by flowing water, drops with safranin coloring solution (Gram D), left for 10-20 seconds and then washed again by flowing water. The result was aerated and added with 1 drop of immersion oil and observed with a microscope with 100x magnification.

Concentration preparation of clove oil microcapsule and clove oil were made from 100% solution stock and then diluted ranging from 75%, 50%, 25%, 12.5% to 6.25%. Clove oil microcapsule was diluted using aquadest, while clove oil was diluted using DMSO10%.

Petri glass with BHA media was prepared, then took 0.1mL of bacterial suspension to be tested, inoculated into the BHA media evenly by spread plate method and leave the surface to dry. Blank disk was soaked for 1-2 minutes with clove oil microcapsule solution and clove oil solution with each respective concentration that has been made

before 75%, 50%, 25%, 12.5%, 6.25% and were left to dry for 1-2 minutes then put onto the surface of BHA media, then the 25µg/mL (k+) of amoxicillin antibiotic disk, negative control disk (clove oil microcapsule using k- aquadest) and clove oil using k- DMSO 10% were being put onto the surface of BHA media. Each of the paper disks was inoculated for a specific amount of distance so that inhibition overlapping zone would not occur. Petri dish was labeled from its bottom. Petri dish was put into a jar with anaerogen, sealed tightly and were left for 15 minutes until the jar becomes heated. The result was incubated at 37 °C for 2x24 hours and each of the inhibition zones on every petri dish was observed using a caliper.

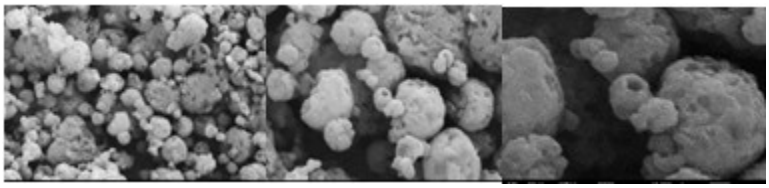
The determination of Minimum Inhibition Concentration **(**MIC) was done by using a solid dilution method. 6 tubes of PCR tube were prepared, then clove oil microcapsule was prepared with the following concentration: 75%, 50%, 25%, 12.5%, and 6.25%). 1mL of BHI media was prepared as positive control. 0.1mL of bacterial suspension is put into the 6 PCR tubes, then vortex it until it has mixed. Petri dish with BHA media on it is prepared, then make a certain pattern on the bottom of each tube to make it into several groups and label them according to its concentration. Clove oil microcapsule solution that has been inoculated with bacteria by using a round ose onto the BHA media surface. The Petri was determined as the minimum inhibition concentration.

RESULTS AND DISCUSSION

The isolated casein was identified as odorless and colorless powder form in the organoleptic test. The soaking result obtained was 1.85% from 1 liter of milk, the soaking obtained were lower compared to the result obtained by the previous research, that theoretically casein could be produced between 3kg/100kg or 3% [2,15]. However, the soaking result of casein did not reach 3% due to a lot of coagulants that stick into the filter paper during filtration resulting in the decrease of the freeze-dried casein.

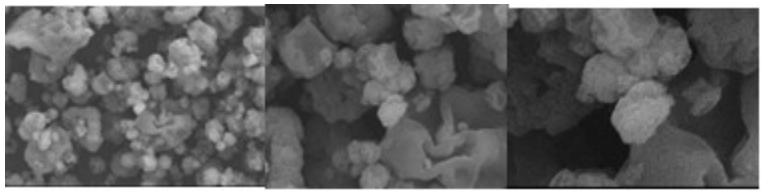
The result from the viscosity test from clove oil suspension shows 14.33 ± 0.97cps and casein suspension with 11.83 ± 0.66cps. Both suspensions have fulfilled the requirement of ≤100cps that serve as a requirement to flow a liquid inside a nozzle of a spray dryer. Clove oil microcapsule was having a powder white-yellowish characteristic with an aromatic smell of clove. The soaking obtained was 41.85%, the soaking value did not reach 100% due to a lot of the spray-dried clove oil microcapsule that is stuck to the evaporation tube and was difficult to be recover.

The particle morphology result of clove oil microcapsule shows that its particle was having a dense round shape with some pores, while spray-dried casein has an irregular round wrinkled shape, that according to the previous research, if a particle is having a wrinkled and deflated shape then it is likely to be caused by a wrinkling of particle during drying and chilling process [16]. Nonetheless, the clove oil microcapsule result was showing round dense shape which indicates clove oils as well encapsulated by the casein. The result could be seen in the Figure 1 and 2.



(a) (b) (c)

FIGURE 1. Clove Oil Microcapsule. (a) 2000x, (b) 5000x, and (c) 10.000x magnification



(a) (b) (c)

FIGURE 2 Spray Dried Casein. (a) 2000x, (b) 5000x, and (c) 10.000x magnification

The particle distribution size result of clove oil shows the size was 1.16µm, the result was in accordance with the B-290 spray drying equipment specification used that could produce a particle with micron-size between 2-25µm.

The loading capacity from clove oil microcapsule was 62.24 ± 2.11%, the loading capacity could determine the weight of clove oil contained in the encapsulation by gravitometer method testing. The loading capacity value was

well enough if compared to the previous research that was making a clove oil microcapsule from leaf and obtain only 57.72% [1]. Therefore, clove stem oil was having a bigger loading capacity compared to leaf clove oil.

The results of the encapsulation efficiency of clove oil microcapsules obtained 97.78%, the encapsulation efficiency was the acquisition of the number of active substances obtained after encapsulation and the results were based on gas chromatography testing. The encapsulation efficiency obtained depends on the active ingredients used, this study uses casein and previous studies made clove oil microcapsules with casein coatings obtained a high encapsulation efficiency value of 99.14% [8]. Casein can be seen that if used as a coating it has good encapsulation efficiency because casein has biopolymer properties and heat stable.

The encapsulation efficiency of clove oi oil microcapsule was 97.78%, the encapsulation efficiency. Encapsulation efficiency obtained depends on the active ingredients used, this research uses casein and the previous research made the clove oil microcapsule using casein and obtain a high encapsulation efficiency value of 99.14%. Casein was known to have a good encapsulate because of biopolymer properties and more stable towards heat [17].

The result of FTIR of clove oil microcapsule shows that it has some functional groups namely the hydroxy bond with a long wave of 3279.95 cm^{-1}, aromatic ring compound 1642.73 cm^{-1}. alkane group 1521.90 cm^{-1}, amine group 1441.81 cm^{-1} and carbon group 1238.23 cm^{-1}. The results show no difference from the previous research [1].

The result of clove oil microcapsule water content was high which is 12.59%, this is caused by the use of spray drying temperature of 110°C which is not optimized and causing the water content of clove oil microcapsule to have a high value.

The solubility in water result shows an average of 91.65 ± 1.64%, clove oil microcapsule during solubility in water test was having a high value, according to the theory with casein as an encapsulate. Casein has an α-S1 component which is hydrophilic and β-casein which is hydrophobic so that in the solubility in the water test, casein works accordingly with its hydrophilic properties.

The bacteria identification using bacterial staining [18], the result of *Streptococcus mutans* staining have a little round size with chain form also did not move and also classified as gram-positive bacteria. From the result of inhibition rate diameter, clove oil microcapsule and clove oil with 0.062g/mL until 0.75g/mL concentration was shown on Figure 3, the result shows an increase in rate.

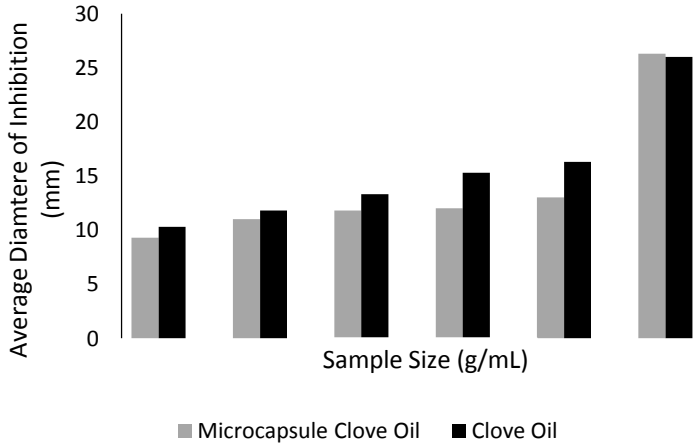


FIGURE 3. Graph of Minimum Inhibitory Concentration

Inhibition rate of clove oil microcapsule was in the strong category which between 10-20mm, yet on the concentration of 0.0625g/mL the category was moderate which is 5-10mm with the result becomes 9.3mm [19]. The inhibition rate of clove oil microcapsule was lower if compared to the clove oil. Clove oil microcapsule has protected the active ingredient release (clove oil). The benefit of clove oil microcapsule, that can also be connected to the working mechanism of a drug such as an encapsulated tablet that is having a long release in the body or a long-acting. The minimum inhibition concentration of clove oil microcapsule was at 0.25g/mL concentration, after observation of incubation for 2x24 hours, there was no growth of bacteria in the media surface. However, at a concentration of 0.0625g/mL and 0.125g/mL shown that there was the growth of bacterial colonies in the form of a small circle that follows the scratching pattern. The concentration of 0.25g/mL could be determined as the MIC that

could inhibit the *Streptococcus mutans* bacteria much as 1663CFU (according to the colony calculation using a colony counter).

CONCLUSION

In conclusion, the clove oil from the trunk could be made into microcapsule with casein as an encapsulate using spray drying. With the efficiency encapsulation is 97.78%, loading capacity is 62.24 ± 2.21%. The clove oil microcapsule particle has a round with pores shape with a size of 1.16µm. It has a water content of 12.59% and solubility in water 91.65 ± 1.64%. Lastly, Clove oil microcapsule has antibacterial activity towards *Streptococcus mutans* with a colony amount of 1.663CFU, inhibition concentration 0.25g/mL with inhibition diameter of 11.8 ± 1.0 mm.

ACKNOWLEDGMENTS

This work is supported by Hibah Publikasi Internasional Terindeks 9 (PIT 9) 2019 funded by DRPM Universitas Indonesia (Grant No. NKB-0067/UN2.R3.1/HKP.05.00/2019).

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