CONTROLLED RELEASE OF ESSENTIAL OILS FROM MICROCAPSULES BASED ON GUM ARABIC AND CHITOSAN PREPARED BY COMPLEX COACERVATION AS MOSQUITO REPELLENT AND ANTIMICROBIAL MATERIAL

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Microcapsules loaded with essential oils, namely *Litsea cubeba*, *Cymbopogon nardus* and *Cymbopogon citratus*, in gum arabic and chitosan were prepared to explore their mosquito repelling effects and their antimicrobial function. The encapsulation ratio, along with the materials characterization and release behavior of the essential oils from the microcapsules were studied, together with temperature and time dependences. Mosquito repellency was assessed against *Ae. aegypti* using the "arm-in-cage" method, and the antibacterial activity was tested against *E. coli* and *S. aureus*. The results demonstrated that microcapsules loaded with essential oils were successfully prepared for all three essential oils. Also, the results showed that the highest encapsulation ratio was found for microcapsules loaded with *Cymbopogon citratus* oil. The microcapsules loaded with all essential oils were effective in prolonging protection time against *Ae. aegypti*, especially in the case of the *Litsea cubeba* oil, while the *Cymbopogon nardus* and *Cymbopogon citratus* oils yielded the highest antibacterial activity against *E. coli* and *S. aureus.*

Keywords: antimicrobial function, complex coacervation, essential oils, microcapsules, mosquito repellency

INTRODUCTION

Mosquitoes are an established vector of infectious diseases in humans throughout the world. The development of methods to repel mosquitoes through topical pharmaceutical intervention by a variety of technologies has been a subject of intense interest. In recent years, mosquito-based diseases have had a large impact on global population mortality, skewed toward higher incidence in poorer nations.

Typical mosquito-repelling active ingredients found in many commercial products formulated to protect humans against mosquito bites consist of synthetic compounds, including DEET (N,N-diethyl-3-methylbenzamide) and Picaridin (2-[2-hydroxyethyl]-1-piperidine carboxylic acid-1-methylpropylester). These ingredients, although very effective repellents, have some limiting characteristics, such as possessing unpleasant odors, and unacceptable medical consequences, including giving rise to brain swelling in children, inducing low blood pressure across patient populations, as well as being environmentally unfriendly, both in terms of product processing and product disposal.[1,2] Alternatively, essential oils are known to have relatively safe toxicological and environmental profiles, and thus have gained prominence in the health, pharmaceutical, cosmetic, food and agricultural industries.[3]

Essential oils are typically extracted from different parts of herbs and spices, such as flowers, seeds, buds, leaves, branches, roots, *etc*., and they have been used in herbal remedies since ancient times. As essential oils contain many

chemical compounds, like flavonoids, steroids, volatile compounds, glycosides, and alkaloids, they exhibit bioactive functionalities, for example, insecticidal, antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.[3,4]

Regarding alternatives to synthetic mosquito repellents, such as DEET and Picaridin, plant-based essential oils can minimize unwanted properties. In particular, essential oils from *Litsea cubeba*, *Cymbopogon nardus* and *Cymbopogon citratus*, found in abundance in tropical countries, contain high concentrations of bioactive compounds. Chemical analysis by gas chromatography reveals that *Litsea cubeba* fruits are rich in essential oils, the dominant components being monoterpenes at 94.4–98.4%, represented mainly by neral and geranial at 78.7–87.4%, while the D-Limonene constituent was at 0.7–5.3%.[5] The essential oils of *Cymbopogon nardus* have been reported to be composed of citronellal (33.06%), geraniol (28.40%), nerol (10.94%) and elemol (5.25%),[6] whereas *Cymbopogon citratus* contains citronellal (83.50%), myrcene (27.83%), geranial (27.04%), neral (19.93%) and geraniol (4.33%).[7] The use of these essential oils for mosquito repellency has been previously reported in the literature. For examples, lemongrass oil (*Cymbopogon citratus*) was formulated as ointment and cream and applied on a bird animal model's skin against a 2-day starved culture of *Aedes aegypti* L. mosquitoes and it demonstrated 50% repellency lasting for 2-3 h.[8] Mixed essential oils from *Litsea cubeba* and *Litsea salicifolia* showed the highest synergistic action (65.5% escape response by *Ae. aegypti*) compared to that of each oil taken alone at the same concentration (LC = 20% and LS = 32.2%), using an excito-repellency test chamber.[9] Citronella oil derived from *Cymbopogon nardus* has been reported for its outstanding insect repellent activity, compared to other oils, in previous studies.[10]

The mosquito repellency and antibacterial properties of these three essential oils need further comparative investigation. In the present work, using micro-encapsulated products to allow controlled release of the active ingredients, the modulation of mosquito repellency and the inhibition of the encapsulated essential oils' degradation were studied as a function of time. The micro-encapsulation technology was developed to enclose and give a time-dependent release profile for the active ingredients, so that the desired properties in pharmaceutic, cosmetic, food, paper products, *etc*., could be optimized for their intended purpose. Their sizes, shapes, and release mechanisms are different, depending on the type of shell materials and the preparation method used.[11-16] The release mechanisms involve dissolution, osmosis, diffusion, disruption, and erosion.[11,14,17-19] In addition, the size and shape of microencapsulated particles can also vary as a function of the materials and methods used in the preparation.[13,15]

Complex coacervation is a method used to prepare microcapsules having a core phase of the active substance and a surrounding shell phase of two polymers, with an opposite charge, where the shell is formed in several sequential steps. Numerous polymers have been used for this purpose, especially biopolymers due to their lack of toxicity and high sustainability. In this work, gum arabic and chitosan were selected as the shell materials to trap essential oils; sodium tripolyphosphate (NaTPP) was used as crosslinking agent, due to its high efficiency and environmental friendliness.[19-22] *Litsea cubeba*, *Cymbopogon nardus* and *Cymbopogon citratus* essential oils were encapsulated in gum arabic and chitosan microcapsule shells using the complex coacervation technique. The characteristics of the microcapsules were analyzed, and the temperature-dependent release of essential oils was studied, along with an assessment of their effectiveness for mosquito repellency and antibacterial activity.

EXPERIMENTAL

Materials

Gum arabic was supplied by Ajax FineChem (Australia) and chitosan (85% DD) was received from SeaFresh Chitosan (Lab) Co., Ltd., Thailand. Sodium tripolyphosphate (NaTPP) (Sigma, Aldrich, USA) and acetic acid (99.8%, RCI Labscan, Thailand) were of analytical grade (>99%). Essential oils, *i.e. Litsea cubeba* (LC), purchased from Aromahub Group Co., Ltd., Thailand, *Cymbopogon nardus* L. (CN) and *Cymbopogon citratus* (CC), from N&B Organizer Co., Ltd., Thailand, were used. Absolute ethanol was obtained from RCI-Labscan, Thailand.

Synthesis of microcapsules

The microencapsulation of each essential oil was performed using the complex coacervation technique. Gum arabic and chitosan were employed as shell materials using a modified procedure.[20-21] Briefly, each essential oil was added into 100 mL of 5% w/v gum arabic solution. An emulsion was formed by

homogenization using a high-speed blender (homogenizer) (T25 digital Ultra-Turrax, IKA, Germany) at a speed of 11,000 rpm for 15 min. Then, 100 mL of 1.25% w/v chitosan solution in 2% v/v acetic acid was added to the gum arabic-chitosan solution to initiate the coacervation process. 100 mL of the crosslinking agent (0.2% w/v NaTPP solution) was subsequently added, and the mixture was then stirred using a magnetic stirrer at 1000 rpm for 2 h to form the microcapsules (MC) of essential oils. The resulting microcapsules were separated by decantation, vacuum filtration, and thoroughly rinsed with deionized water. Finally, the microcapsules were dried to a powder form by freeze-drying (Freeze Dryer, FD 5-4, Gold Sim, Czech Republic). The labels and the description of the microcapsule samples are tabulated in Table 1.

Table 1
Denotation and description of the samples

Sample label	Description
MC	Microcapsules of gum arabic and chitosan as shell materials
MC-CC	Microcapsules of gum arabic and chitosan as shell materials loaded *Cymbopogon citratus* essential oil
MC-CN	Microcapsules of gum arabic and chitosan as shell materials loaded *Cymbopogon nardus* essential oil
MC-LC	Microcapsules of gum arabic and chitosan as shell materials loaded *Litsea cubeba* essential oil
EO-CC	*Cymbopogon citratus* essential oil
EO-CN	*Cymbopogon nardus* essential oil
EO-LC	*Litsea cubeba* essential oil

Characterisation of microcapsules

The encapsulation ratio describes the extent of essential oil incorporation as a percent of the total oil quantity used in the preparation. The total content of essential oils in the microcapsules and the free essential oils (non-encapsulated essential oils) were determined by a modified method.[21,23-26] The amount (g) of the total essential oil content of the microcapsules was determined by reflux extraction of 0.5 g of a dried microcapsule sample in 75 mL of 95% ethanol for 3 h; then the extracted solutions were filtered (Whatman #1). The free essential oil content was determined by adding 0.5 g of dried MC into 50 mL of 95% ethanol, followed by stirring the mixture at room temperature for 15 min, followed by filtration (Whatman #1). After that, the ethanol in the extracted solutions was removed from the essential oils using a rotary evaporator. The resulting essential oils were then weighed. All experiments were carried out in triplicate. The encapsulation ratio (%) was calculated using the following equation:

$$Encapsulation\ ratio\ (\%) = \left(\frac{Encapsualted\ oil\ content(g)}{Total\ oil\ content(g)}\right)x100 \quad (1)$$

The encapsulated oil content was calculated from the difference between total oil and free oil contents. The morphology of the microcapsules was examined using an optical microscope (Olympus CX43 Standard Light Microscope, Japan) equipped with a Canon DS126571 camera (Canon Inc, Japan). The diameter of the wet microcapsules was measured for at least 100 cells within the same image to evaluate the mean diameter of the microcapsules.

Microcapsule sizes and particle size distributions were determined by laser diffraction with a particle size analyzer (Mastersizer Malvern Instruments, Malvern, UK). The measurement of particle size distribution was performed after dispersing the microcapsules in distilled water using ultrasonication for 2 min and 1.5400 as the refractive index of the standard material, gum. Sizes ranged from 0.05-900 µm. The measurement was done in triplicate.

Scanning electron microscopy (SEM, JEOL, JSM-7610F Plus, Japan) was used to investigate the surface morphology of the microcapsules. The microcapsules sample was sputter-coated with a thin layer of gold, and the final micrographs were taken at 15.0 kV.

Thermal stability and weight loss of microcapsules containing essential oils and free microcapsules were analyzed using thermogravimetric analysis (TGA, TG8120, Rigaku, Japan) with a heating rate of 10 °C/min from 30 to 600 °C in a nitrogen atmosphere. In addition, to study the release of essential oils applied on the body under isothermal condition, the release of essential oils was examined by weight loss (%) of microcapsules using TGA (TG8120, Rigaku, Japan) by holding the temperature at 36.5 ± 0.5 °C, replicating the average human body temperature[27] for 180 min under 40 mL/min of nitrogen gas flow.

A Fourier transform infrared spectrometer (FTIR) (Spectrum Two, PerkinElmer Inc., USA) was used to identify the chemical groups of the microcapsules in the range of 500 to 4000 cm^{-1}. The loaded and non-loaded microcapsule samples were pressed into pellet form using a hydraulic press.

Mosquito repellent testing

Essential oils and microcapsules loaded with essential oils were tested for repellent protection time against *Ae. aegypti* using the standard "arm-in-cage" test, according to the recommendations of the World

Health Organization (WHO)[27] (Fig. 1). Also, the study was carried out according to the Declaration of Helsinki guidelines and was approved by the Research Ethics Committee of the Faculty of Medicine of the CMU (protocol code PAR-2558-03391/Research ID: 3391).

Two hundred fifty female mosquitoes aged 5-7 days were randomly selected, placed in a standard mosquito cage (30×30×30 cm), and unfed for 12 h before starting the experiment. Before the test, each volunteer's arms were rinsed with distilled water, air dried, and rubber gloves protected their hands. At the beginning of the test, the mosquitoes' biting activity was checked by introducing bare arms into the mosquito cage. After two or more mosquitoes have come onto their hands, the arm was immediately pulled out of the mosquito cage; this demonstrated that the mosquitoes were ready for the full test to begin. Each volunteer wore rubber gloves containing a 30 cm^2 hole in the test area on the forearm. Then, 0.1 mL of 10% *Cymbopogon citratus*, *Cymbopogon nardus* or *Litsea cubeba* essential oils in coconut oil (EO-CC, EO-CN, and EO-LC) were applied to the test site and left for 5 min. The test arms were then introduced in the mosquito cage for 3 min. If at least two mosquitoes landed and bit in the test area, the test was stopped. It was then considered that such substances could not prevent mosquito bites.

Alternatively, if there were no mosquito bites on the test arm within 3 min, the arm was removed from the mosquito cage. Then, the same procedure was repeated every 30 min, until at least two mosquito bites occurred within 3 min. The elapsed time was recorded. The median values gave the protection time for replicates. This test was carried out using two human volunteers (1 adult female, 1 adult male).

The test was performed in the same manner to test repellent efficiency of the microcapsule samples. In this case, 0.02 g of each essential oil loaded microcapsules, mixed with 0.18 g of coconut oil, were applied as described in our earlier work on DEET and picaridin loaded microcapsules for the study of mosquito repellency.[29] Coconut oil (CCO) was used as a control sample.

Figure 1: Test of mosquito repellency with *Ae. aegypti* by "arm-in-cage" technique according to the WHO standard test method

Antibacterial activity assay

Bacterial culture

The initial bacterial culture was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and the tests were performed against *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25922). Cultures of bacteria were grown in nutrient broth (NB) at 37 °C for 16–18 h using a shaking speed of 150 rpm, and then diluted. The turbidity of the culture suspension was adjusted to 0.5 McFarland standard. Both quantitative and qualitative assessments of the antimicrobial activity of the microcapsule samples were performed.

Growth inhibition assay

The quantitative test was performed on a 96-well plate according to Haase *et al*. (2017) with minor modification. Briefly, 200 µL of turbidity-adjusted cell suspension was added to each well in the presence of 15 mg/mL microcapsules. Each test condition was done in triplicate. The micro-plate was incubated at 37 °C for 24 h using a shaking speed at 200 rpm. After incubation, 100 µL of culture suspension was transferred to a new plate and measured for an absorbance at 600 nm using a microplate reader (Labtech, Germany). The value of 100% viability was determined by the growth of bacteria in the absence of microcapsule samples and 0% viability was defined in the presence of medium alone.

The qualitative method was carried out according to AATCC 147-2004 method, with small adjustments. To do so, 1 mL of bacterial culture was transferred to the tube containing 9 mL of sterilized distilled water. The inoculum loop was dipped in the diluted cell suspension and streaked on the surface of the nutrient agar (NA) to produce five parallel streaks without refilling the loop. The microcapsule samples of 2 x 2 cm were placed on the streaks. The plates were incubated at 37 °C for 24 h.

RESULTS AND DISCUSSION

Characterization of microcapsules

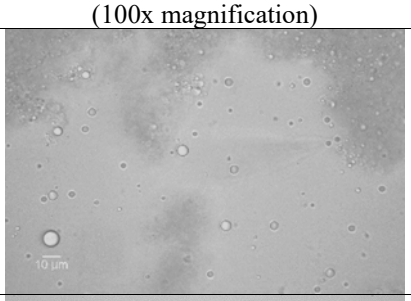
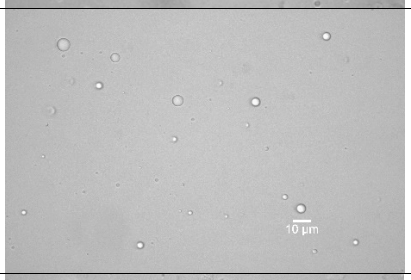
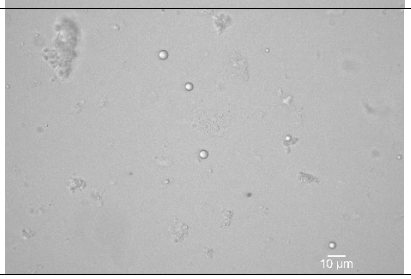
Microcapsules loaded with the essential oils of *Cymbopogon citratus*, *Cymbopogon nardus* and

Litsea cubeba were prepared using complex coacervation and examined by optical microscopy. In wet state, before freeze drying, the resulting microcapsules, namely MC-CC, MC-CN and MC-LC, were all spherical, having essential oils as cores that were surrounded by shell materials, *i.e.* gum arabic and chitosan. Similarly, in another study, microcapsules of gum arabic-chitosan loaded with vanillin and limonene were spherical.[21] The images of microcapsules loaded with *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba* essential oils, as well as their average diameters, are shown in Table 2. Their average diameters are in the range of 1.55–3.82 μm, which are smaller than those of the microcapsules loaded with vanillin and limonene, which were 10.4–39.0 μm.

The dried microcapsules were examined under a scanning electron microscope. The SEM images are presented in Figure 2. As can be seen, the microcapsules loaded with essential oils were highly porous particles, as compared with the microcapsules not loaded with essential oils due to the foam generation during chitosan solution addition and mixing with essential oils, which was not present in the preparation of the microcapsules not loaded with essential oils.

Using a laser particle size analyzer, the size and size distribution of microcapsules loaded with essential oils were measured. The size distribution of the samples is shown in Figure 3, with the size diameter values of 168.77±1.96, 62.16±0.18 and 81.26±0.14 μm for MC-CC, MC-CN and MC-LC, respectively. Thus, according to their size, the microparticles can be arranged in the following order: MC-CC > MC-LC > MC-CN. The sizes of the microcapsules determined by the particle size analyzer are greater than those examined by optical microscopy (which determined the size of a single microcapsule). The discrepancy may be attributed to the collapse and then agglomeration of the microcapsules loaded with different essential oils upon the freeze-drying process. Likewise, freeze-drying in the encapsulation process of curcumin, using alginate and chitosan as shell materials, yielded a collapsed structure of the microcapsules.[30]

Table 2
Images and diameters of microcapsules loaded with essential oils of *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba*

Samples	Optical microscope images (100x magnification)	Microcapsule diameter (μm)
MC-CC		2.17
MC-CN		3.82
MC-LC		1.55

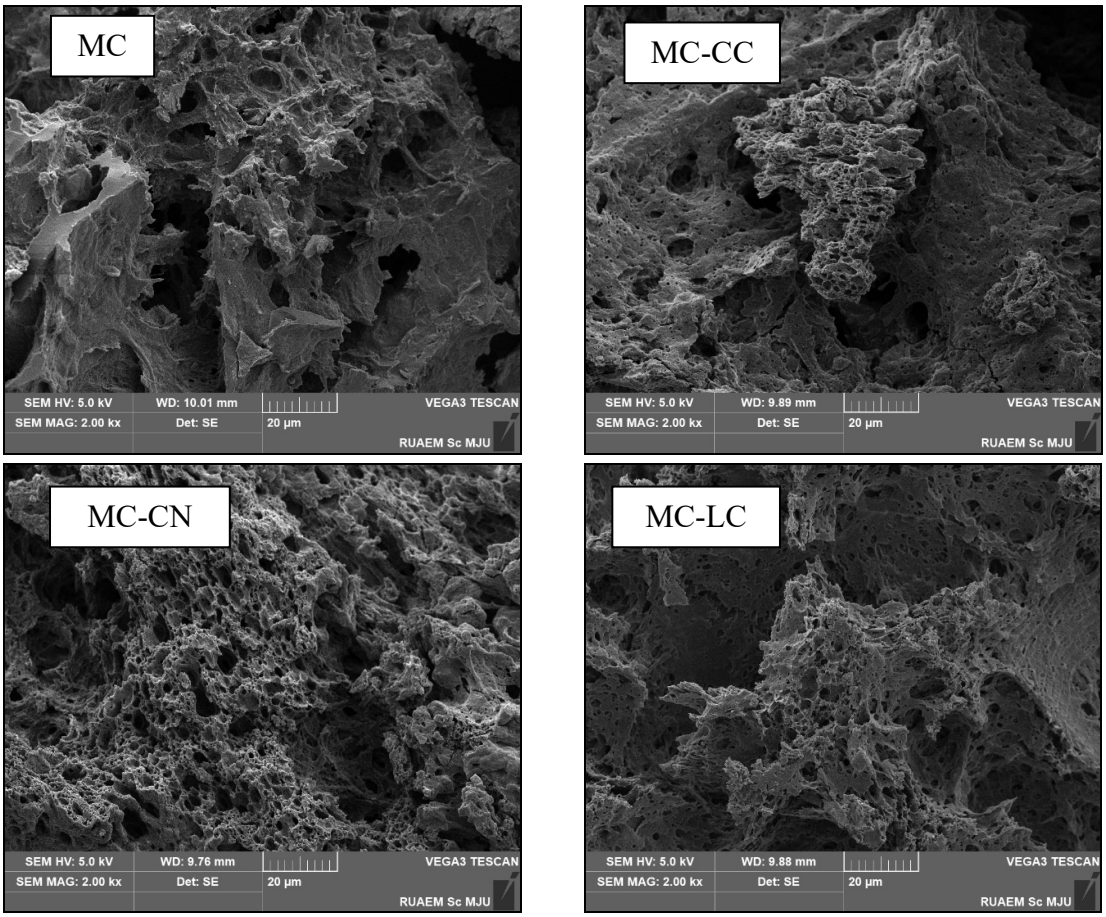


Figure 2: SEM images of microcapsules unloaded and loaded with essential oils

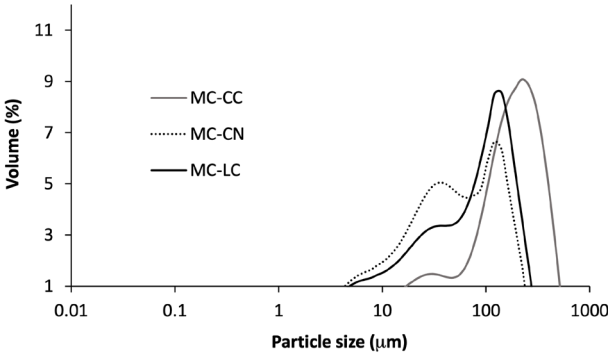


Figure 3: Particle size distribution of microcapsules loaded with essential oils

From FT-IR spectra in Figure 4, it can be seen that microcapsules loaded with essential oils of *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba* show higher intensity of the broad peak at 3010–3600 cm^{-1}, corresponding to O-H stretching, associated with the geraminol and nerol content in the essential oils. Also, C-H stretching bonds are noted around 2850-3150 cm^{-1}, together with the C-H bending at 1380–1450 cm^{-1}, as compared with the spectra of the microcapsules not loaded with *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba* essential oils, these bands being attributed to the hydrocarbon chains in the essential oils composition, such as geraminol, nerol, citronellal, citronellol, nerol, limonene, *etc*. A sharp peak of C=O at 1710 cm^{-1} is obviously dominant in sample MC-LC, which may be attributed to aldehyde constituents.[31]

Figure 5 shows the differential scanning calorimetry (DSC) thermograms for the essential oil loaded and non-loaded microcapsules. All the

samples with and without loaded essential oils have similar thermogram patterns. The first endothermic peak was observed around 100 °C, which is related to the melting behavior of the chitosan polymer chain,[32] whereas gum arabic shows a melting point at around 220 °C, revealed as the second endothermic peak.[33] By increasing the temperature of the microcapsule samples, the degradation of chitosan and gum arabic was observed at 240 °C and 280 °C, respectively.[34] Also, the encapsulation of *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba* essential oils demonstrates changes in thermal characteristics of microcapsules due to shifts of melting temperatures around 100 °C and 220 °C, as well as the degradation temperatures at 240 °C and 280 °C for chitosan and gum arabic, respectively. In addition, CC and LC essential oils in the microcapsules cause small endothermic peaks around 185 °C, associated with their evaporation, as seen in MC-CC and MC-LC thermograms.

Figure 6 presents the thermogravimetric curves (TGA) of microcapsules with and without loaded essential oils, which show similar behavior with two steps of weight loss. A minor weight loss due to moisture evaporation was first observed in the range of 30-130 °C, due to the hydrophilic nature of the respective shell materials. The second loss was found around 200 °C due to degradation of gum arabic and chitosan.[35-36]

Microcapsules not loaded with essential oils have a rapid weight loss in the range of 30-130 °C, while the essential oils loaded samples show gradual loss of weight in both steps. A slow release of essential oils from the microcapsules with increasing temperatures was observed due to thermal stability of essential oils by gum arabic and chitosan shell, in other words, the essential oils were protected from high temperature. By using difference of % weight loss between the loaded and non-loaded microcapsules at 180 °C, essential oil contents were 6.39, 8.09 and 5.46% for MC-CC, MC-CN, MC-LC, respectively. This proves that essential oils were encapsulated in microcapsules of chitosan and gum arabic as shell materials due to the demonstrated slower decline of the weight loss curve with respect to the equivalent non-loaded microcapsules.

The relationship between % weight loss of loaded and non-loaded microcapsules and time at a temperature of 36.5 °C is shown in Figure 7. The high % weight losses after 20 min, meaning high rate of release, are observed for all the samples, especially for MC-LC. These values are associated with the amount of free oil on the microcapsules' surface (the initial gradient to the % (w/w) weight loss *versus* time elapsed curve). MC-LC has the highest free oil amount, so its TGA thermogram shows the most rapidly declining curve. The % weight loss of microcapsules loaded with essential oils is still high within 60 min (with respect to the rest of the plot), revealing a high rate of release, in the order of MC-LC > MC-CN > MC-CC. After 60 min, the rate of release was smaller (with respect to the rest of the plot) and nearly constant until 210 min, for microcapsules not loaded with essential oils (MC).

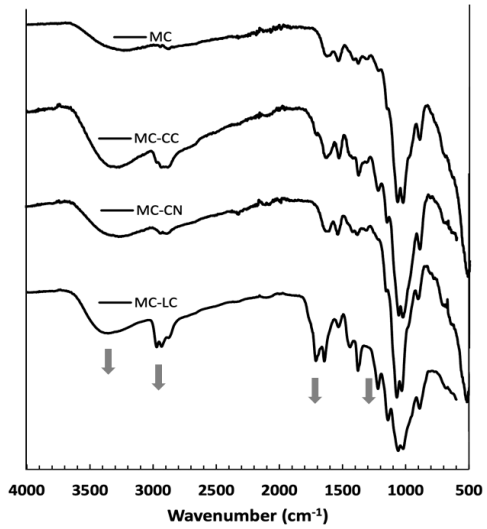


Figure 4: FT-IR spectra of microcapsules loaded with essential oils

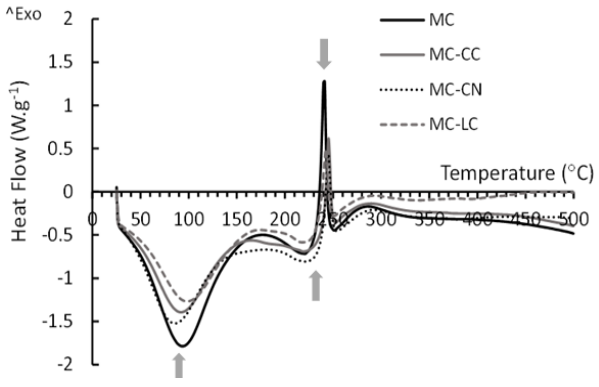


Figure 5: DSC thermogram of microcapsules loaded and not loaded with essential oils

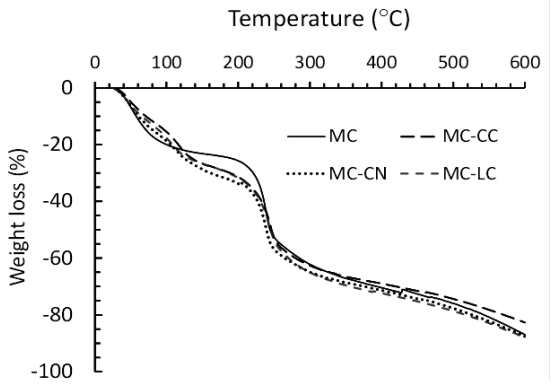


Figure 6: TGA thermogram as temperature dependence of microcapsules loaded and not loaded with essential oils

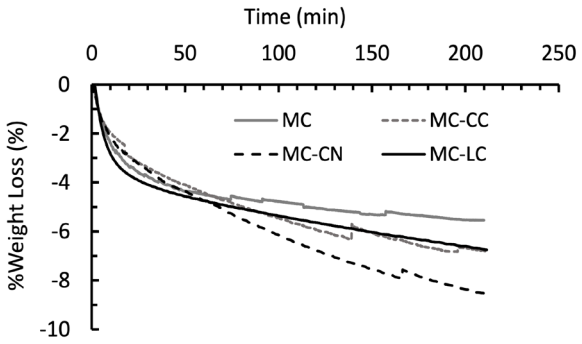


Figure 7: TGA thermogram as time dependence of microcapsules loaded and not loaded with essential oils

Conversely, microcapsules loaded with essential oils are still going in the order of MC-CN > MC-CC ≈ MC-LC (in the time range from 60 min to 210 min). Encapsulation ratios after storage for 210 min (3.5 h) at 36.5 °C were calculated from the difference of % weight loss of each microcapsule at 20 min (amount of free oil) and that at 210 min (amount of encapsulated oil), and were found as 65.56, 56.67 and 45.11% for MC-CN, MC-CC and MC-LC, respectively.

After the freeze drying process, the microcapsules appeared as a yellowish powder, shown in Figure 8 (a). After extraction of encapsulated oils and free oils with ethanol, the essential oils from the microcapsules were dissolved in ethanol, subjected to a rotary evaporator, and the resulting essential oils appeared as a yellow liquid, shown in Figure 8 (b-e). The encapsulation ratios are 66.79, 58.57 and 54.65% (0.8594, 0.5956 and 0.6790 g of encapsulated oils; 0.1711, 0.1556 and 0.1991 g of free oils) for MC-CC, MC-CN and MC-LC, respectively. The encapsulation ratios are slightly higher than those estimated from the TGA thermograms, as mentioned regarding the release of essential oils at 36.5 °C after 3.5 h storage.

As can be seen in Table 3, all essential oils (EO-LC, EO-CC, EO-CN) show significantly higher mosquito repellency than coconut oil (CCO). The values increase by 300% for EO-LC and 500% for EO-CC and EO-CN, as compared to CCO. It has been confirmed in the literature that the essential oils from *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba* exhibit mosquito (*Ae. aegypti*) repellency.[8-9,37]

The encapsulation of *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba* essential oils in gum arabic and chitosan (in the present work) has significantly enhanced mosquito repellency due to the increased repellency values of 700% for MC-CC and MC-CN; and 1400% for MC-LC as compared to CCO, also 275% higher repellency was recorded for MC-LC; and 133% – for MC-CC and MC-CN, as compared to their essential oils alone. These results are not only due to the lower amount of essential oils in the coconut oil (samples EO-LC, EC-CC and EC-CN), as compared to those from microcapsule samples (MC-LC, MC-CC and MC-CN, respectively), but rather due to the fact that microcapsulation ensured a long lasting property of mosquito repellency. The findings in the present work are in agreement with those of Sakulku *et al*., who found that the encapsulation of citronella oil using their nano-emulsion technique to control the release of the citronella oil was efficient in prolonging the mosquito protection time.[38] In addition, the results are in agreement with other literature reports that focused on the use of encapsulation technology in the simple coacervation of gelatin microcapsules with citronella oil trapping and releasing the oil vapor in a controlled and predictable manner over time.[39]

By relating the controlled release function of microcapsules containing essential oils to the median complete protection time against *Ae. aegypti* mosquitos, it can be noted that MC-LC shows the highest protection time, which correlates with the highest release amount in 60 min, as determined by TGA, as mentioned earlier, and the essential oil was maintained in the cage during the test of mosquito repellency for 3.75 h. This agrees with an earlier reported evidence[16] that geraniol encapsulated in gelatin and gum arabic microcapsules resulted in long-lasting retention.[39] Geraniol was mentioned as a commercially important fragrance with insecticidal and repellent properties, as well as

antimicrobial, antioxidant, anti-inflammatory and some vascular effects.[41]

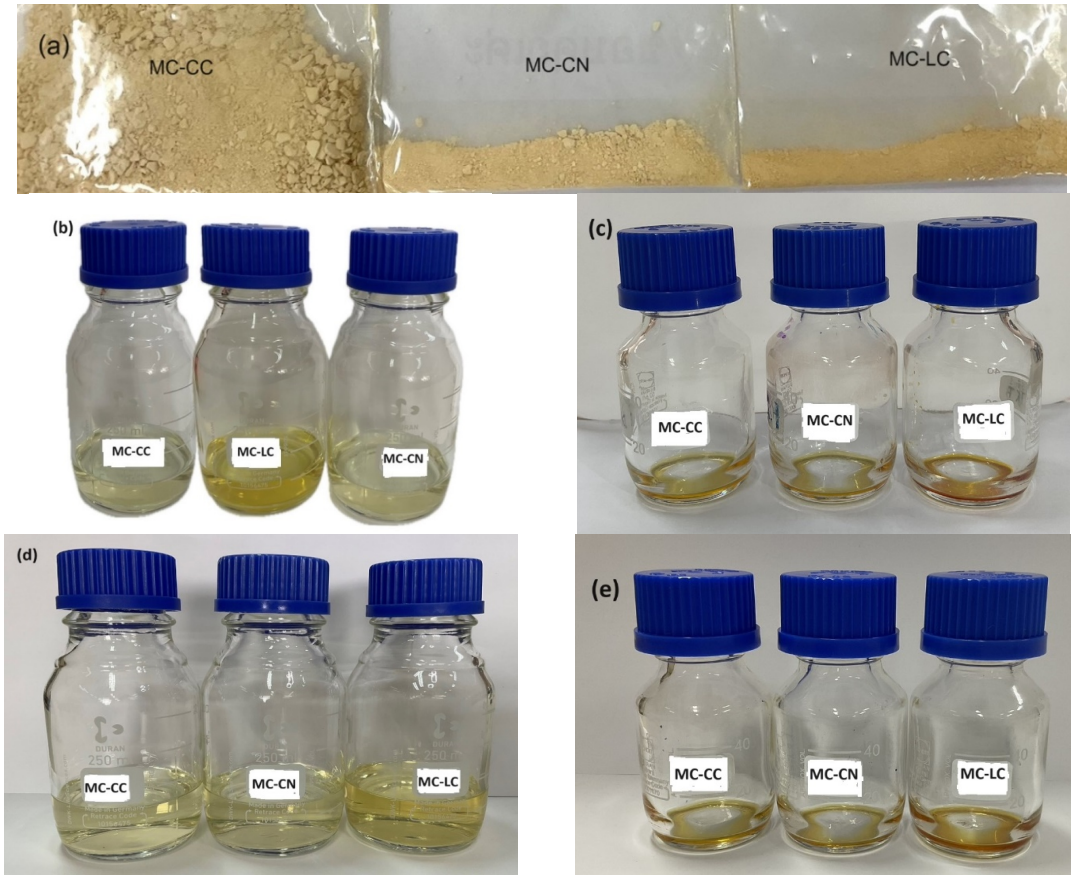


Figure 8: (a) Freeze-dried microcapsules loaded with essential oils, (b) free oils from the microcapsules after being extracted in ethanol, (c) free oils from the microcapsules after ethanol removal, (d) total essential oils in the microcapsules after being extracted in ethanol, and (e) total essential oils in the microcapsules after ethanol removal

Table 3
Mosquito repellency as median complete protection time of essential oils and MCs loaded with essential oils

10% w/v of substance	Median complete protection time (range, h)*
CCO	0.25 (0.00-0.50)
EO-LC[1]	1.00 (0.50-1.50)
EO-CC[1]	1.50
EO-CN[1]	1.50 (1.00-2.00)
MC-LC[2]	3.75 (1.50-4.00)
MC-CC[2]	2.00 (0.50-3.50)
MC-CN[2]	2.00 (1.00-3.00)

*Values followed by different letters in a column were significantly different (Kruskal-Wallis one-way ANOVA, $p<0.05$); [1]Essential oil weight was 0.01 g in 0.1 mL of coconut oil, which was applied to the test site; [2]Essential oil weights were 0.0351, 0.0412 and 0.0300 g in 0.02 g of essential oil loaded MC-LC, MC-CC and MC-CN mixed with 0.18 g coconut oil, which was applied to the test site (the essential oil weights were from 0.0781, 1.0305 and 0.7512 g total oil content in 0.5 g from MC-LC, MC-CC and MC-CN, using the extraction method)

The antibacterial activity of all essential oils and microcapsules containing the essential oils against *E. coli* and *S. aureus* are presented in Table 4. Microcapsules without essential oils (MC) have no activity against *E. coli*, while microcapsules loaded with essential oils exhibit antibacterial activity in the order of MC-CN ~ MC-CC > MC-LC. For *S. aureus*, microcapsules without essential oils do not possess any antibacterial activity, while the set of

microcapsules with essential oils present antibacterial activity in the order of MC-CN ~ MC-LC > MC-CC. The results agree with those of other studies, where it was demonstrated that LC essential oil exhibits antibacterial function against *S. aureus*, as it inhibited even MRSA (methicillin-resistant *Staphylococcus aureus*), through intracellular biological macromolecule leakage.[42] Geraniol was found active against *E. coli* and to possess antifungal activity.[42] Moreover, LC essential oil also inhibited the growth of *Aspergillus flavus*, the cause of aflatoxin release in traditional medicine. The study reported that citral was the main chemical component to reduce the growth rate of colonies and mycelium biomass, and toxin production.[44-45] *Cymbopogon nardus* containing citronellal as the main component was able to inhibit the growth of all 36 bacterial isolates from cultured aquatic animals, as well as *E. coli*. Moreover, *Cymbopogon citratus* oil showed antibacterial properties against *E. coli* and *S. aureus* in foods, such as cream-filled cakes and pastries.[46] Also, an earlier study revealed that *Cymbopogon citratus* exhibited better antibacterial activity against gram-positive bacteria than against gram-negative bacteria, with the exception of *P. aeruginosa*, which agrees with the results of this study, where *Cymbopogon citratus* has higher antibacterial activity against *E. coli* than against *S. aureus*.[47]

Table 4
Antibacterial activity of microcapsules loaded with essential oils against *E. coli* and *S.aureus*

Samples	% cell viability		average % cell viability	% inhibition		average % inhibition
E. coli						
NB+control MC	104.306	103.909	104.11	-4.306	-3.909	-4.11±0.28
NB+MC-LC	50.116	56.409	53.26	49.884	43.591	46.74±4.45
NB+MC-CC	3.279	3.412	3.34	96.721	96.588	96.65±0.09
NB+MC-CN	3.312	2.948	3.13	96.688	97.052	96.87±0.26
S. aureus						
NB+control MC	132.639	118.466	125.55	-32.639	-18.466	-25.55±10.02
NB+MC-LC	7.045	5.919	6.48	92.955	94.081	93.52±0.80
NB+MC-CC	9.045	7.753	8.40	90.955	92.247	91.60±0.91
NB+MC-CN	6.669	5.877	6.27	93.331	94.123	93.73±0.56

*NB = nutrient broth

CONCLUSION

Microcapsules containing essential oils of *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba* were successfully prepared by complex coacervation using gum arabic and chitosan as shell materials. The wet microcapsules loaded with essential oils were spherical, with sizes in the range of 1.55–3.82 μm. The microcapsule sizes were determined as 62–168 μm by using a laser particle analyzer because of the aggregation of the microcapsules after the freeze-drying process, which was confirmed by SEM. FT-IR revealed that essential oils were trapped in the microcapsules of gum arabic and chitosan – the polymeric shell materials. The O-H, C-H, C=O functional groups of chemical constituents in essential oils appeared at 3010–3600, 2850–3150, 1380–1450 and 1710 cm^{-1}, respectively. The %encapsulation ratio determined by the extraction method confirmed that 66.79, 58.57 and 54.65% essential oils were encapsulated in the microcapsules loaded with *Cymbopogon citratus*, *Cymbopogon nardus* and *Litsea cubeba* oils, respectively.

In addition, the TGA with temperature and time dependencies presented in this work show the thermal stability of essential oils due to their slow release from the microcapsules. Despite the lowest encapsulation ratio, the microcapsules loaded with *Litsea cubeba* oil exhibited the highest release rate in the first hour and provided the longest protection time against mosquitoes, compared to the microcapsules loaded with *Cymbopogon citratus* and *Cymbopogon nardus* oils, while the microcapsules loaded with *Cymbopogon nardus* demonstrated the best antibacterial activity against *E. coli* and *S. aureus.*

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