Microencapsulation of lime (Citrus aurantifolia) oil for antibacterial finishing of cotton fabric

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Functional cotton fabric incorporated with antibacterial microcapsules of lime (C. aurantifolia) essential oil (LO) was prepared. The coacervation method, employing two biopolymers of alginate and gelatin as the shells, was preferentially selected to produce the LO microcapsules, whereas immobilization of the LO microcapsules onto the fabric was done using the pad–dry–cure method using various concentrations of citric acid binder. The antibacterial inhibition zone of the functional fabric was subsequently analysed using the Kirby Bauer method. The LO microcapsules were produced with a yield, encapsulation efficiency (EE), and oil content (OC) of 47 ± 4%, 84 ± 11%, and 58 ± 4%, respectively. The homogenous spherical and soft microcapsules (1.554 μm) bonded effectively by 4% citric acid onto the surface of the fabric and detached back by only 3% after 15 cycles of washing. Overall, the optimized functional fabric exhibited the highest antibacterial activities among others against typical skin bacteria, such as S. aureus, E. coli, K. pneumoniae, and S. epidermidis, and thus it can be potentially applied to obtain antibacterial functional textile.

Introduction

Numerous industries harvest and process the primary parts of Citrus aurantifolia, including the pulp and juice, into food and beverages but usually discard the lime peels even though they contain essential oil (LO) that can be utilized as antimicrobials, disinfectants, anti-inflammatory, relaxing, stimulating, antioxidant, anticarcinogenic, antidepressant, and antifungal agents. The extensive activities possessed by LO are because of their substantial amounts of beneficial compounds like α-limonene, γ-terpinene, linalool, linalyl acetate, α-terpineol, geranyl acetate, terpinolene, and β-pinene. However, the direct application of LO is hampered by the properties of these components like high volatility and susceptibility to oxidative deterioration upon exposure to oxygen in the air and changes in temperature and light. Thereupon, many alternative methods have been progressively developed, including microencapsulation, which utilizes shells to protect the active core from outside interferences. The technology is widely applied in health, cosmetics, pharmacy, textiles, and agriculture. In particular, the microencapsulation technology in textiles requires further incorporation of the capsules onto the fabric, employing a binder to prevent the loss upon washing.

Cotton fabrics are susceptible to microbial colonization due to the hydrophilic composition of carbohydrates. Moreover, the pores tend to retain moisture and also serve as a source of oxygen, nutrients, and energy for the growth of microorganisms. The outcome will not only increase the spread risk of diseases but also degrade the quality of the textile products including loss in strength, color damage, and unpleasant odours. To the best of our knowledge, the first study related to the manufacture of functional fabrics immobilized with microcapsule of C. aurantifolia essential oil (LO) was reported by Wijesirigunawardana & Perera in 2018. Large-sized of LO microcapsules coated with biopolymers of chitosan and Arabic gum were developed and subsequently incorporated onto cotton fabrics using succinic acid binder. Overall, the modified cotton fabric exhibited medium antibacterial inhibition against S. aureus, E. coli, Bacillus cereus, and Salmonella typhimurium.

In this study, we developed antimicrobial functional fabrics immobilized with LO microcapsules. To improve the performance, the modification of previous findings was applied, such as employing alginate and gelatin as the shells and optimizing citric acid as the binder of microcapsules with fabrics. In addition, antibacterial activities of the products were evaluated against common skin bacteria like S. aureus, E. coli, K. pneumoniae, and S. epidermidis.

Results and discussion

LO microencapsulation and characterization

The microencapsulation of LO employing the complex coacervation method with two biopolymers of alginate and gelatin...
2% (w/v) as shells produced 47 ± 4% yield with an oil content (OC) and encapsulation efficiency (EE) of 58 ± 4 and 84 ± 11%, respectively. As anticipated, the combined biopolymer shells bearing opposite charges could improve the properties of the microcapsules. Strong intermolecular hydrogen bonds and ionic interactions of the shells presumably existed along with a tendency to cause electrostatic attraction. As a result, the forces tend to decrease the aggregation and precipitation upon encapsulation and finally increase the yield, oil content (OC), and encapsulation efficiency (EE) of the microcapsules.

The average particle size of the capsule was 1.554 μm with a homogenous distribution (mean/median = 0.951), and thus it was categorized as a microcapsule (Fig. 1). Based on the SEM of 1000× magnification, the shape of the microcapsules was observed to be spherical with a smooth surface (Fig. 2).

FTIR studies revealed the functional group bands and their relationship between all materials and LO after the microencapsulation process (Fig. 3). During the coacervation process, the gelatin moieties such as amines at 3321 cm⁻¹, carboxylate at 1652 cm⁻¹ (Fig. 3a), and the hydroxyl group of alginate at 3450 cm⁻¹ (Fig. 3b) seemingly remained the same after the encapsulation (Fig. 3c). A non-covalent interaction apparently occurred between the two biopolymers. According to Saravanan and Rao (2010), polyelectrolyte complexation between the two ionic shells was reported to take place due to ionic interaction between the amino group of gelatin and the carboxylate moiety of sodium alginate at an applied acidic pH. Correspondingly, a signal similar to that of pure LO (Fig. 3d) appeared and overlapped with the coating signal, thereby indicating no significant interaction between the LO and their coatings.

The thermograph of the LO microcapsule (Fig. 4) showed that LO microcapsules experienced a mass loss of 1.5% in the first stage of heating (<100 °C). The loss was presumably due to the evaporation of water/moisture and a small fraction of LO, with boiling point <100 °C. As more heat was applied to the microcapsule in the second stage (100–300 °C), more mass loss occurred, which might be due to the release of more heavy compounds of LO. Finally, the microcapsules started to lose more than 60% of their mass in the third stage (>300 °C). At this stage, the microcapsules initially experienced dehydration and depolymerization before finally suffering from decomposition. The mass loss of the blank microcapsule and LO also occurred upon heating them in the temperature range of 100–750 °C. In particular, the drastic change in the mass of LO below 150 °C was anticipated to be due to the release of the volatile
compounds (Fig. 4). The achievement of good thermal stability of the microcapsules would delay the direct evaporation of LO inside the core and slow down the polymer degradation process upon heating.18

The performance of LO microcapsules against chemical and mechanical interferences was further investigated. Upon chemical extraction at a steady stirring force, the active LO inside the core was freed gradually (Fig. 5). Within 2 h, 79% of LO was found to be released from the microcapsules. Moreover, upon employing Avrami’s kinetic model [eqn (3)], the mechanism and rate constant of LO release were obtained (Fig. 5). The acquired $n$ value of 1.3 ± 0.1 suggested the release mechanism preferentially fits the first order kinetics, while the $R^2$ value of 0.9423 indicated that the LO release from the microcapsules was better fitted to Avrami’s equation. Referring to the computed rate constant of $(2.2 \pm 0.4) \times 10^{-4}$ s$^{-1}$, the release of LO over two shells of alginate and gelatin delayed successfully. LO was implied to be overlaid strong enough inside the microcapsules commonly because of the density of the microcapsule shell material.19 The aforementioned results implicitly suggested that the retention rate of LO inclusion microcapsules might be even slower at normal conditions, where there were no chemical and exaggerated mechanical interferences. Nevertheless, further investigation is necessary, particularly to understand the stability and rate constant of the release of the LO inclusion complex at various temperatures.

**Microcapsule immobilization**

The pad–dry–cure method for the immobilization of the LO microcapsules on cotton fabric was optimized using various concentrations of citric acid as a natural binder (Table 1). In general, the weight of the fabric was affected by the concentration of the binder. The optimum add-on of the fabric was achieved upon addition of 4% binder. The process suggested that such interaction between the carboxylate moieties of citric acid with the –OH group of cotton fibers existed through an esterification reaction (Fig. 6); hence, an increase in total mass was acceptable. Nevertheless, using an adhesive of more than 4% (citric acid) did not increase the add-on, which plausibly means that the reaction reached a saturation point. The curing process became essential as the binding process was induced by heating. Certainly, the appropriate temperature used in the curing process tends to form a stable and continuous film on the fabric surface.20 Thus, perhaps more microcapsules bonded firmly on the fabric and were resistant against the washing cycle.

The successful accomplishment of immobilization was further confirmed by SEM micrographs (Fig. 7). More microcapsules were observed on the surface of the fabric treated with 4% binder (Fig. 7c). The morphology of the microcapsules before (Fig. 2) and after incorporation into cotton fabrics (Fig. 7c) was seemingly different. However, their particle size was confirmed to remain in the range of 14–20 μm, and thus the

| Code of fabric | Binder of citric acid (%) | Add on (%) |
|---------------|--------------------------|------------|
| A             | 2                        | 4.6        |
| B             | 4                        | 6.5        |
| C             | 6                        | 6.0        |

Fig. 5 Oil release of LO microcapsules (mean ± SD/%; $n = 3$) in n-hexane when stirred at 100 rpm, inset graph; proposed Avrami’s kinetic model of the LO release ($n = 1.3 \pm 0.1$; $k = (2.2 \pm 0.4) \times 10^{-4}$ s$^{-1}$).

Fig. 7 SEM micrographs of fabric immobilized with LO microcapsules at 1000× magnification: (a) blank fabric, (b) 2% binder, (c) 4% binder, and (d) 6% binder.
immobilization process did not considerably alter either the size or the morphology of the microcapsules. On the other hand, application of more binder (6%) in the immobilization process caused the microcapsules to be covered by a thin film layer on the surface of the fabric (Fig. 7d). At a certain point, the more citric acid applied in immobilization presumably increased more active carboxylic acid moieties to react with the cellulose of the fabric, and consequently, more binder covered the LO microcapsules. In such a case, excessive binder was not recommended by commercial brands because it changed the fabric hand. Overall, the resultant fabric was found to remain flexible, and natural binders, including citric acid, are known to help control air circulation and elasticity without altering the tensile strength, elasticity, flexibility, air permeability, and fabric softness.

To test the stability of the LO microcapsules immobilized on the fabric, the washing strength test was conducted using a Launder O-meter. The fabrics were tested for 1, 5, 10 and 15 times of standard household washing (Fig. 8). The mass loss of fabric B and C upon 1-time washing was more than that of 3-time washing of fabric A. It was supposed that large particles of microcapsules tend to detach from the fabric in the first cycle of washing, yet the small ones resisted detachment from the fabric. Furthermore, the mass loss tendency of fabric A and C was even more after 10 times of washing. However, after 15 times of washing, fabric B remained firm, and the mass loss was around 3%. This was plausibly due to the 4% binder, which held the LO microcapsules effectively on the surface of the fabric. In general, the results were in agreement with major limitations found in the modified fabric, including the fabric immobilized with LO microcapsules.

Since the fabric immobilized with LO microcapsules using 4% binder showed relatively better resistance against the washing cycle than other fabrics, further SEM analysis was performed to ascertain the morphology of the washed fabric (Fig. 9). The micrographs emphasized that mass loss was observed in the washing strength test. The more the washing cycles applied to the modified fabric, the more LO microcapsules cracked into small (2–10 μm) and irregular shapes.

**Antibacterial activity**

The performance of LO microcapsules immobilized on the fabrics as an antibacterial agent was examined using the Kirby Bauer method (Table 2). The assay was also compared to the activity of LO of *C. aurantifolia* (Table 3). In general, the microcapsules coated on the fabrics with various binders exhibited medium ZoI against all representative bacteria; furthermore, the modified fabrics with 4% binder showed the highest activity among others, especially against *S. epidermidis*. However, antibacterial activities of all modified fabrics were at least 3 times lower than that of LO alone or even limonene, which is the major constituent of LO. The distinctive antibacterial inhibition between LO and encapsulated LO was

![Fig. 8](image.png)  
**Fig. 8** Mass loss (mean ± SD/%; n = 3) of fabric immobilized with LO microcapsules in the washing strength test.

![Fig. 9](image.png)  
**Fig. 9** SEM micrographs of fabric immobilized with LO microcapsules with 4% binder against cycle of washing (a) 1×, (b) 5×, (c) 10×, and (d) 15× (1500× magnification).

Nevertheless, after 15 times of washing, the microcapsules were still firmly bound on the surface of cotton fabric, yet lost their core partially. Beneficially, the organoleptic test on the modified fabric (15 times of washing) confirmed the availability of the LO core, even after 4 months of exposure at ambient temperature. The binder played important roles to prevent the microcapsules from being washed out from the fabric. The decreasing amount of microcapsules on the surface of the fabric suggested that more investigations about stronger binders are necessary. Hitherto, the poor washing resistance of the modified functional fabric is still the main issue.

**Table 2** The inhibition zone of bacterial growth by LO microcapsules immobilized on fabrics at various binder concentrations

| Conc. binder (%) | *S. aureus* | *S. epidermidis* | *E. coli* | *K. pneumoniae* |
|------------------|-------------|-----------------|----------|-----------------|
| 2                | 6.1 ± 0.1   | 9.0 ± 1.0       | 6.3 ± 0.2| 7.0 ± 1.0       |
| 4                | 7.0 ± 0.2   | 12.0 ± 0.0      | 7.1 ± 0.1| 8.4 ± 0.3       |
| 6                | 6.1 ± 0.1   | 8.0 ± 0.0       | 7.0 ± 0.1| 7.2 ± 0.1       |

*a N.a = not active, *mean ± SD; n = 2.*
than the previous fabrics prepared in this study exhibited di-

should be carried out.

microcapsules should exhibit antibacterial activity di-

the fabric compared to the larger one. Capsules could be incorporated more tightly onto the surface of

combination with citric acid as the binder, smaller LO micro-

stronger LO odor released from the cracked microcapsules,

inhibition than before the washing test. This was because of the

sules found on the 15 time washed fabrics should expose higher

test, the antibacterial activity of de

also emphasized the

bacteria plausibly was not homogeneous. The SEM micrograph

the concentration of LO on the fabric surface to inhibit the

excreted slowly upon mechanical and chemical force. Hence,

According to the oil release data, LO inside the shells were

erally added. The mixture was then reheated up to 35

solution. The mixture was further cooled down to 5

sodium alginate solution in distilled water (40 mL) was added

(0.8 g) and LO (2.0 g) were added dropwise. A

further stirred for 3

sodium alginate, and
dihydrate were obtained from Merck. In addition, some tech-

ical grade chemicals such as gelatin, sodium alginate, and

Tween-80 were acquired from PT. Subur Jaya Kimia in Bandung,

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where w = weight of microcapsules; w1 = the actual amount of coated oil, w2 = amount of added oil.

**Release oils and their kinetics.** The LO microcapsules (1 g) was introduced into 100 mL of n-hexane in a glass beaker. The mixture was then stirred continuously at 100 rpm at room temperature. An aliquot samples of 1.5 mL were pipetted from the mixture for every 15 min in 2 hours. The aliquot was filtered and measured by a UV-Vis spectrophotometer at 230 nm. Upon plotting the absorbances to obtain the calibration curve of LO, the cumulative release of oil at various interval times was obtained.

To further evaluate the release mechanism of LO from the shells as well as their kinetic parameters, the Avrami kinetic model was applied (eqn (3)).

\[
\ln(-\ln R) = n \ln k + n \ln t
\]  

The correlation between \(\ln t\) as the x-axis and \(\ln(-\ln R)\) as the y-axis was achieved when the curve was fitted with linear regression, and thus the resulting slope and intercept could be used to calculate the release rate constant k and the release parameter n. If the value of n = 1, the release model was in agreement with the first-order kinetics. However, if n = 0.5, the kinetics was controlled by the diffusion mechanism.

**Particle size measurement.** Particle size measurements were performed using PSA Beckman Coulter LS 13 320, equipped with optical Fraunhofer and water-carrying liquid.

**Morphological measurement.** Morphological measurements were conducted through a JEOL JSM-6510 SEM apparatus with magnifications of 500×, 1000×, and 1500×, as well as 10 kV electricity and BEI (Backscatter Electron Image) detector.

**Fourier transform infrared (FTIR).** FTIR study was carried out on a PerkinElmer spectrum-100 brand in the range of 450–4000 cm\(^{-1}\).

**Thermal stability.** The thermal stability was studied using a thermogravimetric analyzer (TGA) instrument of TG 209 F1 Libra brand, NETZSCH Germany at a temperature range of 30–750 °C and 20 °C per minute heating rate in nitrogen atmosphere.

**Imobilization of microcapsules in cotton fabric.** Microcapsule immobilization onto the fabric was initially performed by preparing a sample of 30 cm × 40 cm cotton fabric. Microcapsule, binder, and catalyst solutions were also formulated. The solution attained a 100 mL volume comprising 20% microcapsules, 2% citric acid binder, 1.5% sodium dihydrogen phosphate dihydrate catalyst, and Tween-80 (0.8 g). In addition, the solution was stirred to reach equilibrium, and then the cotton fabric was introduced into the solution for 5–10 minutes with stirring. Subsequently, the sample was squeezed using a padder (Werner Mathis AG) and dried on a HT steam curing machine (Werner Mathis AG) for 2 minutes at 100 °C, where the curing process also occurred at 130 °C for 2 minutes. The product sample was then characterized by SEM and further tested for washing resistance using a Lauder-O-meter. Furthermore, the material was tested for its antibacterial activity based on the SNI ISO 105-C06: 2010 test standard with two repetitions. The weight gain by the fabric after treatment was calculated using eqn (4).

\[
\text{Add on (\%) } = \frac{w_a - w_b}{w_b} \times 100\%
\]  

where \(w_a\) = weight after treatment; \(w_b\) = weight before treatment.

**Microcapsule immobilization onto the fabric was repeated by using a citric acid binder of 4% and 6%, independently.**

**Antibacterial activity.** For the assay of antibacterial activity, we referred to the method reported by Sumathi et al. (2015), which employed the Kirby Bauer method (SNI ISO 105-C06: 2010 test standard). The assay was not only conducted on the modified fabric with LO microcapsules but also on LO and limonene for comparison. The microbial strains of commonly colonized skin bacteria, such as Gram-positive bacteria; *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 11228), and Gram-negative bacteria; *Escherichia coli* (ATCC 11229), and *Klebsiella pneumoniae* (ATCC 23375) were preferentially selected for the assay. Initially, each microbial strain was cultured overnight at 10° CFU mL\(^{-1}\) in disinfected saline solution. Then, 500 µL bacterial suspension was laid over the agar media containing MHA for Gram-positive bacteria and NA for Gram-negative bacteria. The sample of the modified fabric (6.0 ± 0.1 mm) or 20 µL of LO sample impregnated on a sterile blank disc was placed onto the media plate and incubated at 37 °C for 24 hours, and the antimicrobial activity was then measured based on the zone of inhibition (Zol/mm). In addition, ampicillin of 1000 ppm per disc and n-hexane were used as positive and negative control, respectively.

**Conclusions**

The LO microcapsules were prepared with a yield, encapsulation efficiency (EE), and oil content (OC) of 47 ± 4%, 84 ± 11%, and 58 ± 4%, respectively. The morphology of the capsules was spherical and soft, with an average particle size of 1.554 μm. 79% LO was released in 2 hours upon applying mechanical and chemical force. The immobilization of the LO microcapsules onto the fabric was optimized at 4% citric acid binder with 3% loss of mass after 15 cycles of washing. Antibacterial activities of the optimized functional fabric (4% binder) exhibited the highest inhibition, among others, against *S. aureus*, *E. coli*, *K. pneumoniae*, and *S. epidermidis*.

**Conflicts of interest**

There are no conflicts to declare.

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