Characteristics of microcapsule of red ginger (*Zingiber officinale* var. *Rubrum*) essential oil produced from different Arabic gum ratios on antimicrobial activity toward *Escherichia coli* and *Staphylococcus aureus*

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Abstract

Essential oil has antimicrobial activity. Encapsulation of essential oil might affect its antimicrobial activity. The present study was aimed to study the characteristic of red ginger essential oil microcapsule obtained from varying Arabic gum ratios on the growth inhibition of *E. coli* dan *S. aureus*. Red ginger essential oil from steam distillation was coated using Arabic gum with ratio 1:3, 1:4, 1:5 (w/b). The 1:3 (v/w) ratio of red ginger essential oil and Arabic gum showed the best microcapsule characteristics with average inhibition diameter zones 5.67 mm for *E. coli* and 6.67 mm for *S. aureus*, and reduction of bacterial count for *E. coli* 1.8 log CFU/g and *S. aureus* 2.3 log CFU/g, yield of microcapsule 51.54%, water activity 0.207, water content 3.57%, solubility 97.46%, surface oil 0.08%, and particle size 258.2 µm. The major component of red ginger essential oil was ar-curcumene, zingiberen, β-sesquiphellandrene, and camphene.

Introduction

Essential oil is the volatile oil of secondary metabolite of plants and is known to contain mixtures of various compounds, namely terpenes, alcohols, acetones, phenols, acids, aldehydes, and esters, which are generally used as flavoring agents on food and cosmetics or as functional components in pharmaceutical products. Various types of essential oil from spices are potentially reported to be developed as food preservatives because they have broad-spectrum antimicrobial activity against pathogenic bacteria and food deterioration microorganism.

Red ginger (*Zingiber officinale* var. *Rubrum*) is one of Southeast Asia’s indigenous spices that can produce essential oil. Essential oil of red ginger is the highest (3.90%) among other gingers such as “elephant ginger” (*Z. officinale* var. Roscoe) and “emprit” ginger (*Z. officinale* var. *Amarum*) with 1.62–2.29% and 3.05–3.48% value, respectively. The main obstacle for the storage of fresh essential oil is the volatile characteristic of essential oil that is easily lost which might affect its quality. One way to overcome this problem is to conduct microencapsulation process, so as to obtain powdered microcapsules.

The major bioactive components of the red ginger essential oil of the 6-month-old ginger rhizome are trimethyl-heptadiene-ol, ar-curcumene, camphene, carbaldehyde, β-sesquiphellandrene, and nerol, which showed moderate antimicrobial activity with the value of MIC (minimum inhibitor concentration) from 2.65 to 3.97 mg/mL and MBC (minimum bactericidal concentration) from 3.10 to 5.29 mg/mL to *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*. Furthermore, the results of the application of red ginger essential oil in fresh chicken meat preservation showed a bacteriostatic effect on *B. aureus* and *S. typhimurium* after 8 h storage.
Therefore, it was concluded that red ginger essential oil has the potential to be used as a preservative in fresh food products.\[^5\]

\[^{5}\]E. coli\[^{2}\] and S. aureus\[^{2}\] bacteria are two examples of pathogenic and destructive microbes that can cause infection in humans through contamination in foodstuffs. \[^{4}\]E. coli\[^{2}\] is a gram-negative bacteria and a bacteria indicator of sanitation, where its presence indicates contamination in food and can cause gastrointestinal disorders, while S. aureus\[^{2}\] is a gram-positive bacteria and food-poisoning bacteria causing gastroenteritis from its enterotoxin produced.\[^4\]\ Therefore, the effective effort to inhibit the growth of E. coli\[^{2}\] and S. aureus\[^{2}\] bacteria with an antimicrobial component from red ginger essential oil in foodstuffs is needed.

Spray drying is one method of microencapsulation by changing a liquid into solid by atomizing and drying process.\[^{6}\]\ The microencapsulation effectiveness is affected by the coating material. Arabic gum is known to act as an emulsifier of essential oils and flavor compound, easily soluble in water and compatible with other coating agents such as other hydrocolloid derives from plants such as proteins, carbohydrates, and modified starch.\[^{7,8}\]\ In order to obtain the red ginger microcapsules with high solubility and high antimicrobial activity, different ratios of Arabic gum were studied for essential oil encapsulation. Based on the above description, the antimicrobial activity of red ginger essential oil microcapsule on E. coli\[^{2}\] and S. aureus\[^{2}\] with various Arabic gum ratio was conducted.

\textbf{Materials and methods}

\textit{Materials}

The main ingredient used was 9-month-old red ginger (Zingiber officinale var. Rubrum) obtained from Indonesian Spice and Medicinal Crops Research Institute (ISMCRI), Indonesia. Red ginger essential oil was obtained by steam distillation. Arabic gum was obtained from Bratachem, Indonesia. Suspense of E. coli\[^{2}\] and S. aureus\[^{2}\] bacteria which are equivalent to standard 0.5 Mc. Farland (equivalent to 1.5 x 10\[^{8}\]\ CFU/ml) was obtained from Faculty of Pharmacy, Universitas Padjadjaran, Indonesia.

\textit{Emulsification and spray drying}

Three types of emulsion with various Arabic gum ratios were prepared. The ratio of red ginger essential oil to Arabic gum plus water was set into 1:3, 1:4, and 1:5 (v/w), while the ratio of Arabic gum and water was kept constant (20:80). First, the Arabic gum was added to water and left for 12 h to reach complete hydration. The solution was mixed with the red ginger essential oil using a homogenizer (IKA RW 20 DZM, Malaysia) at 800 rpm for 30 min. The solution then was dried with spray drying Eyela SD-100 (Eyela, China) with an inlet temperature of 95°C and an outlet temperature of 85°C. The composition of three prepared emulsions could be seen in Table 1.

\textit{Phytochemical analysis}

Analysis of the chemical components of oil and microcapsules of red ginger essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS) Agilent Technologies 5975C (Agilent Technologies, USA). The operational conditions were as follows: helium as a carrier gas,

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Ratio} & \textbf{Red ginger essential oil (mL)} & \textbf{Arabic gum (g)} & \textbf{Aquadest (mL)} \\
\hline
1:3 & 62.5 & 37.5 & 150 \\
1:4 & 50 & 40 & 160 \\
1:5 & 41.67 & 41.67 & 166.67 \\
\hline
\end{tabular}
\caption{Composition of emulsion.}
\end{table}
5% HP-5MS phenyl methyl Silox column (30 m x 250 μm x 0.25 μm). Column temperature was set at 50°C, pressure 7.0699 psi, flow rate of 1 mL/min, L, velocity 36.262 cm/s, column flow 1 mL/minute, split ratio 25:1, and split inlet temperature 250°C. Identification of compounds was performed using Willey and Nist National Institute of Technology Library.

**Yield**

The yield of microcapsules of red ginger essential oil was calculated based on the weight of microcapsules produced from the total weight of the microencapsulated material.

**Water content and water activity**

The water content of microcapsules was measured by weighing 1 g microcapsules in a previously weighed cup, and the cup was then heated in the oven at 105°C ± 2°C for ±3 h. The sample was then cooled in the desiccator for ±10 min and the weight was recorded. The drying was carried out until a constant weight was obtained with a difference by less than 0.2% from previous weight. The water activity of microcapsule was measured by \( a_w \)-meter (AquaLab LITE, Decagon Devices, Inc., USA) device, then the value of \( a_w \) on the appliance screen was recorded.

**Solubility**

Three grams of microcapsules were dissolved in water (60 mL), then homogenized over a hotplate with a stirrer at a temperature of 40°C for 20 min. The solution was filtered by a previously weight filter paper. The unfiltered and insoluble sample portion retained a filter paper was dried in the oven for 1 h at a temperature of 105°C. Soluble solid was measured as the different weight of total solid to solid remained in filter paper. Solubility was stated as the ratio of soluble solid to total solid of the microcapsule.

**Surface oil**

6.7 mL hexane was added into 0.5 g microcapsule. The mixture was then shook by hand gently and after that the mixture was filtered with filter paper. The escaped solution was then inserted into the weighted evaporator flask. The filter was washed with the remaining 3.3 mL of hexane (the total hexane used 9.9 mL), then the filtrate was evaporated with a rotary vacuum evaporator at 40°C. The residue remaining in the flask was then weighed.

**Particle size**

The particle size of the microcapsule was analyzed using the Particle Size Analyzer LS 13 320 (Beckman Coulter, Inc., USA).

**Antimicrobial activity (disc diffusion method)**

Sterile Mueller Hinton agar (MHA) was poured into a sterile petri dish and then was left until solidified. Test bacteria was swabbed using a sterile swab onto the surface of the agar. The disc paper was immersed in a 1% microcapsule solution (microcapsules dissolved into 96% ethanol) for 15 min. The other paper disc was immersed in 1% tetracycline–HCl solution (as positive control test) and 96% ethanol solution (as negative control test). The disc paper was placed onto the surface of MHA medium and then incubated at 37°C for 24 h. The diameter of the inhibitory zone formed around the disc paper was then measured using a caliper.
Antimicrobial activity (contact and dilution method)

Microcapsules were dissolved in 96% ethanol to obtain a microcapsule concentration of 1% (w/v). The microcapsule solution was then taken as much as 40 μl and then 4 ml of sterile Mueller Hinton broth (MHB) and 15 μl test bacteria (E. coli and S. aureus) of 10⁸ CFU/ml were added into the test tube, followed by incubation at the temperature 37°C for 18–24 h. The incubation result was then diluted to dilution series up to 10⁻⁵. 1 mL of three last dilutions (10⁻³, 10⁻⁴, and 10⁻⁵) was taken and put into a sterile petri dishes, then eosin methylene blue (EMB) was poured for E. coli and mannitol salt agar (MSA) for S. aureus. The petri dish was then incubated at 37°C for 18–24 h and the growing number of bacterial colonies was observed.

Statistical analysis

Data on antimicrobial activity was statistically analyzed using the F test at 5% significant level. The test was continued to the mean value of each treatment by using Duncan test to determine the significant difference between treatments.

Results and discussion

Phytochemical components of oil and microcapsules of red ginger essential oil

Phytochemical components of oil and microcapsules of red ginger essential oil were analyzed quantitatively using GC-MS. The result of GC-MS analysis of red ginger essential oil in this study had 42 compounds that were identified. Phytochemical components of red ginger essential oil microcapsules still composed of the major components of fresh red ginger essential oil, namely ar-curcumene, zingiberen, β-bisabolene, β-sesquiphellandrene, and camphene with a concentration similar to that of oil after coating (Table 2).

The components of essential oil and microcapsules of red ginger produced were dominated by the monoterpene (hydrocarbon, oxidized) groups, sesquiterpene (hydrocarbon, oxidized) alcohols, aldehydes, acids, and others. Components of monoterpenes and sesquiterpenes are reported to have strong antibacterial activity.\(^{[9,10]}\) The previous research identified 61 compounds of fresh red ginger essential oil at medium harvest age (about 6–8 months) with major components consisting of trimethyl-heptadieneol (7.34%), ar-curcumene (6.77%), camphene (6.18%), carbaldehyde (4.54%), β-sesquiphellandrene (3.80%), nerol (3.47%), and β-bisabolene (3.38%) and other minor components with concentrations of less than 3% each.\(^{[5]}\)

Furthermore, Sivasothy et al. reported that there were 54 compound of red ginger essential oil (Z. officinale var. Rubrum theilade) from Malaysia consisting of 81.9% monoterpenoid compounds with six major components as follows: camphene (14.5%), geranyl acetate (13.7%), geranial (14.3%), nerol (7.7%), geraniol (7.3%), and 1.8-cineole (5.0%).\(^{[10]}\) The different compositions of the reported red ginger essential oil might be influenced by the varieties of plants, soil, growth climate, the way of cultivation, and the rhizome ages.\(^{[10]}\) The components of ginger essential oil are generally dominated by sesquiterpene hydrocarbons namely α-zingiberene, α-curcumene, β-bisabolene, and β-

| No. | Component name       | Essential oil | Oil essentials in various Arabic gum ratios (w/v) |
|-----|----------------------|---------------|--------------------------------------------------|
|     |                      |               | (1:3)                                            | (1:4) | (1:5) |
| 1   | Camphene             | 6.90          | 5.64                                              | 5.22  | 4.94  |
| 2   | Zingiberene          | 13.92         | 13.91                                             | 13.45 | 13.44 |
| 3   | Ar-curcumene         | 17.39         | 15.77                                             | 15.31 | 14.70 |
| 4   | β-bisabolene         | 12.42         | 11.40                                             | 11.24 | 11.21 |
| 5   | β-sesquiphellandrene | 9.82          | 9.69                                              | 9.43  | 8.78  |
Therefore, the components of red ginger essential oil in this study are almost the same as the essential oil component in general.

**Characteristics of red ginger essential oil microcapsule**

The characteristics of red ginger essential oil microcapsule were affected by the encapsulant material ratio. As can be seen in Table 3, the ratio of Arabic gum as an encapsulant material affected the microcapsulate resulted.

**Yield**

The yield of microcapsule is a comparison between microcapsule weight obtained by total solution ingredient and coating agent used. The red ginger essential oil was successfully dried using spray drying with the range 51.54–61.98% (Table 3). The successful spray drying process was indicated by the high yield greater than 50% (solid to solid). The low yield is caused by high amount of sprayed liquid attached and stuck to drying chamber due to the nonoptimal process condition.

Calculating the amount of yield is very important in the drying process because it can be used to determine the efficiency and effectiveness of a process. Different yields are affected by the type and ratio of the coating agent used. The higher the ratio of Arabic gum, the higher the yield of red ginger essential oil microcapsule. This is influenced by the characteristic of Arabic gum as an effective emulsifying agent and stabilizer by increasing viscosity. Arabic gum was reported to be more effective as drying aid in spray-dried microencapsulation process than maltodextrine. The addition of Arabic gum in the feedstock may increase the stability of emulsions oil in water because the release of the material during the drying process can be avoided.

**Water content**

The water content of red ginger essential oil microcapsules increased along with the increase of added Arabic gum. The observed water content of red ginger essential oil microcapsules ranged from 3.57% to 5.19% (Table 3). The relatively high water content of the resulting product might be related to the viscosity of the liquid feed. The lower the viscosity of the feed, the higher the water content of the product. The average water content of the spray dried product was reported in the range 2–6%. The water content of the microcapsules is also influenced by the coating material. The more the addition of Arabic gum at a ratio of 1:5 (v/w), the greater the moisture content in the microcapsules. Arabic gum has a greater molecular weight and more complicated molecular structure; hence, the release of water molecules during the drying process will be more difficult and requires higher energy.

**Water activity**

The water activity of red ginger essential oil microcapsule was increased along with increasing the ratio of added Arabic gum, which was around 0.207–0.276 (Table 3). The stability of the product will be optimal in the range of $a_w$ 0.2–0.3. The value of water activity ($a_w$) is closely related to water

| Ratio (v/w) | Yield (%) | Water content (% db) | Water activity | Solubility (%) | Surface oil (%) | Particle size (μm) |
|------------|-----------|----------------------|----------------|----------------|----------------|-------------------|
| 1:3        | 51.54     | 3.57                 | 0.207          | 97.46          | 0.08           | 258.20            |
| 1:4        | 57.46     | 4.54                 | 0.231          | 98.43          | 0.04           | 334.80            |
| 1:5        | 61.98     | 5.19                 | 0.276          | 98.86          | 0.02           | 318.90            |
content, and higher the water content the higher the $a_w$ value. From Table 3, it can be seen that the higher the water content of microcapsule, the higher the water activity is.

The value of $a_w$ also affects the release of the flavor microcapsules. A high $a_w$ value may cause the water molecules surround the encapsulated flavor product to penetrate into the matrix particles and undergo hydration. The encapsulated flavor product undergoes hydration and the release of flavor or active ingredient will be easier to occur.

**Solubility**

Solubility from red ginger essential oil microcapsules in various Arabic gum coatings ranged from 97.46% to 98.86% (Table 3). Thus, it can be said that red ginger essential oil microcapsules has a high solubility in water. The addition of Arabic gum with high concentrations (>12%) can provide emulsion stability with a uniform or more stable small droplet size. At low concentrations, proteins are not able to cover the droplets of oil perfectly, resulting in clumps that will disrupt the solubility of microcapsules.

**Oil surface**

The percentage of oil surface indicates the number of oil contained on the outer surface of the microcapsules walls. The presence of oil on the outer surface of this microcapsule is undesirable because it can cause the core material to be highly exposed by air, in particular, oxygen and water vapor, thus accelerating product damage.

The surface oil of red ginger essential oil microcapsules decreased with the increasing ratio of Arabic gum added, which ranged from 0.02% to 0.08%. The higher the concentration of the core material contained in the emulsion, the higher the percentage of oil on the surface. The high value of microcapsule surface oil is not wanted due to the product easily deteriorated.

Some factors that affect the amount of encapsulated oil include the ability of coating materials, emulsifiers, and drying conditions. The better ability of the coating material to emulsify the active ingredients will be more effective in maintaining and defending the active ingredient so that the surface red ginger essential oil can be minimized. Several previous studies have suggested the effectiveness of Arabic gum as a coating agent in maintaining oil in the capsule. This is due to the 2% Arabinogalactan protein (AGP) of Arabic gum compound having emulsification capability. During the process of spray drying, the films might form to trap oil as an active component while water molecules whose molecular weight is low will be evaporated. In addition, high viscosity causes a strongly formed film layer to reduce oil migration to the outer surface of the microcapsules.

**Particle size**

The particle size of red ginger essential oil microcapsules increased in fluctuation along with increasing the ratio of Arabic gum added, ranging from 258.20 to 334.80 $\mu$m (Table 3). The size range of these microcapsule particles was still in the range of the size of the general microencapsulated powder that is between 1 and 5000 $\mu$m.

**Antimicrobial activity**

**Inhibition zone diameter**

The ratio microcapsule with various Arabic gum ratio showed different effects on the inhibit zone diameter of *E. coli* and *S. aureus*. The average inhibitory zone diameter of red ginger essential oil tested on *E. coli* showed a range of 4.17 to 5.67 mm, and that on *S. aureus* showed a range of 4.50–6.67 mm, both of which were categorized as an intermediate antimicrobial activity (Table 4).
The 1% concentration of red ginger essential oil microcapsule can reduce the total number of *E. coli* and *S. aureus* bacteria by 1.3–2.3 log CFU/g from the initial control number after 48 h of incubation period. The effect of red ginger oil and Arabic gum ratios on the reduction of the total number of *E. coli* and *S. aureus* bacteria after incubation for 48 hours (Table 5). The average decrease in the total number of *E. coli* bacteria was 1.5 log CFU/g, while for *S. aureus* it was 1.9 log CFU/g. The 1:3 (v/w) ratio of red ginger essential oil and Arabic gum showed the highest amount of microbial reduction, 1.8 log CFU/g for *E. coli* and 2.3 log CFU/g for *S. aureus*. This is in line with the observation of the inhibitory zone test, where the inhibiton to *S. aureus* bacteria was relatively bigger than *E. coli*.
Derivative compounds such as geranial, neral, geraniol, 1,8-cineole, α-caryophyllene, α-pinene, and camphor are suspected to be involved in various mechanisms such as bacterial cytoplasmic membrane damage, coagulation of cell components, and disruption in the proton motive force (PMF). The essential oil with antibacterial compounds such as thymol, eugenol, and carvacrol can cause cell membrane damage and release intracellular ATP and other components of microbes.

## Conclusion

The microcapsule of red ginger essential oils in various Arabic gum coating ratios contains major phytochemical components, ar-curcumene, zingiberene, β-bisabolene, β-besquiphellandrene, and camphene, and has the characteristics of yield 51.54–61.98%, water activity 0.207–0.276, water content 3.57–5.19%, solubility 97.46–98.86%, surface oil 0.02–0.08%, and particle size distribution 258.2–334.80 μm. The 1:3 (v/w) ratio of red ginger essential oil and Arabic gum are selected as the best ratio in inhibiting the growth of test bacteria by producing the highest mean inhibitory zone diameter of 5.67 mm against *E. coli* and of 6.67 mm against *S. aureus*, and reduction of total colony of *E. coli* and *S. aureus* by 1.8 and 2.3 log CFU/g, respectively.

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