Optimization of microspheres containing virgin coconut oil and hydrolyzed virgin coconut oil as antimicrobial

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INTRODUCTION

Virgin coconut oil is a clear liquid oil produced by various techniques with many varieties to generate low water content and free fatty acid. Fermentation using Saccharomyces cerevisiae bacteria efficient technique which produces content meet the Asian Pacific Coconut Community (APCC) standard.

VCO have antibacterial effect due to the presence of various high-medium chain saturated fatty acids. Lauric acid has strong antibacterial activity against bacteria. Lauric acid, caprylic acid, and capric acid can fight Candida species and other fungi. VCO liquid oil causes difficulties in transportation and storage. Converting VCO into powder form with encapsulation in β-cyclodextrin increased flexibility of VCO. Hee et al., in 2017, microencapsulated VCO with efficiency of 73%–80%.

VCO is suspected cannot generate antibacterial activity if not in hydrolyzed form. If VCO is still in the form of triglycerides, it must be broken down first either using catalyst or enzymatic. Silalahi’s research et al. proved that hydrolyzed VCO (HVCO) was active against bacteria. HVCO can inhibit Clostridium difficile of 99.9% whereas 0.15% HVCO gave 50% inhibition. Other researchers tried using papaya enzyme. This study compared potency of VCO and HVCO. Alginate microspheres have been successfully encapsulated several drugs and proteins.

Abstract

Virgin Coconut Oil (VCO) with potential fatty acid content has a strong antibacterial effect for drug and cosmetics. VCO was hydrolyzed as hydrolyzed VCO (HVCO) to increase its activity. This study aims to optimize VCO and HVCO microspheres. Examination was included fatty acid content, density, and organoleptic. VCO and HVCO microspheres were characterized by yield, moisture content (MC), morphology, and size. Fatty acid content was done through gas chromatography mass spectrometry whereas for microspheres, effect of alginate concentration (1%–2%), and addition of poly ethylene glycol (PEG) were studied. VCO and HVCO microspheres formulas showed that an increase in polymer concentration, increasing yield, and improving MC. The most optimal 2% alginate with the addition of PEG increased yield up to 59% and reduced size. F4 microspheres resulted the highest yield, low MC, and high fatty acids. Morphology was spherical and smooth with small size. Optimization of HVCO microspheres showed its potential which recommend for antibacterial and antifungal activity.

Key words: Antibacterial, antifungal, hydrolyzed VCO, microspheres, VCO

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The research will optimize VCO and HVCO alginate microspheres.

MATERIALS AND METHODS

Materials
Kopyor coconut was from kopyor plantation in Purwokerto; Chemicals were sodium alginate (pharmaceutical grade), CaCl2 (pharmaceutical grade), Tris-HCl buffer, Rhizomucor miehei lipase enzyme and Aquadest (PT. BrataChem).

Production of virgin coconut oil
VCO was prepared from separation of two layers of coconut milk which first formed from a squeezed mixture of coconut meat and water (1:1). The top layer was taken followed by centrifugation at 1000 rpm for 60 min. After centrifugation, three layers were formed then oil layer was removed to avoid mixing of oil pulp and water until VCO is generated.[19]

Hydrolyzed virgin coconut oil
Hydrolysis of VCO was conducted using Rhizomucor miehei lipase enzyme as previous.[13]

Physical characteristics and IR evaluation
Characteristics of VCO and HVCO test were done including organoleptic, density, and content of lauric acid which compared to the reference.[7] Infrared spectra was compared to the reference.[20]

Fatty acid of virgin coconut oil/Hydrolyzed virgin coconut oil (USP 35)
A sample of 50 mg was added with 1 ml of NaOH 2% in methanol, then is closed tightly and heated at 90°C for 5 min. After cooling, 1 ml of BF3 was added and reheated for 30 min and extracted. The upper phase was taken for analysis by gas chromatography/mass spectrometer (GC/MS) Agilent 6980N USA network GC system with auto sampler.

Production of microspheres
Sodium alginate is dissolved in water according to Table 1 by stirring at 500 rpm for 1 h, then VCO/HVCO is dripped slowly while stirring at 700 rpm for 15 min. Alginate-VCO/HVCO emulsion is sprayed into CaCl2 solution (2%) with a distance of 8 cm from the surface (15 psi, 1000 rpm, 2 h). Microspheres were washed twice and freeze dried at −60°C for 28 h.

Moisture content and yield
Moisture content (MC) was measured using MC analyzer. To determine % MC and yield, the following equation was used.

\[
\% \text{MC} = \frac{\text{weight before being analysed} - \text{weight after being analysed}}{\text{weight before being analysed}} \times 100\%
\]

\[
\% \text{Yield} = \frac{\text{weight of formed microparticles} (mg)}{\text{weight of constituent components} (mg)} \times 100\%
\]

Particle size and morphology
Diameter of 300 particles was measured using optical microscopy completed with software size analysis.[21] Morphology was examined using Scanning Electron Microscopy at 20.00 kV.

Statistical analysis
One-way ANOVA was applied to determine the significant differences between formulas and confidence level of 0.95 was used (\(\alpha = 0.05\)).

PRODUCTION OF VCO

Optimization of microspheres by centrifugation generated high yield better than by heating or fermentation.[22] Organoleptic showed a clear oil with a distinctive smell of coconut. Lisna and Purnama (2010) revealed that centrifugation method produced a distinctive aroma, while the odors of VCO by fermentation produces slightly sour taste and taste of cooking oil by heating method.[22] Yield of VCO was 4.21% w/w. This study compared VCO and HVCO which according to the literature can increase antibacterial activity.[13]

Density and fatty acid content
According to the APCC, resulting VCO of 0.920 ± 0.002 g/cm³ met the standard. Results of fatty acid content are shown in Table 2.

Table 1: Formula of virgin coconut oil microspheres

| Components       | Function          | VCO microspheres (%) | HVCO microspheres (%) |
|------------------|-------------------|----------------------|-----------------------|
|                  |                   | Formula 1 | Formula 2 | Formula 1HVCO | Formula 2HVCO | Formula 3HVCO | Formula 4HVCO |
| VCO              | Active agent      | 5         | 5         | -           | -             | -           | -             |
| Hydrolyzed VCO   |                   | -         | -         | 5           | 5             | 5           | 5             |
| PEG              | Cosolvent         | -         | -         | -           | -             | 1           | 1             |
| Sodium alginate  | Polymer           | 1         | 2         | 1           | 2             | 1           | 2             |
| CaCl2            | Cross-linker      | 2         | 2         | 2           | 2             | 2           | 2             |
| Aquadest         | Ad 100            | Ad 100    | Ad 100    | Ad 100      | Ad 100        | Ad 100      | Ad 100        |

VCO: Virgin coconut oil, HVCO: Hydrolyzed VCO
Fatty acids were consisted of lauric acid (21.73%), myristic acid (20.43%), palmitic acid (19.29%), oleic acid (17.71%), and linoleic acid (17.71%). Lauric acid was found of 21.73% which was below the APCC specifications. Fatty acid content of HVCO showed that the fatty acids with some content had increased according to the hypothesis when compared to the VCO. Lauric acid content was lower than the APCC, which is 43.0%–53.0%. However, the content of unsaturated fatty acids such as palmitic, oleic, and linoleic acid is higher than the APCC. High content of saturated fatty acids tends to make oil more stable to oxidation reactions. The distinction could be due to differences in production method, coconut varieties, and height of the growth place.

Virgin coconut oil and Hydrolyzed virgin coconut oil microspheres

The formulation of VCO and HVCO microspheres has many advantages, especially in terms of stability and acceptability. VCO contains saturated fatty acids which tend to solidify at room temperature. Soliman et al. explained that the use of 5% essential oil using alginate polymer (1%–2%) was able to produce high adsorption efficiency and loading capacity. Aerosolization technique has many advantages. Freeze drying was chosen for drug stability.

Organoleptic of microspheres

Organoleptic of VCO microspheres was white voluminous light powder with a characteristic smell of coconut oil.

Moisture content

MC of VCO and HVCO microspheres is shown in Table 3. Concentration of oil and polymer did not have significant effect on the similar to study conducted by Frascareli et al., the lowest was indicated by the obtained particles with higher oil content which could be attributed to the higher hydrophobicity of the microparticles.

IR spectra

IR spectra of VCO microspheres as predicted from literatures is shown in Table 4. Vibrations of the-NH and OH groups are the characteristic of alginates and presence of a peak of 1418 cm⁻¹ (COO-symmetric). Wavenumber of 1092 cm⁻¹ and 1032 cm⁻¹ is caused by vibrations of CO and CO-C in the mannuronic and guluronate units. There is a wavenumber shift of 1549 cm⁻¹ in the VCO into a larger wavenumber (1595 and 1600 cm⁻¹) which indicated presence of C = O. The wavenumber shift showed intermolecular bonds such as electrostatic bonds that can form and shorten the bond length of the-NH functional group, thereby increasing the wavenumber. The same condition was appeared in HVCO, which also confirmed all the specific groups showed that VCO and HVCO remain stable during microspheres production.

Yield and particle size

Yield and particle size are shown in Tables 5 and 6. The higher the concentration of alginate, the greater the yield. Yield is a determining factor for the successful usages of a method. Karaaslan et al. in 2021, mentioned that the increase in maltodextrin in the emulsion increased yield. Similar to this research, yield

Table 2: Qualitative of virgin coconut oil’s fatty acid content

| Components         | VCO (%) | HVCO (%) |
|--------------------|---------|----------|
| Caproic acid       | 0.77    | 1.10     |
| Octanoic acid      | 3.78    | 5.29     |
| Decanoic acid      | 4.63    | 5.99     |
| Undecanoic acid    | 0.04    | Do not appear |
| Lauric acid        | 21.73   | 25.74    |
| Tridecanoic acid   | 0.07    | Do not appear |
| Myristic acid      | 20.43   | 22.50    |
| Palmitic acid      | 19.29   | 15.99    |
| Linoleic acid      | 3.53    | 2.40     |
| Oleic acid         | 17.71   | 14.53    |
| Elaidic acid       | 0.31    | 0.2      |
| Searic acid        | 7.07    | 5.87     |
| 11-Eicosenoic acid | 0.13    | Do not appear |
| Aric acid          | 0.30    | 0.23     |
| Behenic acid       | 0.07    | 0.06     |
| Lignoceric acid    | 0.12    | 0.09     |

VCO: Virgin coconut oil, HVCO: Hydrolyzed VCO

Table 3: Moisture content of virgin coconut oil and hydrolyzed virgin coconut oil microspheres

| Formula | MC±SD | VCO microspheres | HVCO microspheres |
|---------|-------|------------------|-------------------|
| F1      | 5.27±2.76 | 4.15±1.16        |
| F2      | 5.28±2.28 | 3.09±2.02        |
| F3      | -      | 3.89±2.83        |
| F4      | -      | 3.59±1.54        |

MC: Moisture content, SD: Standard deviation, VCO: Virgin coconut oil, HVCO: Hydrolyzed VCO

Figure 1: Morphology of VCO microspheres at ×20,000 magnification. VCO: Virgin coconut oil
increased along with higher polymer concentration. Yield is also affected by the drying temperature but depends on the oil content.[35]

Average diameter of microspheres of <5 µm which suitable for topical and oral drug. Increase in polymer concentration did not affect the size. Diameter is very dependent on stirring speed, the higher the stirring, the smaller the size.[36]

**Morphology**

Morphology of microspheres is shown in Figures 1 and 2.

Microspheres morphology in all formulas showed a spherical shape, small, and smooth surface. This research recommends further investigation on the activity, stability of HVCO microspheres in storage and its usage in pharmaceutical fields.

**CONCLUSION**

The increase of alginate concentration up to 2% affected VCO microspheres’ size. Hydrolyzing VCO using enzymatic was able to produce HVCO supported by its potential antibacterial and antifungal activity. HVCO microspheres produced better physical characteristics with addition of poly ethylene glycol and potential to be tested for stability and activity.
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Conflicts of interest

There are no conflicts of interest.

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