Nanoencapsulating of Kaffir Lime Oil with Coacervation Method using Arabic Gum and Maltodextrin as Encapsulant

*K. Triyono¹, N. Suhartatik², Y.W. Wulandari³

¹Departement of Agrotechnology, Faculty of Agricultural, University of Slamet Riyadi, Jl. Sumpah Pemuda no.18, Surakarta, Central Java 57136, Indonesia
²,³Agriculture Product Technology, Faculty of Industrial and Food Technology, University of Slamet Riyadi, Jl. Sumpah Pemuda no.18, Surakarta, Central Java 57136, Indonesia

Kaffir lime oil is an essential oil from Citrus hystrix leaves. The product is generally volatile when exposed by air, kaffir lime oil covering is used, made with the nanocapsule. The technique used is coacervation method. This experiment aims to identify the ratio of arabic gum and kaffir lime oil, and also by maintaining the optimal crosslinking time. Nanoencapsule making process starts with mixing arabic gum into the kaffir lime oil on various mixing ratio. The coacervation process is done by dropping encapsulant mix and kaffir lime oil with various concentration into glutaraldehyde. After the coacervation, next is the process of adding maltodextrin into the mix with homogenization process and lastly is spray drying emulsion. Analysis is done by observing the result of encapsulation efficiency, particle distribution, and morphology profile using Scanning Electron Microscope. The result shows that the best nanoencapsulation efficiency is between 70.71% - 80.75%. The optimum condition for the highest value of total essential oils content in on 1:3 ratio (b/v) and the optimal time of crosslinking is 13 minutes. The nanocapsules had spherical shape with dips in the surface with average size of nanocapsules of 457.87 nm.

Keywords: kaffir lime oil, coacervation, arabic gum, maltodextrin, nanoencapsulation

INTRODUCTION

Kaffir lime oil is essential oil from kaffir lime leaves (Citrus hystrix. DC). That essential oil possesses some important bioactivities such as antioxidant, antileukemic, antiinflammatory, antihemorrhage, antioxidative stress properties, and antibacterial properties. Nowadays, essential oil of kaffir lime is in demand as a fragrance in the food, perfumery and cosmetic industries. Citrus hystrix leaf is rich in vitamin E. Among 62 edible tropical plants analysed for α-tocopherol content citrus hystrix leaves (398.3 mg/kg edible portion) ranked second behind saurupus androgynus (426.8 mg/kg edible portion) (Ching and Mohamed, 2001). Thirty-eight constituents were identified in the leaf essential oil of kaffir lime representing 89% of the total oil (Waikedre et al., 2010).

The oil was rich in monoterpenes (87%) with β-pinene as major component (10%) and low limonene (4.7%). The essential oil of citrus hystrix was characterized by high content of terpinen-4-ol (13.0%), α-terpineol (7.6%), 1,8-cineole (6.4%), and citronellol (6.0%). Twenty nine compounds were found in the essential oil of kaffir lime leaves (Loh et al., 2011) β-citronellal was the major compound amounting to 66.85% of total oil. Kaffir lime is identified as volatile compound and that product is generally volatile when exposed by air. therefore, protection in the way of encapsulation is needed.

*Corresponding author: Kharis Triyono, Departement of Agrotechnology, Faculty of Agricultural, University of Slamet Riyadi, Jl. Sumpah Pemuda no.18, Surakarta, Central Java 57136, Indonesia. E-mail: kharistriyono464@gmail.com Tel: +62271853839
Encapsulation is the process of coating liquid core material using certain encapsulant which makes the core particles have desired fisikokimia characteristic (Zuidam and Nedovic, 2010). Encapsulation can produce particle with micrometer down to nanometer diameter (Carvajal et al., 2010). And nowadays, encapsulation is done in nanometer size to make better carrying system (Ali et al., 2013). Low efficiency in encapsulation has become one main problem in the encapsulation process. The efficiency of the encapsulation is determined by the method and the materials used during the encapsulation process. Various kinds of natural polymer has been known for their ability to be used in producing nanoparticle (Bertolini et al., 2001) In this study, gum arabic and maltodextrin are used as the encapsulant of the combination coacervation and spray drying in the encapsulation process.

Gum Arabic is chosen because of its ability to form protecting coat with the coacervacion method because of its good emulating ability. Besides, during the process of spray drying, gum arabic can form a coat protecting the core material from oxidation, absorption, and evaporation (Pandy and Patel, 2013). Fajriani (2015) was able to make nanocapsule using gum arabic and gelatin with coacervationand freeze drying method resulting 64,81% efficiency. Maltodextrin was chosen for its ability to protect the core material from oxidation and its low viscosity on high solid concentration. Therefore, it is good to be used as the encapsulant material using spray drying method. Coacervation is done because of its simplicity. Moreover, it doesn’t need heat treatment, so it will prevent damage to the core material. Coacervation makes use of the macromolecule characteristic, containing kation and anion substances, to react electrostatically, forming the capsule (Tono et al., 2010). The use of hydrophilic materials in the encapsulation process has been known for producing the material will be separated easily from the coating material because of the same hydrophilic characteristic (Ahmed et al., 2009). The coating process in this study using gum arabic as the first coating and crosslinked with glutaraldehyde, maltodextrin as the coating will be used so that it will protect the surface essential oil. That is expected that encapsulation with high efficiency will be obtained. Furthermore, the aim of this study is to identify the best ratio between essential oil and encapsulant, and the best cross linking time to produce the high efficiency of nanoparticles.

MATERIALS AND METHODS

Sample kaffir lime (Citrus hystrix DC) leaves were colected from local orchid in Klaten, Central Java. Upon arrival at the laboratory, mature leaves were sorted out manually based on the size and color, dark green color with a glossy sheen and bright green. The leaves were washed under running tap water and used as experiments without crushing. The species was colected during March-April 2017.

The materials used in this research are kaffir lime oil, obtained from extraction in laboratory from this research. Maltodextrin (MD), and gum Arabic (GA). The chemicals used are glutaraldehyde 25% (Merck, Germany) as the cross linking agent. And the chemicals used to analyze are alcohol, ethanol, Galatic Acid (Merck, Germany), FeCl₃ (J.T Baker, USA), and Folinciocalteau (Merck, Germany). Kaffir lime oils staining is observed using microscope (olympus CX21LED biological microscope) and obitlab application (Advance, Indonesia), Spectrophotometer UV-Vis (Spectronic 200, Thermo Scientific) is used to test the total essential oil and the surface essential oil to calculate the encapsulation efficiency. Particle size is analyzed using Distribution Size Particle (Horiba SZ-100), and the nanocapsule morphology is observed using SEM (Scanning Electron Microscope JEOL JSM-5310 LV, Japan).

Procedure

Kaffir lime oils staining

Kaffir lime oil staining is conducted based on Ali (Bertolini et al., 2001) with modification. Yellow FeCl₃ 5% solution is added to the nanoparticle solution with 5% ratio of FeCl₃ solution: nanoparticle solution (3 drops: 1 ml). The mixture is then stirred slowly for 30 minutes. The colored nanoparticles solution is then observed with the light microscope (Advanced, Indonesia) using OPTILAB application.

Total essential oil

Total essential oils analysis method is conducted based on Ali (Bertolini et al., 2001) with modification. 0,1 gr of kaffir lime oil nanocapsule is weighed and diluted to 100 ml using aquadest, taken as much as 1 ml (100x dilution) to put in the reaction tube then 1ml of saturated NaCO₃ solution is added to the test tube and incubated for 10 min at room temperature. Then 0.5 ml of the folinciocalteau (Chemix CV, Yogyakarta) reagent and 7.5 ml aquadest were added, the mixture homogenized using vortex and then incubated for 30 min at room temperature under dark environmental conditions. Absorbance of the sample was then measured using a UV-vis spectrophotometer at a wavelength of 770 nm. The total phenol content of the sample was interpreted to be equivalent to gallic acid based on the standard curve of obtained gallic acid.

Surface kaffir lime oils

Surface essensial oil content analysis is based on the study of Cilek (Young et al., 1993) with modification. A total of 100 mg of nanocapsule kaffir lime powder sample is weighed and dissolved into 1 ml of ethanol solution: methanol (50:50), then the mixture was homogenized using vortex (Maxi Mix II) for 1 minute. After that, the mixed solution is filtered using filter paper. The solution that
passed from filtration was then taken and weighed as much as 100 mg then diluted to 10 ml (100x dilution). One ml of solution from dilution was taken and inserted into the test tube, then added 1 ml saturated Na2CO3 solution and incubated in room temperature for 10 min. After that, the folinciocalteu reagent (Chemix CV, Yogyakarta) was added as much as 0.5 ml and the sample was incubated for 30 min at room temperature with dark environmental condition. The sample absorbance was observed at a wavelength of 770 nm using a UV-Vis spectrophotometer and the results of the data were interpreted in the level of gallic acid equivalent based on the obtained standard error curve of the gallic acid.

**Encapsulation efficiency**

The efficiency of encapsulation is calculated by comparing the essential oil concentration trapped in encapsulation with the total essential oil concentration and interpreted in percentage form. The encapsulation efficiency is calculated by the following formula:

\[
\text{Efficiency} = \frac{\text{total essentialoil concentration (mg / g sample)} - \text{surface essentialoil (mg / g sample)}}{\text{total essentialoil (mg / g sample)}} \times 100\%
\]

**Characteristics of Encapsulation Morphology**

The morphological characteristics of kaffir lime nanocapsule were observed by using Scanning Electron Microscopy (SEM). The kaffir lime nanocapsule powder is shown on a two-sided adhesive placed on an aluminum cradle. Then the sample is coated using gold and then observed under SEM with 1000x magnification.

**Particle Size Distribution**

The particle size distribution of kaffir lime oils nanocapsules was analyzed using the Horiba SZ - 100 Nanopartica tool. The nanocapsules and nanoparticles were dispersed into aquades by dilution of 10 x and then inserted into the tube and measured into the Horiba SZ-100 tool.

**RESULT AND DISCUSSION**

**Essential Oil Staining**

One method that can be used to prove the compounds in kaffir lime oils is successfully encapsulated is by essential oil staining with ferric chloride (FeCl3) compounds. Ferric chloride will react with several organic compounds and produce a certain color according to the type essential compounds in the organic compound (Soloway and Wilen, 1952).

The results of the phenol staining analysis show the reaction between phenol contained in kaffir lime. The reaction is shown that in non-ferric chloride-added aqueous essential oil is not reddish yellow color only transparent round spots, while in ferric chloride- Round spots with reddish color. Observations on kaffir lime solutions which have been added with gum arabic coating and maltodextrin or not added ferric chloride formed only clear circle with a slightly vague wall, but in figured can be seen when it has been given the addition of ferric chloride visible sphere to reddish color inside and has a real wall. The observations indicate that the formula used can trap essential oil in nanocapsules.

**Nanoencapsulation Efficiency**

Efficiency nanoencapsulation obtained is proportionate with concentration increase of the coating toward the total solid kaffir lime oils. Bigger ratio of the concentration results in bigger efficiency. At the concentration comparison of 1:0 between the kaffir lime and the encapsulant, the average efficiency is 73.56%, at the concentration comparison of 1:1.5, the average efficiency is 74.03 %, and at the concentration comparison of 1:1.5 between the kaffir lime and the encapsulant 1:3 the highest average efficiency obtained at 79.07%. ANOVA test result significance rate (p<0.05) shows that between the 1:0 and 1:1.5 concentration comparison, there is no significant result, and at the concentration comparison of 1:3, significant result toward efficiency is obtained. The result obtained is appropriate with stating that the usage of gum arabic 15% results in higher encapsulant efficiency compared to the usage of gum arabic10%. Young et al., (1993) and Yanuwar et al., (2007) explain that encapsulant efficiency decreases along with the increase of the core material. The increase of the core material cause the decrease of the encapsulant viscosity.

The duration of cross-linking happens on gum arabic by glutaraldehyde affects on the efficiency of nanoencapsulation like in Table 1. Base on Table 1 shown that the duration of cross-linking affects differently on different concentration comparisons. From the results, the highest efficiency is obtained at 13 minutes cross-linking duration with concentration comparison of 1:3. ANOVA test result at significance rate (p<0.05) shows that on 13 minute duration with 1:1.5 concentration comparison and 1:3 concentration comparison have higher efficiency compared to the 0 minute and 26 minute duration. Therefore on the 13 minute duration with 1:0 comparison concentration, significantly lower efficiency is obtained. This occurs because the encapsulant doesn't react with glutaraldehyde, so the wall protecting kaffir lime oils is not
formed. While, on 13 minute duration with 1:1.5 concentration comparison and 1:3 concentration comparison, the highest efficiency is obtained because the cross-linking process on the proper condition to produce the desired level of hardness in which during the homogenization process.

The longer the cross-linking duration between gum arabic and glutaraldehyde, the harder the encapsulant wall would be, this would result in longer core material release duration Chang et al., (2006) and (Pandya and Patel, 2013). Furthermore, on 0 minute cross-linking duration, it can be concluded that the cross-linking process doesn’t happen which makes small efficiency because of high amount of uncapsulated essential oils. Besides, on 26 minute cross-linking duration, the process has been going for too long which also makes small efficiency because the encapsulant wall will be too hard to be broken down. When the polymer cannot be broken down during the homogenization process, it would result in bigger size and the core release duration would be longer. It will also make the maltodekstrine, as the second wall, unable to react with the gum arabic. Smaller efficiency is also caused by improper coating. At 1:1.5 and 1:3 concentration comparison, efficiency obtained is bigger compared with 1:3 concentration comparison in 0 and 26 minute durations. Hence, in this study, the amount of cross-linking agent is not varied. Still, the amount of gum arabic being cross-linked is varied, this indirectly result in varying amount of cross-linking agent reacting with gum arabic. Alvim and Grosso (2009) explained, with higher amount of cross-linking agent, the harder the cross-linking would be made.

Table 1: The effect of time on efficiency at various concentrations

<table>
<thead>
<tr>
<th>Time (minute)</th>
<th>Kaffir lime concentration: gum arabic (b/v)</th>
<th>Efficiency (%)</th>
<th>Kaffir lime concentration: gum arabic (b/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:0</td>
<td>71,21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70,71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1:1.5</td>
<td>73,69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79,94&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>75,79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71,65&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>-<sup>e</sup> values with different superscript within a column are significantly different (P<0.05)

**Particle Distribution**

The size of overall nanoencapsulated before spray drying has a size of 457.87 nm. The results of the particle distribution measurements prove that the nanoencapsulation formula using coacervation of gum arabic coated with maltodextrin succeeds in producing nanometer-sized particles. This study looks at the comparison of particle size distribution after and before spray drying.

The particle distribution graph of the kaffir lime nanoencapsulation formula after spray drying. Nanoparticles as a whole has a size of 318.45 nm. The size of the nanoparticles smaller than that of nanocapsule is due to the fact that at the time of the manufacture of kaffir lime nanoparticles, homogenization is done so that the resulting average particle size is smaller. After spray drying the nanocapsule has an average larger size spread due to the formation of aggregation between the powders. Agglomeration occurs due to maltodextrin. Used of maltodextrin as an ingredient will result in poor wall structures, since maltodextrins can form agglomeration and caking on flour products.

**Nanocapsule Morphology**

The nanocapsule morphology affects the properties of the nanocapsules formed. One example of an affected trait is the rate at which the active compound is released. The observation of nanocapsule morphology was done using Scanning Electron Microscopy (SEM) to find out the form of nanocapsule. This morphological analysis was performed only on samples with the highest efficiency in the ratio of kaffir lime oils and gum arabic concentration in 1:3 at cross-linking time of 13 minutes. Observations can be seen in figure. 1. From the results of observation nanocapsule with the best efficiency can be known form nanocapsule produced from nanoencapsulation formula by conservation and spray drying method.

![Figure 1. Nanocapsule morphology with highset efficiency](image)
From figure 1 shown that the nanocapsule of the optimum treatment has a round or shrink shape. The shrinking form can occur because the encapsulation can’t sustain the form when there is rapid evaporation during the spray drying process. In chamber spray drying the air temperature used reaches 140°C and the evaporation of water is very fast. Similar results can be found in the research of (Ali et al., 2013) where the capsule formed is also round and waved due to very rapid solvent evaporation. In nanocapsule morphological observations, a broken capsule is not found, this proves by preceding the process of encapsulation with coacervation make the capsule wall becomes strong.

An uneven form of the surface of the nanocapsule indicates that the second coating or maltodextrin does not react well with gum arabic as the main coating. Not reacting maltodextrin with gum arabic makes maltodextrin stand alone to maintain evaporation of water when drying with spray drying. No reaction between maltodextrin and gum arabic could well be caused by long cross-linking, so that within 13 minutes of cross-linking resulting in the highest efficiency, excessive cross-linking is still occurring.

CONCLUSION

Nanocapsule with uneven surface morphology, a mean size of 457.87 nm with an efficiency of 79.07%. was obtained through the incorporation of coacervation and spray drying methods, by comparison of concentration and cross-linking time between gum arabic and glutaraldehyde for 13 minutes and the ratio of kaffir lime and gum arabic 1:3.

ACKNOWLEDGEMENTS

This research was supported by Terapan Reseach research grant funded by Ministry of Research, Technology, and Higher Education of Indonesia (No. 218/S9/AK/2017).

REFERENCE


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