Food Research 8 (Suppl. 1) : 60 - 66 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Ultrasound-assisted encapsulation of citronella oil (*Cymbopogon nardus*) in alginate/carrageenan beads using ionic gelation method

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Article history:
Received: 7 March 2023
Received in revised form: 11 October 2023
Accepted: 17 December 2023
Available Online: 3 March 2024

Keywords:
Encapsulation,
Ultrasound,
Citronella oil,
Alginate,
Carrageenan

DOI:
https://doi.org/10.26656/fr.2017.8(S1).9

Abstract

The objective of this research was to determine the effect of ultrasound-assisted encapsulation of citronella oil using alginate/carrageenan (Alg/Carr) with the presence of SDS surfactant. The functional groups of beads particles were analyzed by fourier transform infrared spectroscopy (FTIR) and morphological was characterized by scanning electron microscope (SEM) analysis. Fourier transform infrared spectroscopy (FTIR) results showed a sharper peak at 2922 cm^{-1} as the C=C vibration of the methyl group of aromatic compounds in citronella oil, indicating an increase in encapsulation efficiency with ultrasound treatment. The ultrasonication process also causes the C-H bond (1426 cm^{-1}) to break, resulting in polymer degradation. The SEM results show that ultrasonication causes the presence of cavities or pores in the cracked wall. In this study, the use of ultrasound during the encapsulation of citronella oil in Alg/Carr enhances the encapsulation efficiency to 95-97%.

1. Introduction

Citronella (*Cymbopogon nardus*) belongs to the family Panicodiae of Graminales and is widely used in pharmaceuticals, food/beverage industries, fragrances and cosmetic ingredients (Shah *et al*., 2011; Olorunnisola *et al*., 2014; Pari, 2020). Citronella oil is yellow and has the main compounds, namely citronellal (37.87%), geraniol (19.88%) and citronellol (9.11%) (Loko *et al*., 2021). Citronellal has pharmacological activities, such as antifungal, antibacterial, anti-inflammatory, antioxidant and antivirus (Bota and Martosupono, 2015; Ekpenyong, Akpan and Nyoh, 2015; da Silva *et al*., 2020; Prasad *et al*., 2022). In addition, it can withstand two compositions of platelets and cure diabetes, gastrointestinal infections, depression or anxiety, pneumonia, and malaria. In industry, it functions as a flavoring agent, additive and preservative in food and beverages (Akono Ntonga *et al*., 2014; Avoseh *et al*., 2015; Chukwuocha *et al*., 2016; Nivetha *et al*., 2016; Oladeji *et al*., 2019). However, citronella bioactive compounds also have unstable properties such as poor bioavailability, low solubility, rapid release, and easily damaged in case of environmental stress, as well as conditions related to processes such as temperature, pH, exposure to light, oxygen and time storage (Rouf *et al*., 2020; Smaoui *et al*., 2021). Encapsulation is a method of entrapment of a compound on the outer layer or matrix. This method is beneficial for increasing the bioavailability of bioactive and solubility and is a well-accepted method for increasing efficacy (Patel *et al*., 2021).

Encapsulation technology can deliver bioactive components and improve their handling properties. The encapsulating substance is called the external phase, coating material, or carrier substance or shell, while the encapsulated substance is called the internal phase, active agent or core material. Polysaccharides are the most widely used material for encapsulation in food applications among all materials (Nedovic *et al*., 2011). Zhang *et al.* (2016) report that encapsulation of β-galactosidase enzyme into carrageenan-based bead hydrogels improved the stability and activity of the enzymes. The ionic gelation method is widely used for encapsulating bioactive compounds. This technique has advantages compared to other forms, which are convenient, easy and inexpensive (Hosseini and Varidi, 2021) In conventional ionic gelation encapsulation, emulsion droplet sizes are large and non-uniform. Using ultrasound as a homogenization tool can effectively reduce the size of emulsion droplets down to the nano-size level resulting in better emulsions than conventional emulsion production methods, resulting in higher efficiencies (Silva *et al*., 2015).

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

Ultrasound can promote emulsification and depolymerization to form biopolymer cross-links and microspheres (Leong *et al*., 2017). Several applications in emulsion formulations have been successfully performed using ultrasound, resulting in size reduction, high precision, low polydispersity (homogeneity), and no particle agglomeration (Abbas *et al*., 2013). The sonochemical experimental technique is relatively simple, and stable core–shell materials can be produces quickly. In addition, this technique offers flexibility in selecting different shell and core materials.

The main objective of this study was to encapsulate citronella oil into polysaccharide (alginate and κ-carrageenan) particles to increase its stability and bioavailability using ultrasound treatment. These polysaccharides were ionically cross-linked with calcium chloride. The structure (functional groups) of obtained beads was analyzed using Fourier transform infrared (FTIR), and the morphological was characterized by scanning electron microscopy (SEM). The encapsulation efficiency and swelling behavior evaluation were performed in buffer solution pH 7. The porosity of the polymer matrix can be controlled by varying the concentration of the citronella oil and surfactant. On the other hand, we also evaluated the effect of ultrasound-assisted encapsulation using different time variables.

2. Materials and methods

2.1 Materials

Citronella oil was obtained from Darjeeling Sembrani Aroma Ltd., Bandung, Indonesia, with specific gravity specifications at 25°C: 0.876-0.890 with CAS Number 8000-29-1. The chemical used include sodium alginate (CAS Number 9005-38-3 with a molar mass of 216.12 g/mol obtained from SIGMA-Aldrich, USA), carrageenan, sodium dodecyl sulfate (SDS), calcium chloride ($CaCl_2$), sodium hydroxide (NaOH), hydrochloric acid (HCl), and buffer solutions were obtained from Merck Chemical Co. (Darmstadt, Germany). All reagents were analytical grade.

2.2 Alginate and carrageenan solution preparation

The bead-forming materials, such as sodium alginate powder and kappa carrageenan, were each dissolved in 100 mL of distilled water (with a ratio of 2% w/v and 1% w/v, respectively), stirred at 500 rpm for 45 mins and the temperature was adjusted at 30°C.

2.3 Encapsulated citronella essential oil

The alginate and carrageenan solution were made as in the previous step. Followed by mixed alginate solution with surfactant sodium dodecyl sulfate (SDS) 0.5% (w/v) and citronella oil (8%, 10%, 12%, 14% and 16% v/v) rotated using a magnetic steering wheel for 20 mins. Then the mixed solution is sonicated using an ultrasonicator. Different times for ultrasonication were used: 0 min, 4 mins, 6 mins, 8 mins, 10 mins and 12 mins. The results of ultrasonication were added with 1:1 (v/v) carrageenan and rotated using a magnetic stirrer until homogeneous for 15 mins, then dripped the mixture with a syringe into a $CaCl_2$ solution (0.2 M). The droplets of the mixture form beads in $CaCl_2$ solution. Wait 30 mins. Strain the beads, and dry them at 30°C for 48 hours.

2.4 Encapsulation efficiency

Encapsulation efficiency was calculated by substracting the amount of bioactive losses from Alg/ Carr beads after being dropped into $CaCl_2$ solution from the initial amount of bioactive added to alginate/ carrageenan mixture. This measurement was evaluated by indirect method. Added 2 mL of $AlCl_3$ and 2 mL of KCH_3COO to the 2 mL of $CaCl_2$ solution after gelation process. Then, the amount of bioactive compounds that was successfully encapsulated was determined using the Spectrophotometer Genesys 20 UV-Vis at a wavelength of 367 nm:

$$\%EE = \frac{Qt - Qr}{Qt} \times 100\%$$

Where *Qt* is the total quantity of citronella oil's bioactive, and *Qr* is the quantity of bioactive contained in the citronella oil that is lost into the $CaCl_2$ solution after the ionic gelation process.

2.5 Swelling analysis

The swelling ratio was calculated by weighing citronella loaded alginate/carrageenan dried beads, then 0.2 g of citronella loaded alginate/carrageenan beads were immersed into 30 mL of buffer solution with pH 7. After 30 mins, the swollen beads are weighed and the swelling ratio of citronella loading alginate/carrageenan beads was calculated using the equation.

$$Swelling = \frac{Ms - Md}{Md}$$

Where *Ms* and *Md* is the mass of the swollen beads and dried beads, respectively.

2.6 Scanning electron microscopy

The results of encapsulation before and after ultrasonication were analyzed for surface morphology using the scanning electron microscope JEOL JSM-6510LA. SEM magnification is 7500×.

https://doi.org/10.26656/fr.2017.8(S1).9

2.7 Fourier transform infrared

The composition (functional group) of citronella loaded alginate-carrageenan beads before and after sonication were analyzed using FTIR (Perkin-Elmer PC1600, US). Scanning was performed in the range of 4000-450 cm^{-1}.

3. Results and discussion

3.1 Encapsulation efficiency

In this research, the effect of variables such as the concentration of sodium dodecyl sulfate and citronella oil concentration and sonication timer on encapsulation efficiency were analyzed and discussed. Table 1 shows that the encapsulation efficiency of citronella oil with the addition of SDS has increased. The encapsulation efficiency of citronella oil with the addition of SDS showed an increase. The lowest encapsulation efficiency is 94.98% at 0.1% SDS concentration, and the highest is 96.86% at 0.9% SDS concentration. As the sodium dodecyl sulfate (surfactant) concentration increases, so does the amount of solubilized citronella oil in the mixture, resulting in higher encapsulation efficiency. The encapsulation efficiency of citronella oil into alginate-carrageenan beads was remarkably increased by modifying the alginate-carrageenan beads with SDS addition. It was principally in consequence of the interaction between citronella oil and SDS, which was expected to slow down the diffusion of citronella oil from the depth of the cavity (Kahya and Erim, 2019).

The addition of citronella oil also improved the hydrogel encapsulation efficiency as shown in Table 1. In this study, the encapsulation efficiencies obtained were in the range of 97%, with the addition of 16% citronella oil yielding the highest encapsulation efficiency of 97.48%. This can be ascribed to the increased hydrophobic interactions between citronella oil and alginate-carrageenan, which makes citronella oil tightly packed by the wall materials. A previous study by Ren *et al*. (2022) showed that encapsulation efficiency increased with increasing concentration of curcumin. The research of Guo *et al*. (2018) also showed that variations in the concentration of the encapsulated compound would increase the results of encapsulation efficiency, although it is not too significant. The concentration of citronella in accordance with the matrix made may increase the value of the encapsulation efficiency.

Table 1 also shows that sonication time greatly influences the encapsulation efficiency. Encapsulation efficiency is improved by checking at the exact time from 0 to 10 mins. At 10 mins, the highest encapsulation efficiency reached 97.74%. The encapsulation efficiency decreased as the sonication time increased from 10 to 12 mins. It is because the sonication process opens up the polysaccharide structure and breaks more glycosidic bonds. A potential explanation for this is that excessive sonication may induce polysaccharide unfolding and aggregation, resulting in reduced encapsulation efficiency (Ma *et al*., 2021). Sonication causes a decrease in particle size and an increase in surface area, which makes the coating process easier and provides high encapsulation efficiency (Ameer and Maraie, 2019). On the other hand, increasing the sonication time to 12 min could lead decrease in encapsulation efficiency (97.54%), indicating that excessive sonication could lead to polymer degradation leading to a decrease in its ability for complete coating of the particles. Prolonged application of ultrasonic treatment is not recommended as it may damage the bioactive compounds present in the

Table 1. Experimental details of the encapsulation of citronella oil in alginate-carrageenan wall material in the presence of ultrasonic irradiations

Run	SDS Concentration (% v/v)	Citronella oil concentration (% v/v)	Sonication time (min)	% Encapsulation efficiency
1	0.1	10	8	94.98
2	0.3	10	8	95.64
3	0.5	10	8	95.72
4	0.7	10	8	96.22
5	0.9	10	8	96.86
6	0.7	10	8	96.22
7	0.7	12	8	97.09
8	0.7	14	8	97.31
9	0.7	16	8	97.48
10	0.7	16	4	97.17
11	0.7	16	6	97.41
12	0.7	16	8	97.48
13	0.7	16	10	97.74
14	0.7	16	12	97.54

https://doi.org/10.26656/fr.2017.8(S1).9

formulation.

3.2 Swelling

The addition of SDS will reduce the swelling of the hydrogel. As shown in Figure 1, SDS concentrations between 0.1 and 0.9% yielded swelling ratios between 10.01 and 7.13. The swelling concentration of 0.9% is lower than 0.1%. SDS has a critical micelle concentration (CMC) value of 8 mmol/L (0.2% w/v). In this study, the concentration of SDS used was above the critical micelle concentration (CMC). When SDS is added to the medium above the CMC value, alginate acquires electrophoretic mobility and the density of molecules containing negative charges in the Alg/Carr/SDS association increases. This additional charge provides a larger surface area for adsorption of divalent cations and more cross-linking than beads without SDS (Kahya and Erim, 2019). The increase in SDS concentration causes a decrease in the swelling ratio due to the presence of a stronger cross-link, and many beads will be challenging to swell (Hariyadi *et al*., 2014). When swelling is low, the beads' disintegration rate decreases and shows more stable behavior, which relates to the formerly reported study that SDS increased the stiffness of alginate beads (Kaygusuz *et al*., 2016). The quantity of loaded citronella reduces the swelling ratio of the beads. Beads with low levels of citronella have abundant hydroxyl groups as hydrophilic groups that provide to water absorption. Nevertheless, increasing the citronella loading within the beads results in less swelling, as it can occupy part of the hydrogel space structure, which will limit the water absorption capacity (Chen *et al*., 2019).

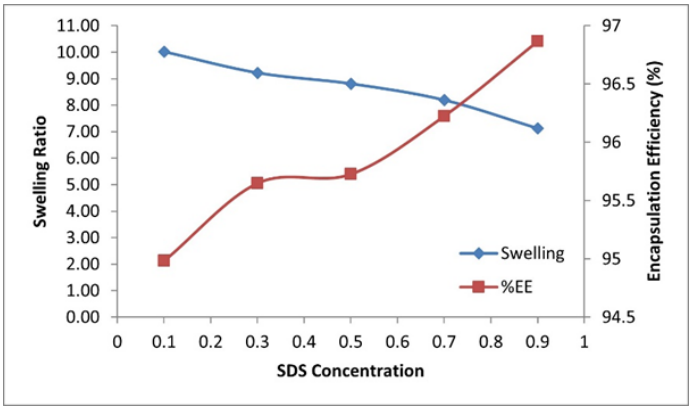

Figure 1. Effect of SDS Concentration on swelling ratio.

3.3 Scanning electron microscopy analysis

Surface morphology of citronella-loaded alginate/carrageenan beads with and without ultrasound treatment were examined by SEM at 7500× magnification. In Figures 2A and 2B (SDS added), the SEM results show voids in the crack walls. Beads show that the surface is leafy with many cavities (Kahya and Erim, 2019). The structure arises because the interaction between the biopolymer (Alg/Carr) chains and sodium dodecyl sulfate is under the critical agglomeration concentration and forms a chain-like complex. In this study, it can be assumed that the exact interaction between alginate and carrageenan biopolymers and SDS led to profound morphological changes and increase the stiffness of alginate/carrageenan beads (Kaygusuz *et al*., 2016).

The figure shows that alginate and carrageenan encapsulants can trap citronella oil. The resulting particles treated by ultrasound (Figure 2C and 2D) showed a different morphology than those prepared by traditional methods (Figure 2A and 2B). On the surface of the bead, dark pores and longitudinal cracks are detected. It exhibits significant damage to the bead surface due to the excessive energy released by collapsing the ultrasonic cavitation bubbles, which induces stress and shear forces. The forces causing mechanical degradation of the external layer of alginate and carrageenan, thereby reducing the particle size, mainly results in multiple channels, fissures, and cracks on the grain surface. The ultrasound breaks the glycosidic bonds and loosens the tightly packed particle structure, providing larger area for the penetration of surrounding material (Falsafi *et al*., 2019).

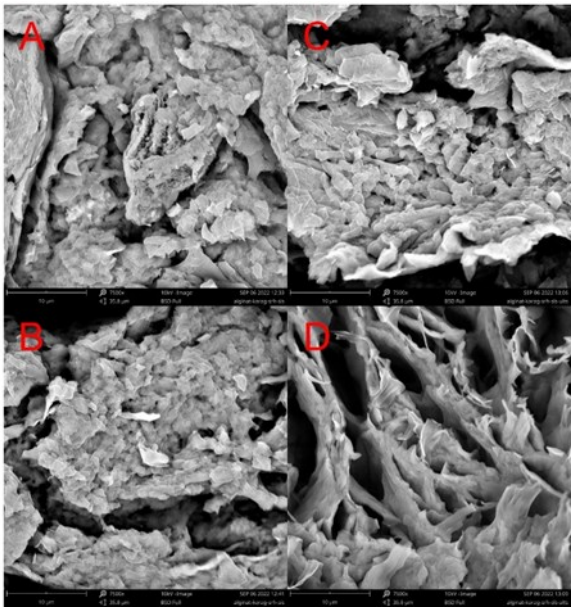

Figure 2. Scanning electron microscopy images (SEM) of encapsulation citronella oil bead. A and B: non-ultrasound, C and D: ultrasound.

3.4 Fourier transformed infrared

The spectra of microcapsules containing citronella oil are presented in Figure 3. The FTIR spectra of ultrasonically treated microcapsule granules were compared to conventional microcapsules (with and without the addition of surfactants), empty Alg/Carr granules, and citronella oil.

https://doi.org/10.26656/fr.2017.8(S1).9

In the spectrum of citronella oil from Figure 3a, 1730 cm^{-1}, 2368 cm^{-1}, and 2922 cm^{-1} were associated with symmetrical and asymmetric C=C vibrations and C-H strain of methyl groups of aromatic compounds in citronella oil. The absorption band at 2368 cm^{-1} indicates the presence of an aldehyde group. In the spectrum of Alg/Carr from Figure 3b, the absorption at 891 cm^{-1} was assigned to vibration of galactose-6-sulfate which is Carr. There is also an intense band at 1094 cm^{-1} caused by the valence vibration of the C–O bond. An absorption band is also seen at 1527 cm^{-1} due to the asymmetric and symmetrical vibrations of the carboxylate anion of the Alg. The peak of the 1020 cm^{-1} wave is the C-O absorption band, a group owned by Alg.

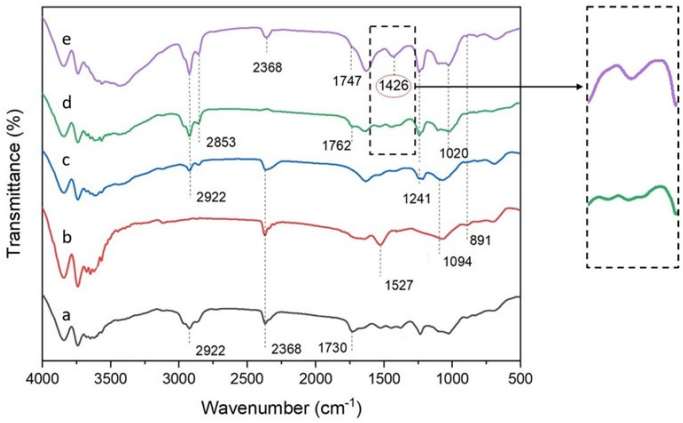

Figure 3 FTIR spectra for a) citronella oil; b) Alg/Carr; c) Alg/Carr/SDS; d) Alg/Carr/SDS/citronella oil (Non ultrasound); e) Alg/Carr/SDS/citronella oil (Ultrasound).

In Figure 3c, with the addition of SDS into Alg/Carr, a peak spectrum is formed at 1241 cm^{-1}, which is the stretch of the sulfate group S=O belonging to SDS containing a spectrum of beads. In Figure 3d and Figure 3e, it is known that the adsorption bandwidths between 2853 cm^{-1}, 1762 cm^{-1}, and 1747 cm^{-1} are the vibrations of the C=C and C-H bonds stretching the methyl groups of aromatic compounds in citronella oil and proved that citronella oil smells good and is successfully packaged in Alg/Carr wall material. As shown in Figures 3d and 3e, the properties of citronella oil were found to have little effect on functional groups by ultrasound treatment. In the spectrum, Alg/Carr/SDS/citronella oil using the US from Figure 3e shows a sharper peak at 2922 cm^{-1}, which is the C=C vibration of the methyl group of aromatic compounds in citronella oil. Indicates that using ultrasound increases citronella content in the beads, which increases encapsulation efficiency. There is also sharp band at 1426 cm^{-1}, which shows the C-H bond. The ultrasonication process causes the C-H bond to break, resulting in polymer degradation.

Furthermore, citronella oil was encapsulated in the Alg/Carr wall material in the presence of ultrasound. Therefore, ultrasonication does not significantly affect the FTIR spectrum of encapsulation of citronella oil. This is in accordance with research by Liang *et al.* (2018), where stronger hydrogen bonds and electrostatic interactions cause changes in the secondary structure of zein in resveratrol encapsulation.

4. Conclusion

In this study, citronella oil encapsulated microspheres were successfully encapsulated using ultrasound-assisted in alginate-carrageenan with the presence of SDS. The encapsulation efficiency of citronella oil using ultrasound-assisted in alginate-carrageenan increased to 95-97%. The FTIR spectrum shows that ultrasonication increases encapsulation efficiency. A sharper peak indicates it at 2922 cm^{-1}, the C=C vibration of the methyl group of aromatic compounds in citronella oil. The ultrasonication process causes a change in the spectrum at the peak of 1426 cm^{-1} where the C-H bond is broken and causes polymer degradation. The SEM images show that the microparticles have a leafy structure with the addition of SDS and particles undergoing ultrasonic treatment are more porous, and the particle size is smaller and more uniform. The swelling ratio decreases with increasing SDS concentration which causes the rate of disintegration of the beads decreases and they show more stable behavior.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors acknowledge the financial support from the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, through PDUPT research program.

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