



Encapsulation of Clove Oil within Ca-Alginate-Gelatine Complex: Effect of Process Variables on Encapsulation Efficiency

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ABSTRAK

Karena memiliki khasiat seperti analgesik, minyak cengkeh biasa digunakan sebagai obat, antibakteri, antioksidan, dan antimikroba. Kemungkinan enkapsulasi minyak cengkeh sebagai makrokapsul padat dipelajari dengan pembuatan makrokapsul Ca-Alginate-Gelatine. Variabel proses yang digunakan adalah variasi konsentrasi alginat 1% dan 1,5% b / v, dan perbandingan massa antara alginat-gelatin divariasikan antara 1: 4, 1: 6, dan 1: 8 w / w. Selain itu, variasi konsentrasi CaCl₂ (10%, 20% dan 30% w / v) sebagai cross-linking agent pembentukan kompleks Ca-Alginate juga digunakan sebagai variabel proses. Peningkatan konsentrasi alginat, gelatin dan CaCl₂ nampaknya menurunkan efisiensi enkapsulasi karena terbatasnya volume ruang bebas yang terbentuk pada matriks Ca-Alginat-Gelatin. Efisiensi enkapsulasi tertinggi (93,08%) diperoleh pada penggunaan Alginat 1% w / v, dengan perbandingan alginat dengan gelatin 1: 4 dan ikatan silang dalam larutan CaCl₂ 10% w / v selama 15 menit.

Kata kunci: enkapsulasi, minyak cengkeh, Ca-Alginate-Gelatine, mikrokapsul.

ABSTRACT

Owing to the properties such as analgesic, clove oil is commonly used as medicine, antibacterial, antioxidant, and antimicrobial drugs. The possibility of clove oil encapsulation as a solid macrocapsule was studied by making Ca-Alginate-Gelatine macrocapsules. The process variables used were variations in Alginate concentration of 1% and 1.5% w/v, and the mass ratio between alginate-gelatine was varied between 1: 4, 1: 6, and 1: 8 w/w. In addition, variations in the concentration of CaCl₂ (10%, 20% and 30% w/v) as a cross-linking agent for the formation of Ca-Alginate complexes were also used as process variables. The increase of alginate, gelatine and CaCl₂ concentration seems to decreased the encapsulation efficiency because of the limitation of the free space volume formed in the Ca-Alginate-Gelatine matrix. The highest encapsulation efficiency (93.08%) was obtained in the use of Alginat 1% w/v, with a ratio of alginate to gelatine 1: 4 and cross-linking in a 10% w/v CaCl₂ solution for 15 minutes.

Keywords: encapsulation, clove oil, Ca-Alginate-Gelatine, macrocapsules.

1. INTRODUCTION

Clove essential oil is known to have various uses, one of which is its anti-microbial, antifungal and antioxidant properties. But with its many uses, clove essen oil has a weakness in its application because it is very easily oxidized, evaporates or even reacts with other compounds. In addition, clove oil is at risk of evaporating when it is related to

its volatility so that the clove aroma is feared to decrease. The technology of clove oil encapsulation or powdering can maintain the quality and aroma of cloves in cigarettes so that the taste and quality of cigarettes is maintained.

Encapsulation is the process of trapping an active compound in a matrix in the form of particulates to achieve one or more of the



desired properties [1]. The oil is encapsulated various for reasons, such as the conversion of liquid forms into solids, to facilitate transportation. Other reasons include maintaining taste/protection, protection from evaporation or oxidation, and controlled release applications. The encapsulation of various oils for nutrition, therapy, flavouring or aroma has been extensively studied, with examples including fish oil, alpha-tocopherol, wheat germ oil, lemon oil, and lemongrass oil. The choice of material and encapsulation process is arranged by three main criteria: application, economy and safety. At present, spray drying is the most commonly used method for oil microencapsulation, and wall materials used are usually carbohydrates, proteins and gums. The weight ratio of oil to wall material usually ranges from 0.1 to 1.0, however 0.2 to 0.5 is more common, and the composition of the encapsulated oil can reach 50% of the final weight of the product [2–4]. However, in some food and cosmetic products, higher oil content may be needed to achieve a high oil content (around 65-70%) using a simple extrusion process [5], in which oil is encapsulated in Ca-alginate sphere coated with chitosan. But now, the effect of process variables to achieve this goal is still poorly understood. Study conducted by Chan [6] shows that palm oil can be encapsulated at high levels by first making oil-water emulsions and dropping in bath settings to obtain Ca-Alginate spheres with oil encapsulated in them. Then the sphere obtained is dried in two ways, using an oven at 70 °C or using a freeze dryer for 24 hours.

2. EXPERIMENTAL

Encapsulation of oil was performed using emulsion extrusion technique. Emulsion extrusion is considered as the most common approach of encapsulation and might be achieved by emulsifying or dispersing the hydrophobic components in an aqueous solution where gelation occurred.

2.1. CLOVE OIL ENCAPSULATION

Clove oil encapsulation was carried out using an extrusion emulsion technique [7]. Where, sodium alginate was dissolved in demineralized water to produce alginate solution with a concentration of 1 and 1.5 w/v%, the solution was stirred for 15 minutes at a temperature of 70°C with a speed of 1000 rpm to release the trapped bubbles before use. Then the gelatine with a ratio of 1: 4, 1: 6 and 1: 8 from the mass of Na-Alginate was added to the solution while stirring until homogeneous solution formed. After that, clove oil (10 g) was dripped slowly into the solution followed by stirring at a speed of 1000 rpm for 45 minutes. In this step the oil was gradually added to the alginate solution to form a good emulsion (marked with the colour change to cloudy). Oil-alginate emulsions were then dropped into a solution containing calcium chloride (10, 20, or 30 w/v%). The resulting macrocapsules were allowed to harden in CaCl₂ solution for 15 minutes. The oil-alginate granules were then separated from the solution using a filter, rinsed twice with demineralized water, and to absorb excess water on the surface tissue paper was used. The macrocapsules were then dried using a freeze dryer for 24 hours and grinded to obtain a fine powder.

2.2. ENCAPSULATION EFFICIENCY TEST

Quantification of the amount of encapsulated oil was carried out by extracting oil from 0.5 g capsules through a dissolution process with 5 mL of sodium citrate solution (0.055 M) and 5 mL n-hexane for 2 hours. The amount of encapsulated oil in the hexane layer was determined using Gas Chromatography (GC). Encapsulation efficiency (EE%) was calculated by the following equation

$$EE(\%) = \frac{V_o}{V_i} \times 100\% \quad (1)$$

Where,

V_o = Volume of Clove oil in the macrocapsule.

V_i = Initial volume of clove oil.

2.3. PARTICLE MORPHOLOGY ANALYSIS

Particle morphology test was done for clove oil macrocapsule. Samples were observed using SEM. The magnification used is between 500x-2,000x. The morphology test was performed in the Research Laboratory of Material and Metallurgical Engineering, Faculty of Engineering, Institut Teknologi Sepuluh Nopember, Surabaya.

3. RESULTS AND DISCUSSION

3.1. CHARACTERISTICS OF EUGENOL AND CLOVE OIL

A typical histogram was obtained from gas chromatography with two main peaks which indicate eugenol (Peak 1) and caryophyllene (Peak 2) as can be seen in Figure 1. In

general, the steam distillation method is used for extracting clove oil where the oil produced contains the lowest amount of eugenol acetate which, but has a high amount of caryophyllene and humulene. A study conducted by Kegley et al. [8] reported that eugenol acetate in clove oil could reach levels as low as 0.5%. The oil used in this study comes from leaves, and the method used to obtain extracted oil is steam distillation. This could explain unidentified eugenol acetate, and other factors, as previously explained. In some literature [9], there are a number of compounds reported, for example 11-19 compounds in Indonesian clove oil. However, all studies show that the main chemical compound in clove oil is Eugenol.

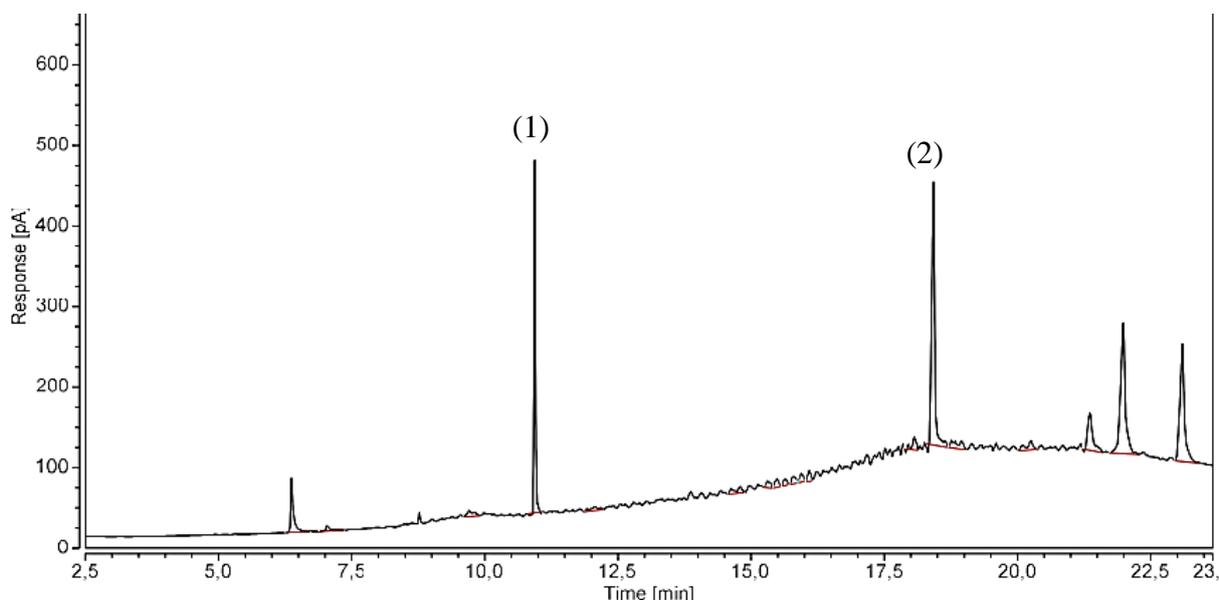


Figure 1. Typical GC-histogram of clove oil used in this work

3.2. CHARACTERISTICS OF MACROCAPSULE

Morphological studies in this work have been conducted using Scanning Electron Microscopy of the powder obtained from macrocapsules. Polymeric materials like the alginates used in this study produced skin-forming particles with a continuous non-liquid phase. The micrograph of the alginate powder/ macrocapsules of the best formulation in Figure 2 show the surface of

the capsules appear much wrinkled and is accompanied by a crater-like basin. This wrinkled surface can occur due to the drying process. Loss of water during the drying can empty the space previously filled with water and will make the surface wrinkled. The irregular craters scattered on the capsules become traps for oil and several lumps were found. According to Soliman et al [7], lumps are formed due to the deposition of oil droplets towards the outside of the capsules

which causes plasticization of the capsule structures. The appearance of the obtained macrocapsule indicate the structure of polycore encapsulation model. The volume of this cell cavity is affected by the amount of Alginate, Gelatine and CaCl_2 as a cross linker. The increase in number of these three ingredients generally will reduce the pore volume. An explanation of this can be discussed in the effect of each variable.

3.3 EFFECT OF ALGINATE CONCENTRATION

To get the better understanding of the effect of alginate concentration on the encapsulation efficiency, different alginate concentrations (1% and 1.5% w/v) were used at various concentrations of calcium chloride which were kept constant at 10%, 20% and 30% w/v and 15 minutes crosslinking time. The results are presented in Figures 3, which indicate that the encapsulation efficiency decreases with increasing alginate concentration.

A further increase in alginate concentration causes a decrease in loading capacity. This can be attributed to the formation of dense tissue structures with smaller void (pores) so that the ability to trap clove oil droplets is reduced. In other word increasing the concentration of alginate has a decreased free volume in the polymer matrix (compact structure with smaller pore size), and then the amount of oil that can be trapped inside the pores this will decrease. This assumption is supported by Sevda and Rodrigues's [10] findings which indicate that higher alginate concentration causes a decrease in the size of the pores formed in the resultant macro beads [11].

The use of alginate with a concentration of 1% w/v provides encapsulation efficiency greater than the concentration of 1.5% w/v. The highest efficiency of 93.08% was obtained at the concentration of alginate 1% w/v. Meanwhile, the highest efficiency for 1.5% w/v alginate concentration, efficiency was 38.04%.

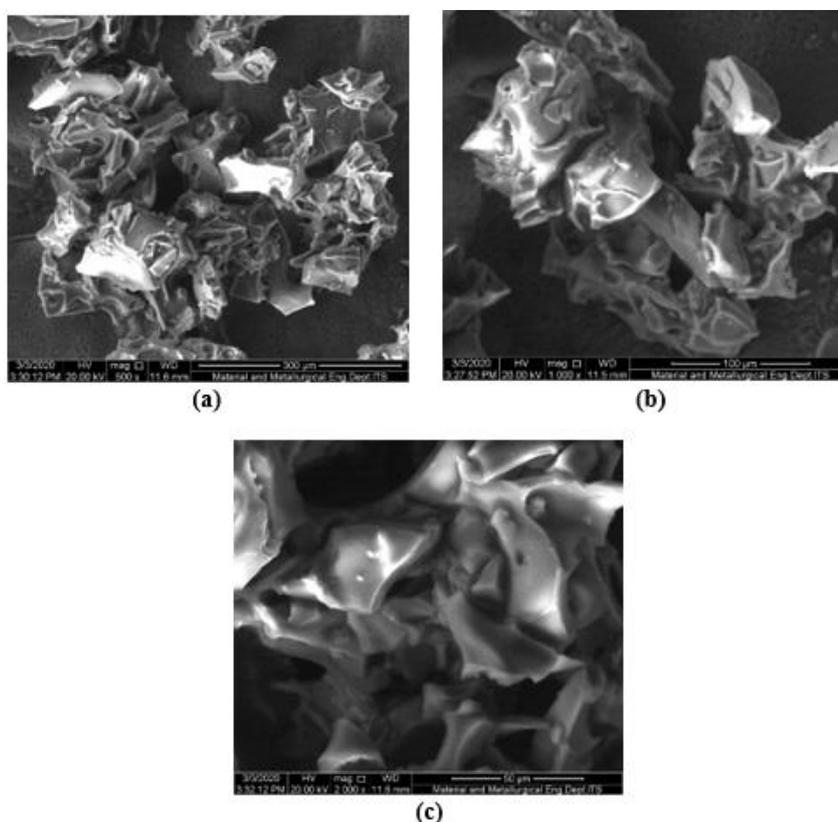
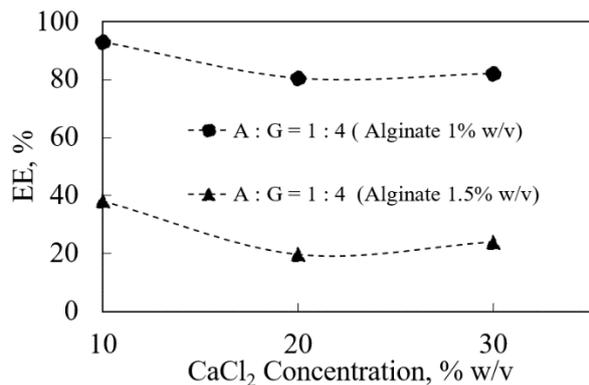
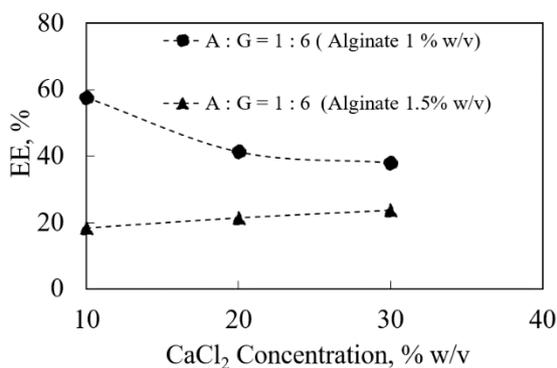


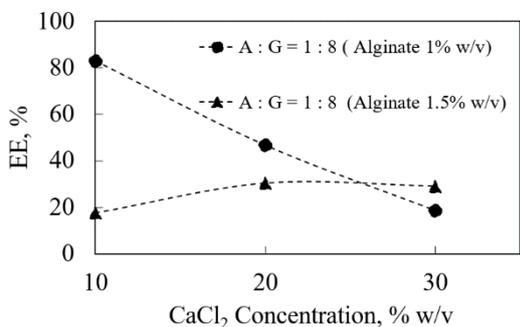
Figure 2. SEM graphs of Ca-Alginate-Gelatine Matrix loaded with Clove Oil with magnification of (a) 500x; (b) 1000x; (c) 2000x.



(a)



(b)



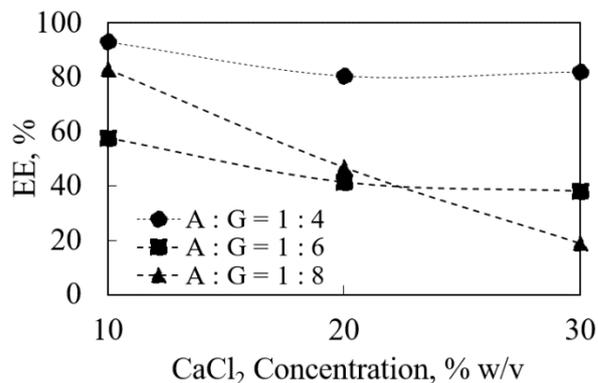
(c)

Figure 3. Efficiency of clove oil encapsulation using 1% and 1.5% w/v Alginate concentrations with a ratio of Alginate to Gelatine (a) 1: 4; (b) 1: 6; (c) 1: 8. at various CaCl₂ concentrations

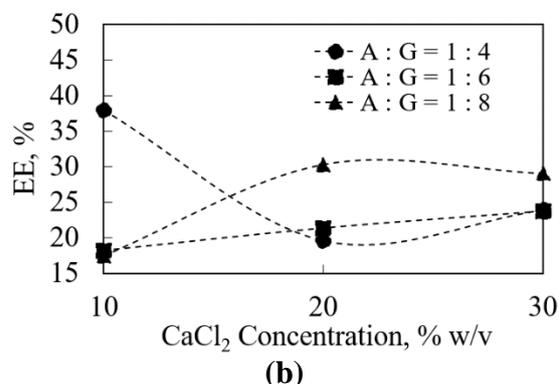
3.4 EFFECT OF GELATINE CONCENTRATION

The effect of gelatine, an anionic biopolymer, on the encapsulation efficiency has been studied by varying the amount of gelatine in the range of 1: 4, 1: 6 and 1: 8 w/w of the

alginate. The effect of variables on encapsulation efficiency is shown in Figure 4.



(a)



(b)

Figure 4. Efficiency of clove oil encapsulation with (a) 1% w/v; (b) 1.5% w/v Na-Alginate concentration at various Gelatine and CaCl₂ concentration.

The decrease in encapsulation efficiency can be attributed to gelatine which resulting in increased interaction between functional groups on gelatine and alginate molecules which consequently increases the degree of cross linking so that the capsule becomes more compact and the available free space becomes smaller [11].

3.5 EFFECT OF CaCl₂ CROSS-LINKER CONCENTRATION

Calcium chloride, the crosslinking agent for alginate matrix shown in Figure 5, is assumed to act by complexing the carboxylic anion in alginates with bivalent calcium ions, thereby

forming a three-dimensional network (egg-box model).

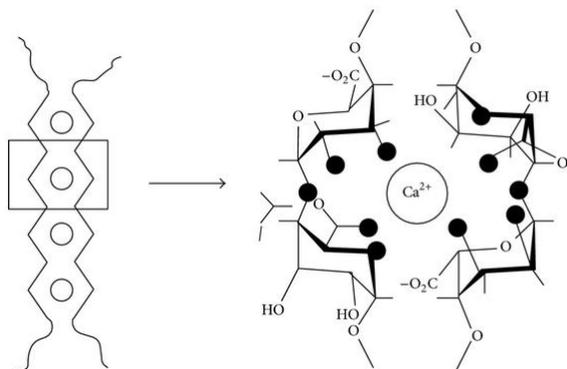


Figure 5. "Egg-box" model for Calcium Alginate.

In our work the cross-linker effect on encapsulation efficiency was studied by varying the concentration of CaCl_2 at the range of 10%, 20% and 30% w/v. The results shown in Figure 3 and 4 clearly show that efficiency continues to decrease by increasing the concentration of cross-linkers. Whereas at the Alginate concentration of 1.5% w/v with a ratio of alginate to gelatine 1: 6 and 1: 8 encapsulation efficiency increased but the amount was not significant. What is worth noting is that a decrease in efficiency may be due to the fact that with increasing amounts of calcium ions in cross-linking solutions, alginate capsules containing smaller cavities are produced that accommodate less amount of water and thus reduce swelling of the polymer capsule [9].

3.6 MACROCAPSULE STABILITY

The stability of the obtained macrocapsule was observed with changes in shape when placed in environmental conditions. The observations show that the obtained macrocapsule product has a fairly low stability. After being left in the room for 3 weeks, deformation occurs and the encapsulated oil comes out of the capsule. It seems that there is a mismatch between the oil and the chosen wall material so that it cannot maintain the macrocapsule structure for a long time.

4. CONCLUSION

The use of higher alginate, gelatine and CaCl_2 concentrations will reduce the efficiency of clove oil encapsulation because it decreased the free space / pore volume formed in macrocapsules. In this study, the highest concentration obtained at the concentration of alginate 1% w/v was 93.08%. Meanwhile, the highest efficiency for 1.5% w/v alginate concentration, efficiency was 38.04%. The stability of the macrocapsule were rather low, deformation occurs and the encapsulated oil comes out of the capsule after 3 weeks being left in room condition.

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